2009-2010

वार्षिक रीपोर्ट

Annual Report

VACCINATE YOUR CHILDREN
GIVE THEM A HEALTHIER LIFE

Global warming

राष्ट्रीय कॉलरा और आंत्र रोग संस्थान

(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES

(INDIAN COUNCIL OF MEDICAL RESEARCH)

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(INDIAN COUNCIL OF MEDICAL RESEARCH)

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

सत्यमेव जयते

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
(स्वास्थ्य अनुसंधान विभाग)
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
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 extremely pleased to extend my whole-hearted appreciation of the significant research work being carried out at National Institute of Cholera and Enteric Diseases, Kolkata on various aspects of diarrhoeal pathogens and other organisms such as *Helicobacter pylori*, *Salmonella typhi* and HIV/AIDS. The enthusiastic efforts of all the scientists and staff are noteworthy. May the institute continue to aspire for greater heights and succeed in publishing the research work in journals of repute and high impact factor and focus on research efforts which should ultimately benefit the marginalized sections of our society.

As in the past, the Council shall extend utmost support for the future endeavours of NICED to reach greater milestones in health research aimed at achieving the goal of a healthy, happy and prosperous India.

(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 कोलकाता स्थित राष्ट्रीय हैजा तथा आंत्ररोग संस्थान द्वारा अतिसारीय रोगजनों और *हेलिकोबैक्टर पाइलोरी, साल्मोनेला टाइफ़ी* जैसे अन्य जीवों तथा एच आई वी/एड्स के विभिन्न पहलुओं पर किए जा रहे महत्वपूर्ण शोध कार्य की सराहना करने में हार्दिक प्रसन्नता है। सभी वैज्ञानिकों और स्टाफ़ के उत्साहजनक प्रयास उल्लेखनीय हैं। आशा है यह संस्थान नवीन ऊंचाइयों को प्राप्त करने की दिशा में निरन्तर प्रयासरत रहेगा तथा प्रतिष्ठित एवं उच्च इंपैक्ट फ़ैक्टर वाले जर्नलों में शोधकार्य को प्रकाशित करने में सफ़ल रहेगा और अंततः अपने शोध प्रयासों को समाज की सीमान्तवर्ती आबादी को लाभान्वित करने की दिशा में केन्द्रित रखेगा।

विगत वर्षों की भांति, परिषद राष्ट्रीय हैजा तथा आंत्ररोग संस्थान के भावी प्रयासों को अधिकतम सहायता प्रदान करेगी जिससे एक स्वस्थ, सुखमय और समृद्ध भारत के लक्ष्य को प्राप्त करने के उद्देश्य से स्वास्थ्य अनुसंधान में उत्कृष्ट सफ़लता प्राप्त की जा सके।

(विश्व मोहन कटोच)

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

From the Director's Desk

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

Since its humble inception in 1962 in four rooms on a floor rented in central Kolkata, the National Institute of Cholera and Enteric Diseases (NICED) has pushed ahead with the daunting task of harmonizing basic and applied research as a single functional entity. Initially, the thrust was on operational research, for example implementation of oral rehydration therapy in hospitals and community and studying the transmission of cholera in community, both of which were widely acclaimed research and had tangible implications for the national and global policy on the control of diarrheal diseases. This singular thrust in the 1970s and 1980s resulted in a major reduction in diarrhoeal death especially among children. Alongside the Divisions of Epidemiology and Microbiology, which catered to the core interest of NICED, Divisions like those of Immunology, Biochemistry, Pathophysiology, Virology and Electron Microscopy were created to pursue more fundamental research on the molecular and genetic characterization of enteric pathogens and their virulence factors like toxins, colonization factors and on the innate and adaptive immune response in enteric infections. Currently, NICED with several high-end equipments like Scanning and Transmission Electron Microscopes, Atomic Force Microscope, Confocal Microscope, Fluroscence Activated Cell Sorter (FACS), Differential Scanning and Isothermal Titration Calorimeters has one of the best infrastructures in Eastern India for doing in-depth research in biomedical sciences. I am happy that the enrichment in infrastructure is reflected in the marked improvement in the quality of publications from the NICED in recent years. The ideal situation would be the utility of this basic research to address real-time problems on diarrhoea in this country, may it be creating a vaccine or may it be understanding virulence factors and their mechanisms of action at the fundamental level. The debate between basic and applied research has been a tenuous one but at NICED we have attempted to harmonize these two arms of research to derive maximum benefit and this is an effort which goes on.

A glimpse of some of the on-going projects that are of more visible relevance to public health include a major initiative to strengthen diarrhea surveillance that covers both inpatients and outpatients in hospitals, urban community and travelers and involves monitoring of a total of 26 bacterial, viral and parasitic pathogens associated with diarrhea. We are planning to expand the surveillance to include rural population as well and a proposal to develop a multi-center National Diarrhea Surveillance is in the evaluation stages. A clinical trial of a heat- and formalin-killed combination of bivalent *Vibrio cholerae* O1 and O139 strains, the two serotypes associated with epidemic outbreaks of cholera, has been successfully conducted and the vaccine is now licensed and is commercially available in India. A live oral recombinant vaccine, developed by three Institutes in India is in the process of Phase III clinical trial. It is worthwhile to remember that development of new vaccines is a continuous rather than a one-time venture. This is so, partly because new pathogens emerge and the old pathogens change themselves for survival in the environment and human host.

Diarrheal diseases have continued to be the main focus of the NICED. In 1984 when the first AIDS case was detected in Kolkata, the mandate of NICED was expanded to include basic and operational research on the human immunodeficiency virus (HIV). As in previous years, NICED had been called upon to strengthen the efforts of the Central and State Governments by lending our manpower and expertise in emergency-like situations arising from outbreaks of diarrheal diseases and occasionally, of some non-enteric viral diseases like avian influenza H5N1 and pandemic H1N1. We are happy to be associated with such national exigencies.

In the year under report, NICED along with Indian Institute of Chemical Biology and with Bose Institute celebrated the 50th year of the discovery of cholera toxin by Professor Sambhu Nath De in 1959 by conducting an International Symposium. Dr. De's work sang but he remained largely unsung. This was a high point of the year and the symposium was very well attended. As in the last two years, we continue to strive for developing participatory management involving all staff, especially the scientists and students who form the core of any research Institute so that the Institute can evolve into a thriving multi-centric organization. The growth of the Institute has been phenomenal in the last decade and it has indeed stepped into adulthood. To ensure involvement of the staff in running such an organization is not merely a matter of opinion but a necessity.

What NICED is today would be impossible without the encouragement and support of the Director-General, ICMR and the Secretary, Department of Health Research, Ministry of Health and Family Welfare, Government of India. Finally, it is my pleasure to acknowledge the Faculty, students and Staff of NICED for their efforts and dedication.

निदेशक की मेज से

हमारे संस्थान की विनम्रशुरूआत सन् 1962 में हुई थी। सेंट्रल कोलकाता में चार कमरे किराए पर लेकर इसकी स्थापना हुई थी। कोलरा और आंत्र रोग (एनआइसीईडी) के नेशनल इंस्टीट्यूट आगे एक संयुक्त कार्यात्मक एकाई के रूप में बुनियादी और अनुप्रयुक्त अनुसंधान के चुनौतीपूर्ण काम के साथ आगे बढ़ा। प्रारंभ में, परिचालन अनुसंधान पर जोर दिया था अस्पतालों और समुदाय में हैजा के संचरण के अध्ययन में मौखिक पुनर्जलीकरण चिकित्सा के उदाहरण के कार्यान्वयन के लिए जो दोनों के व्यापक रूप से प्रशंसित थे, अतिसारीय रोगों के अनुसंधान और नियंत्रण पर राष्ट्रीय और वैश्विक नीति के लिए ठोस प्रभाव पड़ा सन् 1970 के दशक में यह विलक्षण जोर दिया गया और सन् 1980 में बच्चों में विशेष रूप से एक अतिसारीय मौत में प्रमुख कमी के परिणामस्वरूप जानपदिक रोग विज्ञान और सूक्ष्म जीव विज्ञान, एनआईसीईडी के मुख्य ब्याज, इम्यूनोलॉजी, जैव रसायन, पैथोफिसियोलॉजी, विषाणु विज्ञान और इलेक्ट्रॉन माइक्रोस्कोपी के तरह के प्रभागों के लिए और उनके द्वेष की आणविक और आनुवांशिक लक्षण के बारे में अधिक मौलिक शोध को आगे बढ़ाने के लिए बनाये गये थे। टॉक्सिन जैसे कारकों, बसाना कारकों और सहज और अनुकूली आंत्र में संक्रमण प्रतिरक्षा प्रतिक्रिया पर वर्तमान में, स्कैन और द्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोप, परमाणु शक्ति सूक्ष्मदर्शी, कॉनफोकल सूक्ष्मदर्शी, फलूरोसेंस सक्रिय सेल सॉटेर (एफ ए सी एस) विभेदकों स्कैन आइजोथर्मल टाईट्रेशन के कैलोरीमीटर्स और उच्च अंत उपकरणों के साथ एनआईसीईडी सर्वोत्तम संस्थाओं में से एक है। विज्ञान में गहन अनुसंधान मुझे खुशी है कि बुनियादी ढांचे में संवर्धन से प्रकाशनों की गुणवत्ता में उल्लेखनीय सुधार में परिलक्षित होता है। हाल के वर्षों में एनआईसीईडी की आदर्श स्थिति इस बुनियादी इस देश में हैजा पर वास्तविक समय समस्याओं से निपटने के अनुसंधान की उपयोगिता है, यह एक टीका बनाने या इसे बुनियादी स्तर पर विषैलापन कारकों और कार्रवाई के अपने तंत्र को समझने में हो सकता है बुनियादी और अनुप्रयुक्त अनुसंधान के बीज एक कमजोर बहस है। हम अनुसंधान के इन दो हथियारों के अनुरूप करने के लिए अधिकतम लाभ प्राप्त करने के लिए रत है।

चालू परियोजनाएं अधिक प्रासंगिकता के सार्वजनिक स्वास्थय के लिए कर रहे हैं। कुछ की एक झलक एक प्रमुख दस्त निगरानी है कि दोनों दाखिल मरीज/रोगी और अस्पतालों में बहिरंग विभाग, शहरी समुदाय और यात्रियों और 26 जीवाणु के कुल की निगरानी शामिल किया गया है। यह मजबूत पहल में शामिल हैं। वायरल और परजीवी दस्त रोगजनकों के साथ जुडे हम निगरानी के विस्तार की योजना बना रहे हैं।

ग्रामीण जनसंख्या के रूप में अच्छी तरह से और एक के लिए एक बहु - राष्ट्रीय केंद्र अतिसार निगरानी विकसित प्रस्ताव शामिल मूल्यांकन के चरण में है। विबरीयो कोलरा O1 और O139 उपभेदों, दो हैजा की महामारी फैलने के साथ जुडे सीरमप्रकारों की गर्मी और फॉरमेलिन मारे संयोजन का एक नैदानिक परीक्षण, सफलतापूर्वक आयोजित किया गया है और टीका के लिए अब लाइसंस प्राप्त है और भारत में व्यवसायिक रूप से उपलब्ध है। एक जीवित मौखिक टीका पुन संयोजक, भारत में तीन संस्थानों द्वारा विकसित चरण की प्रक्रिया में नैदानिक परीक्षण है यह महत्वपूर्ण है कि नए टीकों का विकास एक बार उधम के बजाय एक निरंतर प्रकिया है। यह तो है,

आंशिक रूप क्योंकि नए रोगजनकों उभरने और पुराने रोगजनकों स्वयं पर्यावरण और मानव अस्तित्व के लिए मेजबान में बदल जाते हैं।

अतिसारीय रोगों पर एनआईसीईडी का मुख्य ध्यान केंद्रित किया जाना जारी रखा है। सन् 1984 में जब पहली बार एड्स का मामला कोलकाता में पाया गया, एनआईसीईडी के जनादेश के लिए ह्यूमन इम्यूनो डेफिसियेंसी वायरस (एचआईवी) पर बुनियादी और आपरेशनल रिसर्च में शामिल विस्तार किया गया था। जैसा कि पिछले वर्षों में, एनआईसीईडी को हमारे मानव शक्ति और आपातकालीन तरह अतिसारीय रोगों और एवियन इन्फलूएंजा (एच 5 एन 1) जैसे कुछ गैर अंत्र वायरल रोगों (एच 5 एन 1, एच 1 एन 1) के कभी कभी, के प्रकोप से उत्पन्न होने वाली स्थितियों में विशेषज्ञता उदाहरण देकर केन्द्रीय और राज्य सरकारों के प्रयासों को मजबूत करने के लिए बुलाया गया था। हम इस तरह के राष्ट्रीय आपात संकट के साथ जुडे होने से खुश हैं।

आलोच्य वर्ष में, रासायनिक जीवविज्ञान और बोस संस्था के साथ एनआईसीईडी प्रोफेसर शम्भू नाथ दे द्वारा हैजा विष की खोज (सन् 1959) के 50 वें वर्ष में एक अंतर्राष्ट्रीय संगोष्ठी का आयोजन द्वारा मनाया। डॉ. दे की प्रसंशा की गई पर उनके ऊपर चुप्पी रही। इस वर्ष संगोष्ठी बहुत अच्छी थी और कई देश विदेश के वैज्ञानिकों ने उसमें भाग लिया। जैसे कि पिछले दो वर्षों में, हम भागीदारी जारी रखने के लिए सभी कर्मचारियों को शामिल प्रबंधन, विशेष रूप से वैज्ञानिकों और छात्रों को, जो किसी भी शोध संस्थान के मुख्य विकासशील ताकि संस्थान एक संपन्न बहु - केंद्रित संगठन में विकसित हो सके इसके लिए प्रयास करते हैं। संस्थान कां विकास पिछले दशक में अभूतपूर्व रहा और इसने वास्तव में वयस्कता में कदम रखा है। कर्मचारियों के इस तरह के एक संगठन चलाने में भागीदारी सुनिश्चित करने के लिए केवल राय की बात नहीं है बल्कि एक आवश्यकता है।

एनआईसीईडी आज अंनुसंधान के क्षेत्र में अंन्तराष्ट्रीय स्तर पर जो ख्याति प्राप्त कर रहा है। उस के लिए भारत सरकार स्वास्थय अनुसंधान के सचिव तथा भारतीय आर्युविज्ञान अंनुसंधान परिषद के महानिदेशक के समर्थन एंव प्रोत्साहन के बिना संभव नहीं था।

अंत में सभी वैज्ञानिकों, छात्रों एंव कर्मचारियों के प्रयास एंव समर्पण की सराहना करते हुये मुझे अत्यंत खुशी है।

जी. बालाकृष नायर

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

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Molecular Epidemiology

Research & Training
on diarrheal diseases
(WHO collaborative centre)

ADMINISTRATION

CONTENTS

RESEARCH ACTIVITIES

BACTERIOLOGY

Research in 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 by applying molecular genetic and classical microbiological techniques. Recently molecular methods have been established for the rapid identification of enteric pathogens from stool specimens that were negative by conventional assay systems. The Division also provides referral services for identification and characterization of different enteric bacteria and laboratory support during investigation of outbreaks/epidemics of diarrhoeal diseases in West Bengal and other parts of the country. Data on clonality of El Tor hybrid strains from Indian and other Asian countries are being shared with members of the PulseNet Asia-Pacific. A laboratory network CholDInet has been initiated, and a Global *V. Cholerae* data base is being set up.

Scientist	**:**	S.K. Niyogi, Scientist F T. Ramamurthy, Scientist F S. Dutta, Scientist E A. Palit, Scientist E B.L. Sarkar, Scientist E R.K. Nandy, Scientist C A.K. Mukhopadhyay, Scientist C S. Basu, Scientist C H. Koley, Scientist B
Staff	**:**	J. Kharwar, Technical Officer S. K. Bhowmik, Technical Officer S. K. Dutta, Technical Officer A.K. Mondal, Technical Officer N.K. Sikdar, Technical Assistant

S.R. Ghosh, Technical Assistant
A. Ganai, Technical Assistant
S.C. Saha, Sr. Laboratory Assistant
S. De, Sr. Laboratory Assistant
R. B. Balmiki, Sr. Laboratory Assistant
M. L. Gupta, Laboratory Assistant
R.B. Bose, Sr. Technical Assistant
A. K. Saha, Attendant
K. Roy, Laboratory Attendant
S. Mondal, Laboratory Attendant
V K Singh, Chowkidar
K. Ghosal, Sweeper

Senior Research Fellows : Goutam Chowdhury
Jawed Alam
Hemanta Mukherjee
Parijat Das
Sanjucta Dutta
Soumik Barman
Tapas Patra
Jayeeta Banerjee (Ghosh)

Junior Research Fellows : Piyali Mukherjee
Sayantani Patra
Soma Mitra

Research Assistants : Anuradha Sinha
Devarati Dutta
Prachetash Ghosh
Rituparna De
Santanu Ghosh
Sourav Sengupta
Subhashree Roy
Sucharita Guin
Suman Nandy
Arindam Naha
Kazi Shaiful Haque

1. Relationship between climate variability and occurrence of diarrhea and cholera: A pilot study using retrospective data from Kolkata, India.

Principal Investigator : G. B. Nair

Co -Investigators : A. Palit, A. Deb and S. Kanungo

Objectives

General:

To determine the effects of climate variability on changes in the occurrence of diarrheal diseases over time.

Specific:

To find out relationship between climatic factors and occurrence of diarrhea and cholera.

To identify and test a range of non-climatic factors that can potentially influence the above relationship.

Work accomplished:

This protocol has been developed and the project was carried out as a follow up of the generic research protocol developed to assess negative health impact of climate change on diarrhoeal diseases with emphasis on cholera and capacity of health system to cope with the consequences.

The generic protocol was prepared for WHO-SEARO as an agreement for performance of work between SEARO and National Institute of Cholera and Enteric Diseases, Kolkata (NICED). At NICED, the document was jointly prepared by Dr. G.B. Nair, Director, NICED and Microbiologist, Dr. A. Palit, Microbiologist, Dr. A.K. Deb, Epidemiologist and Dr. S. Kanungo, Epidemiologist.

This same project work was carried out by the same team, in collaboration with World Health Organization, Kobe, Japan who has funded the project. The main purpose of the project was evaluation of association of the following variables through collection and analysis of 10 years (1999 – 2008) retrospective data.

Conclusions:

The main target was to determine the effects of climate variability on changes in the occurrence of diarrheal diseases over time and to understand changes in disease patterns due to climate changes.This study involved retrospective analysis of 10-year hospital-based data on diarrhea and cholera, as well as data on meteorological and remotely-sensed data (SST) that highlighted some characteristics about the climatic variabilities during this period and the possible relationships with occurrences of diarrhea and cholera in Kolkata. The data indicated that over the past 10 years in Kolkata, there had been an increase in the number of hotter days, along with cooler nights – possibly an indication of extremes of temperatures in the coming years. Simultaneously, there was a drop in relative humidities (morning and evening), resulting in lesser amounts of rainfall despite an increase in number of rainy days. The number of cholera cases increased in recent times. Moreover, cholera cases surged to abnormally higher levels during 2004 – indicating occurrence of an outbreak at that time in Kolkata. It was demonstrated that the previous month's number of diarrhea and cholera cases were the best predictors for occurrences of diarrhea and cholera in the following month; moreover, the relative humidity in the evening and the sea surface temperature also significantly predicted occurrence of diarrhea and cholera respectively. However, these results should be interpreted very cautiously. Since no definitive positive / negative “trend” was noticed for any of the variables (climate or disease), except for “Cholera”, which showed an upward trend over last 10 years (probably indicating influence of factors other than those under consideration so far). As these 10 years data did not reflect any significant change over time, this time period (10 years) probably was not sufficient to assess changes in disease patterns due to climate changes; at best the data could only indicate how disease occurrence changes with “season” (within each year).

Recommendations:

We strongly recommend conducting well-designed prospective studies that may give us better control over data collection and enhance the opportunity of examining the putative relationships between climate changes and occurrences of diarrhea and cholera.

2. Diarrhoeal outbreak caused by vibrios after cyclone Aila in Coastal Areas of West Bengal

T. Ramamurthy

On May 25, 2009, cyclone Aila tore through West Bengal causing deaths and devastation in a vast swathe of land close to the world's largest mangrove forest Sundarbans. Aila is the first storm to hit West Bengal and its suburbs in 28 years affecting 18 out of the 19 districts, especially in South and North 24 Parganas districts. At least 5.1 million people were displaced, with more than one million people stranded in the Sundarban islands alone. Overall, the cyclone Aila has claimed 275 lives. Due to heavy rainfall and flooding the affected areas were submerged and about 7000 tube-wells and fresh water ponds were engrossed by sea water affecting clean drinking water supply. Of the 275 deaths, 30 were due to diarrhoea and more than 85,000 people were affected with many ailments in the districts of North and South 24-Parganas soon after Aila. Acute diarrhoea was reported from Sandeshkhali, Hingalganj blocks in North 24-Parganas and Gosaba, Basanti, Raidighi, Pathar Pratima, Namkhana, Kultali and Sagar Islands of South 24-Parganas district in the state. In Sandeshkhali, Ghospara, more than 10,000 people suffered from diarrhoea and 325 cases representing all age groups were admitted in the hospitals.

One hundred and sixty two stool specimens/rectal swabs were collected from diarrheal patients by the Health officials and NICED team that investigated the diarrhoeal outbreak and thereafter processed for pathogenic vibrios. As shown in Table. 1, the isolation of *Vibrio cholerae* from Aila affected regions was high and isolates were identified as El Tor hybrid strains that produces classical cholera toxin. Pulsed-field gel electrophoresis (PFGE) profiles of *V. cholerae* O1 has shown that the isolates from diarrhoeal patients of Aila affected areas are closely related to each other and similar to the strains isolated before the cyclone from the same area as well as with Kolkata strains (Fig. 1). These results indicate a prevailing clone of *V. cholerae* O1 is responsible for this outbreak.

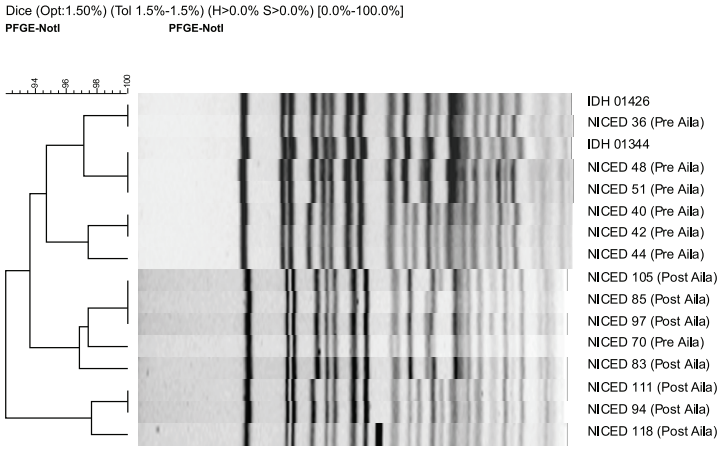


Fig.1 : Dendrogram of *Not*I PFGE profiles of *V. cholerae* O1 isolated during pre and post Alia period and comparision with isolates from IDH, Kolkata.

Table 1 : Isolation profiles of vibrios during and after Aila in different areas of North and South 24 Parganas, West Bengal.

Status	Place	*V. cholerae* O1 Ogawa	*V. cholerae* O1 Inaba	*V. parahaemolyticus*	*V. fluvialis*	*V. cholerae* non-O1 non-O139
Before Aila	N-24 Pgs	1				
(n=25)	S-24 Pgs	2		1	1	
	Total	3		1	1	
After Aila	N-24 Pgs	25		4	4	5
(n=162)	S-24 Pgs	44	2	2	8	2
	Total	**69**	**2**	**6**	**12**	**7**

Seven *V. cholerae* non-O1, non-O139 strains belonging to different serogroups were isolated from Aila affected areas and exhibited different PFGE profiles. *V. parahaemolyticus* was isolated from 6 diarrhoeal cases. All the O3:K6 serovar strains were clonal in the PFGE, including the one that was isolated from a diarrhoeal patient in the Infectious Diseases Hospital (IDH), Kolkata (Fig.2). The other serovar O4:K8 was different from the one that was isolated in the IDH (Fig. 2). Twelve *V. fluvialis* were identified among diarrhoeal patients from Aila affected areas. In the PFGE, all the *V. fluvialis* strains displayed different profiles. Inundation by seawater, lack of hygiene and contamination of drinking water sources would have facilitated spread of these marine vibrios infecting the coastal population. Due to the timely investigation of the outbreak and proper management of diarrhoea cases by health authorities, the outbreak was controlled in two weeks.

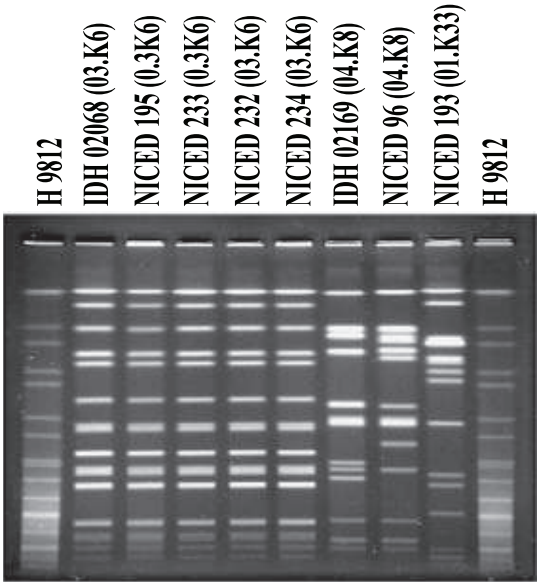


Fig. 2 : *Not*I PFGE profile of *V. parahaemolyticus* strains isolated from diarrhoeal patients of Aila affected area

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

Shanta Dutta

The classical diagnosis of typhoid fever is done by isolation of the organism using standard blood culture, which has only 50% sensitivity and requires about one week. The available serology based tests like The Widal, Typhidot and Tubex kit tests are neither specific, sensitive nor could they fulfill criteria of an ideal test. Therefore molecular methods have been directly used on clinical samples for better performance. PCR was the most commonly used method and we validated one PCR method amplifying *flic-d* gene for rapid diagnosis of typhoid fever even when the patients came after having taken antimicrobials.. Although culture confirmed cases are usually considered as gold standard, yet lack of growth of the organism after antimicrobial therapy limits the use of conventional method.

So far 169 blood samples were collected from clinically suspected typhoid fever cases and processed by three methods e, g, conventional culture, serology and PCR based method. Total 12 samples were positive by culture (11 *S. Typhi* and 1 *S. Para A* isolates), 60 by Widal and 95 by typhoid specific PCR showing better performance of PCR method. More samples are being collected and will be tested for obtaining scientifically valid result. Anti microbial resistance pattern showed increasing quinolone resistance (>50%) among the isolates.

We have included a number of *Salmonella Typhi* isolates from Kolkata in a typhoid vaccine trial study during 2003 to 2007, for molecular typing and transmission analysis. SNP typing was carried out using standard protocol. A total of 378 isolates were subjected to SNP analysis and the following haplotypes (shown in the Tables) were obtained. H58 was the predominant haplotype, followed by H42. H58B was the major subtype. The Figure shows the phylogenetic tree of the Kolkata *S. Typhi* isolates. The PFGE profile showed circulation of more than one clone of *S. Typhi* in Kolkata. Single pulsotype (P1) was predominant (30%) among the isolates.

Haplotype level – all

	2003	2004	2005	2006	2007
H14	3	7	5	10	0
H16	1	3	1	0	0
H37	0	0	1	0	0
H42	4	27	13	21	0
H50	3	1	2	0	0
H52	0	1	0	1	0
H55	0	1	0	0	0
H58	36	83	72	71	4
H8	0	1	0	0	0
H85	1	3	1	0	0

H58 - grouped

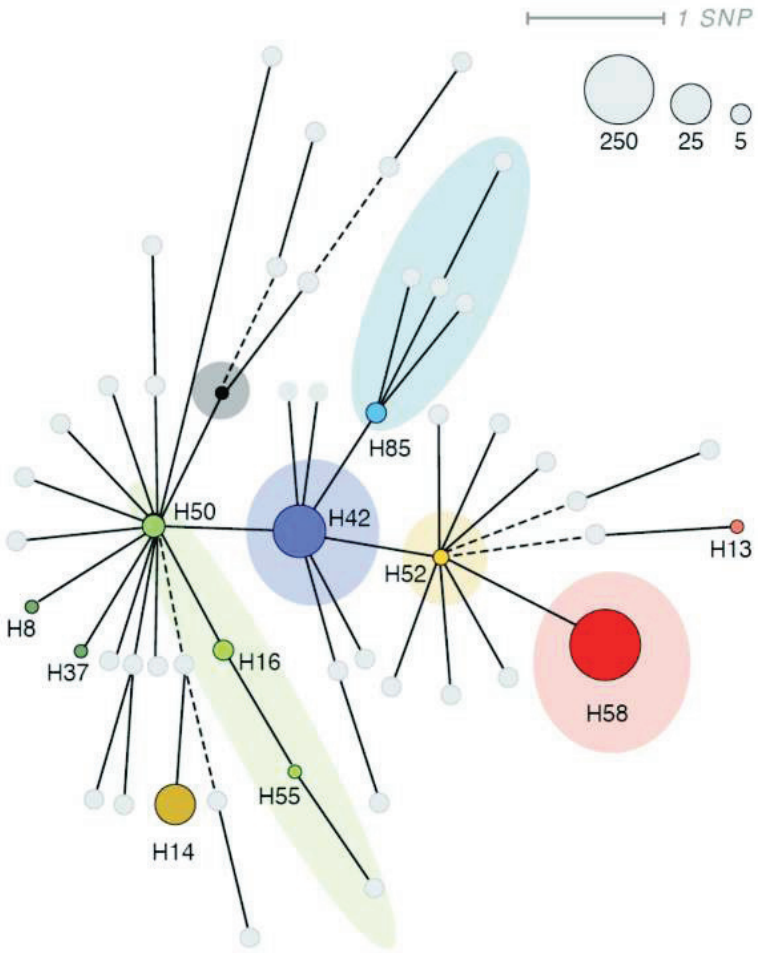
	2003	2004	2005	2006	2007
H58 A	3	8	5	4	1
H58 B	18	44	46	41	1
H58 G	12	21	10	24	0
H58 H64	1	5	8	2	2
Other	2	4	2	0	0

4. Evaluation of Anti-Typhoid and Anti-Diarrhoeal Activity of three Ethnomedicinal Plants of Tribal use from different parts of India.

Shanta Dutta

Primarily anti-Typhoid (*Salmonella enterica* serovar typhi*)* activity of the decoction and crude alcoholic extract of *Shorea robusta* L. (Dipterocarpaceae) as practiced by the Kaatabhai tribes of Maharashtra, was determined. Anti-typhoid activity of crude aqueous and hydroalcoholic extracts of two more medicinal plants viz. *Achyranthes aspera* Linn. (Amaranthaceae) and *Ephedra ciliate* Fisch. (Ephedraceae) used by the tribes of Uttar Pradesh, Madhya Pradesh and Rajasthan were also determined. Secondarily the antidiarrhoeal and antidysenteric activity of decoction and hydroalcoholic extract of all three medicinal plants as practiced by some tribal population was determined.

The preliminary *in vitro* antibacterial study revealed that both aqueous and methanolic extract of *Shorea robusta* has considerable antibacterial activity against *S. typhi*, and *Shigella flexneri* and *Shigella dysenteriae* with MIC90<1000 µg/ml. Considerable synergistic activity was observed when tested *in vitro* by 1000-2500 µg and 5000 µg of the extract along with 30 µg of chloramphenicol. Both TLC and repeat study with HPLTC revealed that at least two major compounds were present in the aqueous extract of *S. robusta*. Baseline and peak display also revealed the same.



5. Role of seasonality on the distribution, abundance and diversity of *Vibrio* organisms in estuaries of West Bengal: relation with cholera incidence

Dr. Anup Palit

The study was planned with the following objectives: to monitor some physico-chemical features of the estuarine water along the same transect & culture of *Vibrio*s and monitoring its abundance along the transect; to record the direct viable counts of *V. cholerae* to reveal the VBNC state of the pathogenic bacteria; to study biochemical differentiation of isolated *Vibrio* strains to observe its diversity change in different parts of the estuary; to identify different O1, O139 and non-O1 non-O139 strains of *V. cholerae* by immunological methods to study the relationship of seasonal change and cholera incidence patterns in the Kolkata region; to study the influence of river bank erosion and sediment resuspension - i.e. benthic inputs, by e.g. cyclones or peak rainfall on *Vibrio* diversity and abundance in the water column.

The field and laboratory studies/observations in this project is summarised in brief.

There are 4 study sites. Viz. Namkhana (Narayanpur, S. 24 Parganas dist.) - Site 1; Basonti (Basonti subdivision, S. 24 Parganas dist., W. Bengal) - Site 2; Howrah (across the River Hooghly near Kolkata) - Site 3 and Diamond Harbour (Nurpur) - Site 4.

The observations are as follows:

Site-2 showed a comparatively higher salinity (range was between 26-28.5 ppt) in comparison to Site 1 (range was between 20-24 ppt).

Total *Vibrio* count seems to be very high at Site 2 (ranging between 1.5- 4.5 X 10^3cfu/ml) in comparison to Site 1 (ranging between 0.6-3.2 X 10^2 cfu/ml). In Hooghly river, the salinity ranges between only 0.1-0.3 ppt. and the *Vibrio* load is too low. However, the high coliform predominance has been observed.

The samples of site-4 showed a little bit higher salinity (0.2-1.0).

121 samples have been analyzed so far. (33 from site-1; 29 from site-2; 47 from site-3; 12 from site-4). Predominance of *V. cholerae* has been detected mostly from Site 2 and Site 1.

Sl no.	Study site	pH	Salinity (ppt)	Conductivity (ms/cm) or (µs/cm)	Total dissolved solids (mg/l)	Total Bacterial Count (TBC) (cfu/ml)	Total *Vibrio* Count (TVC) (cfu/ml)	Total Coliform Count (TCC) (cfu/ml)	Total *E.coli* Count (TEC) (cfu/ml)
1	Namkhana, (S.24 Pgs) – Site 1	7.54 -8.84	13-28.8	30.4-44.4 ms/cm	92.17 -152.08 (mg/l)	1.3 X 10^2- 4.8 X 10^4	3 x 10^1-3 X 10^3	0-500	0- 20
2	Basonti, Gosaba, (S.24 Pgs) – Site 2	8.04-8.97	26.8-28.6	41.9-44.3 ms/cm	<1000 (mg/l)	8 x 10^1 -5 x 10^4	6 X 10^1- 6 X 10^3	0-2700	0-1500
3	Diamond Harbour (Nurpur) (S.24 Pgs) – Site 4	8.51-9.29	0.4-4.7	414-8440 µs/cm	20.3- 413.9 mg/l	8 X 10^2 -8 X 10^3	0-20	10-300	0-50
4	Kolkata Hooghly river – Site 3	8.38-9.73	0.1-0.2	291-549 µs/cm	15.4- 19.5 (mg/l)	5 x 10^{-} -4 x 10^{-}	nil	280-2930	10-100

6. Nationwide screening of phage types of *V. cholerae* O1 and O139

B. L. Sarkar

Our laboratory receives, on average, more than 900 strains of *V. cholerae* O1, O139 and non-O1, non-O139 annually from all parts of the country and abroad for phage typing, biotyping and serotyping at our National Phage Typing Centre, NICED. During the period, no O139 strains were received from any Indian laboratory. We are also collaborating with Brazil, Japan and Austria on *V. cholerae* strains and phage typing.

During the period under study, a total of 862 strains of *V. cholerae* were received from different parts of the country for serotyping, biotyping and phage typing. Of these, 688 (79.8%) representative strains were confirmed as *V. cholerae* O1 biotype ElTor and were included in phage typing study. This year, highest number of strains was received from Maharashtra state followed by Gujarat. Majority of the strains belonged to *Ogawa*. For the last couple of years, *Ogawa* was the dominant serogroup. A total of 20 (2.9%) 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 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- 551(80.0%) followed by type 21-23 (3.3%), type 26-21 (3.0%), type 13-14 (2.0%), type 25-12 (1.4%) respectively. It has been observed that type 27 is the predominant phage type circulating in India.

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

B. L. Sarkar

Vibriophage N4 (ATCC 51352-B1) occupies a unique position in the new phage typing scheme currently under routine use in our laboratory. Successful completion of whole genome sequencing explored that vibriophage N4 comprised one circular, double stranded chromosome that was 38,497 bp in size. Homology search revealed maximum identity between the genomes of vibriophage N4 and VP4 and also found close relation to enterobacteriophage T7. Out of 47 ORFs of the N4 genome, 30 were supposed to predict function by homology search. DNA-DNA hybridization data indicates two evolutionarily distinctive branches of possible phylogenetic origin of O1 and O139 vibriophages.

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

B. L. Sarkar

A study was undertaken to observe the host lysis activity with selected vibriophage in animal model and to challenge phage therapy in RITARD model. This study showed that the cocktail phage could control the proliferation of *V. cholerae;* possibly the cocktail phages lysed the infecting bacterial cells in RITARD model. The studies are underway to confirm that cholera phage can be the alternate to antibiotic as phage therapy.

9. Carbohydrate utilization pathways of *Vibrio cholerae* and their relevance in regulation of expression of genes including virulence determinants.

Ranjan Kumar Nandy

Vibrio cholerae O1 and O139 strains colonize in the human gut, express a number of toxigenic factors that lead to massive diarrhea in the form of Cholera. During *in vivo* survival in the human intestine, *Vibrio* can utilize different types of carbohydrates. Among the different form of sugars, abundance of gluconate in the intestinal milieu is well known and the gluconate utilization through Entner-Doudoroff (ED) pathway is also known to exist among certain other human pathogens. With this background the study was initiated to understand gluconate catabolism by *V. cholerae* and also through ED pathway. Genome search in *V. cholerae* revealed that the genes of ED pathway and the associated regulatory genes are localized within a cluster which was considered to have a potential correlation

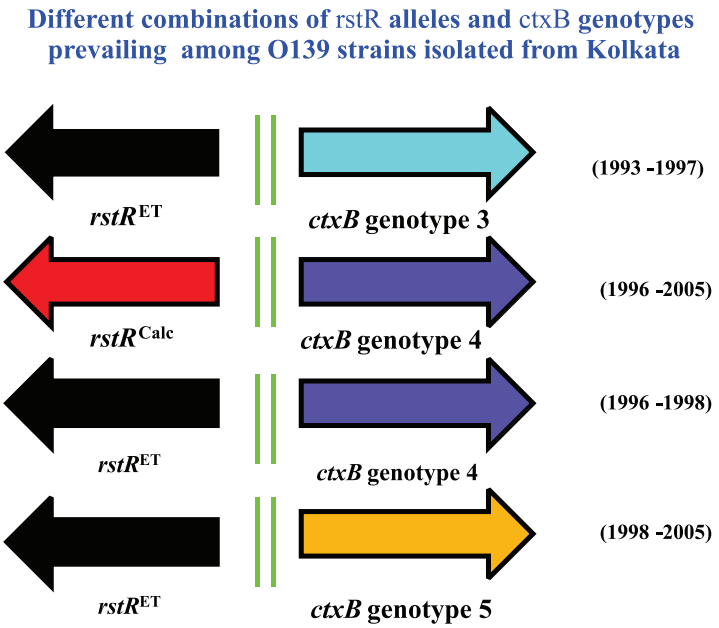
between ED pathway and gluconate utilization system in *V. cholerae*. In fact, growth of *V. cholerae* strains in media supplemented with gluconate increased expression of virulence genes like *ctxA* and *tcpA*. This study was extended by generating in-frame deletion mutation of *edd* gene, one of the prime genes in ED pathway. The Δ*edd* mutant showed reduced level of *ctxA* and *tcpA* expression at the transcriptional level. This observation was further supported through animal model studies where Δ*edd* mutant showed severe attenuation of virulence. Therefore, our data indicate an important relationship between the catabolism of gluconate and bacterial pathogenesis, stressing the relevance of the utilization of the resources available in the host's environment. This study may help to initiate a situation for drug designing or probiotic therapy for treatment of diarrheal patients in future.

10. Complex O139 CTX prophages: Facing a phase-out

Asish K. Mukhopadhyay

The genesis of *V. cholerae* O139 in 1992 attracted worldwide attention. A quiescent period followed in the history of *V. cholerae* O139, and it was thought that the appearance of O139 cases was a one-time event. But a resurgence of serogroup O139 occurred in August 1996 in Kolkata. From 2000- onwards the incidence of O139 has gradually decreased over the years.

Chronological analysis of 125 *Vibrio cholerae* O139 strains isolated during 1993 - 2005 from Kolkata revealed the prevalence of two new genotypes of cholera toxin (CT) along with the different combinations of *ctxB* and *rstR* alleles resulting in variant CTX prophages.

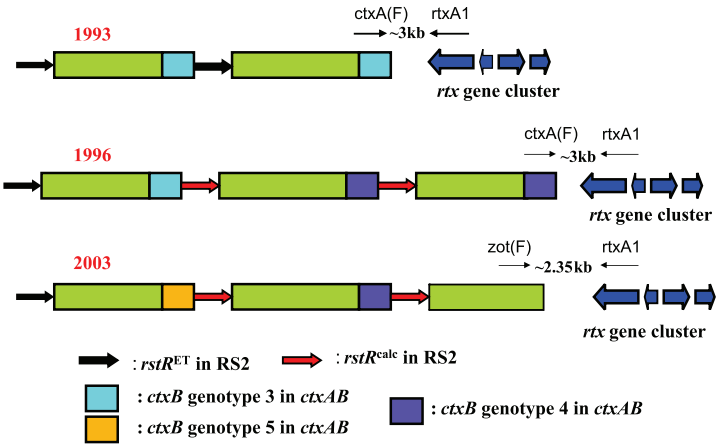


One of the new genotypes of *ctxB* first appeared in 1996 with the reemerged *V. cholerae* O139 strains that had CTX Calcutta phage was designated as genotype 4. In 1998, another new genotype designated as genotype 5 was detected that prevailed mostly in CTX phages with El Tor *rstR*.

The prototype El Tor CTX phage with genotype 3 gradually disappeared in O139 strains and the predominant CTX prophages in O139 since 2002 are Calcutta phages with genotype 4 and El Tor phages with genotype 5. Results showed that *V. cholerae* O139 strains of Kolkata isolated over a decade harboured CTX prophages in the large chromosome having no RS1 downstream of CTX prophage.

During the course of its intermittent incidence over a decade, five types of O139 strains have been detected based on CT genotypes. The diverse genotypes of *ctxB* as well as *rstR* alleles occur among *V. cholerae* O139 strains along with the variations in other genetic segments of O139 strains which are not yet ascertained, perhaps consequence of the temporal variation of incidence and prevalence of O139. Such abrupt genetic changes thus acquired by *V. cholerae* O139 strains since its genesis may have an impact in their declining prevalence in cholera endemic areas like Kolkata.

Schematic diagram showing copy number of CTX prophage and allelic combinations of rstR and ctxB in O139 strains isolated in different time frames

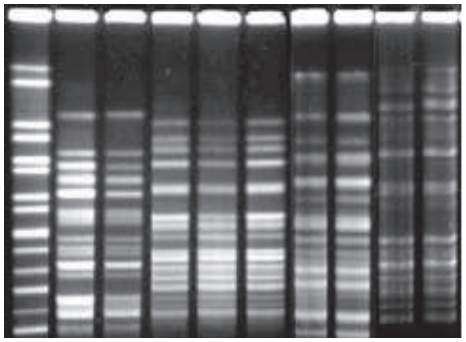


11. Role of gut microflora in neonatal sepsis with special reference to gram-negative bacteria

Sulagna Basu

The gut of a neonate is colonized by bacteria immediately after birth. Among the hundreds of bacteria that colonize the gut there are some potential pathogens. While not all colonization leads to infection, the pathogenicity of the aerobic Gram Negative Bacilli (GNB) may predispose the babies towards infection. With this in view, the study examines the pattern of colonization of the neonatal gut by aerobic Gram negative bacilli (GNB) and evaluates the association between gut colonization and sepsis. This deserves attention because of the high incidence of sepsis and the differences in hygienic environment in developing countries compared to the developed world.

A prospective study of neonates without any surgical intervention, with and without clinical sepsis, was carried out for a year in a tertiary care hospital in India. The gut (gastric aspirate & stool) and the blood samples of the babies



Analysis of XbaI digested genomic DNA of the *E.coli* isolated from the blood and gut of neonates

were analyzed. Antibiogram and Pulsed Field Gel Electrophoresis were carried out to evaluate the relatedness of the gut and blood isolates.

A diverse array of GNB was isolated, *Klebsiella pneumoniae* being most common. Results further showed that colonization was influenced by environmental factors like the baby's stay in the neonatal intensive care unit or use of an enteral feeding tube. GNB was the cause of septicemia in majority of the cases, *K. pneumoniae* being the most frequently isolated GNB from the blood. Babies with GNB in the gut have higher incidence of clinical sepsis than those without. In 50% cases the genotypes of the organisms found in the blood were indistinguishable from their gut counterpart.

An association of gut colonization with neonatal sepsis was observed. In this context, the surveillance of the gut flora could be considered to reduce neonatal infections.

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

Hemanta Koley

Pathogenic *Vibrio cholerae* strains are the etiologic agents of cholera. Pathogenic O1 and O139 isolates typically encode two critical virulence factors, cholera toxin and toxin coregulated pilus. CT is primarily responsible for diarrheal purge whereas TCP is an essential intestinal colonization factor of *V.cholerae*. TCP has been shown to be critical for colonization both in animal models and in humans. Most pathogenic non-O1, non-O139 strains are CTX^{-ve} TCP $^{-ve}$ but can still cause diarrhea.

Comparative studies on the colonization ability of *V.cholerae* of diverse serogroups with a variety of combinations of different virulence factors were made. In the present study, we investigated the role of individual virulence factors and its influence on the colonization ability as well as diarrhoeagenicity and colonization ability in RITARD model.

All the strains examined could colonize in both the ileum and the jejunum. However, interstrain differences in colonization ability were explicit and some strains showed better colonization capacity in the ileum than in the jejunum. Kelly et al (2006) and Tam et al (2008) worked on EPEC and EHEC organism and showed that structural components of a type III secretion system has important role on colonization.

Therefore, we started screening *V. cholerae* and found that K11857, *V. cholerae* O139 strain was T3SS gene positive. Then target specific vcsC2 gene knockout was done in collaboration with Dr. Santasabuj Das. T3SS deleted mutant showed relatively lower colonization ability in Mouse model. RITARD model support our data that T3SS deleted mutant showed relatively lower colonization and no diarrhea.

This study showed T3SS has an important role in colonization in RITARD and mouse model.

13. Development and evaluation of a heat killed multi-serotype oral *Shigella* vaccine

Hemanta Koley

Enteric bacterial infection causes diarrhoea throughout the world, especially in developing countries. Among them *Shigella* infection is a major cause of infant morbidity and mortality in developed as well as developing countries

At present control measures for shigellosis include only antibiotic and oral therapy. Unfortunately due to malpractice of use of antibiotics, *Shigella* has developed multidrug resistance and also rehydration therapy is not in practice for shigellosis.

There is lack of therapeutic measurements at present; good sanitation practices and candidate anti dysentery vaccines will be ideal to combat *Shigella* infections. But in a developing country like India, sanitation is an acute problem. However, for more than 50 years, scientists are trying to develop suitable candidate vaccines against shigellosis.

After four successive oral immunizations with heat killed single strain, on 21st day of immunization, intra-rectal challenge was done according to the method of Shim *et al.* 2007 with wild type Sereny positive *S. dysenteriae, S.boydii* 4 and *S.flexneri 2a, S.flexneri 3a S.flexneri 6 and S. sonni* strains; most of the unimmunized PBS treated control guinea-pigs appeared to be sick and their rectal temperature was persistently elevated whereas in case of the guinea-pigs immunized with heat killed S*higella* strains, no such symptoms were observed. Most of the unimmunized PBS treated control guinea-pigs developed diarrhoea and their perineal region remained constantly wet and soiled with faeces. Within 48 h thick liquid stool of the guinea-pigs became mucoidal with occasional presence of blood indicating features of acute shigellosis. However no such symptoms like diarrhoea and presence of mucous and blood in stool or death were observed among the guinea-pigs immunized with heat killed immunogen. In this representative protection study, 100% protection was observed in the immunized group of guinea-pigs.

AWARDS AND HONOURS

Dr. G. B. Nair

Dr. G. Balakrish Nair, Scientist 'G' Director of this Institute was awarded the distinction of Fellow of the American Academy of Microbiology in the year 2009, in recognition of his outstanding contribution as a Microbiologist.

Dr. Anup Palit

Member, Drinking Water sectional Committee, FAD25, Ministry of Consumer affairs, Food and Public distribution, GOI, 2009-10.

Reviewer, STS (ICMR), 2010 programme.

PRESENTATIONS AND VISITS

Dr. G. B. Nair

Attended Foundation Day celebration of Indian Institute of Chemical Biology, Kolkata as Guest-in-Chief on April 2, 2009.

Participated in the 13th International Conference on Emerging Infectious Diseases in the Pacific Rim - Focus on Enteric Diseases at the Hotel Oberoi Grand, Kolkata from April 6-9, 2009.

Attended "Policymakers meeting on the introduction of cholera vaccination using new generation oral vaccines in India" at Hotel Taj Mahal, New Delhi on April 10, 2009.

Attended meeting on 'Focus on Neglected Tropical Infectious Diseases: Integrating Low Cost Vaccine into Global Cholera Control' at Annecy, France during April 14-17, 2009.

Attended and delivered a talk on "Climate change and the potential impact on diarrhoeal diseases" at the First Meeting of the Regional Technical Advisory Group for Integrated Control of Acute Diarrhoea and Respiratory Infections at NICED, Kolkata during April 23-24, 2009.

Participated in the Second Informal Consultation Series meeting conducted by WHO/IHR Global Laboratory Directory for the establishment of a specific agenda on laboratory network and define its role at Geneva, Switzerland during June 28-30, 2009.

Attended Apex Committee meeting of Vaccine Grand Challenge Program of DBT held at CSIR Science Centre, New Delhi on July 22, 2009.

Attended meeting as Member of the Advisory Committee for recommending CSIR Young Scientist Award for the year 2009 in Biological Sciences at CSIR Complex, New Delhi on July 28, 2009.

Participated at the Informal Consultation on Research to Assess Communicable Disease Impact of Climate Change convened by WHO Regional Office for South East Asia (WHO-SEARO) at ITC Sonar Bangla, Kolkata during August 24-26, 2009.

Attended a meeting on "Diarrhoeal Diseases and Enteric Vaccines Advisory Committee (DEVAC)" as WHO Temporary Adviser in Benalmadina, Malaga, Spain during September 7-8 , 2009.

Presented a talk to the 44th Joint Meeting and Conference of the United States-Japan Panel on Cholera and Other Bacterial Enteric Infections in San Diego, California during October 12-14, 2009.

Attended 3rd India Probiotics Symposium on "Probiotic Foods in Health & Disease" held in New Delhi from November 21-22, 2009.

Delivered a lecture to the student of the Dept. of Human Rights, Loreto College, Kolkata on November 24, 2009.

Attend the Sixth Meeting of the PulseNet Asia Pacific Network at the Department of Medical Sciences, Nonthaburi, Thailand during December 15-17, 2009.

Delivered a talk on "Public Health Considerations - Developing Countries" at the FAO Expert Workshop on Application of biosecurity measures to control Salmonella contamination in sustainable aquaculture during January 19-21, 2010 at UNESCO MIRCEN for Marine Biotechnology, College of Fisheries, Mangalore.

Attended the First meeting of the Committees of the project "Introduction of Cholera Vaccine in Bangladesh; ICVB" at the ICDDR, B, Dhaka, Bangladesh on January 28, 2010 and followed by a meeting of the investigators with the Gates team on January 30, 2010.

Attended 1st Organizing Committee meeting of the 4th India Probiotics Symposium chaired by Prof. N.K. Ganguly on February 6, 2010 at New Delhi.

Attended Governing Board meeting of the National Institute of Biomedical Genomics (NIBMG) at Toshali Sands, Puri, Bhubaneswar on February 13, 2010.

Attended Third Task Force meeting on ICMR-DBT "Guidelines for evaluation of probiotics in food" at ICMR Hqrs., New Delhi on February 24, 2010.

Invited by Glenmark Pharmaceuticals Ltd. to attend scientific discussion on "Perspective on the need for novel antisecretory antidiarrheal agents" at the Orchid Hotel, Mumbai on February 27, 2010.

Attended WHO "SEA Regional Conference on Epidemiology" at Taj Palace Hotel, New Delhi during March 8-10, 2010.

Dr. S. K. Niyogi

Visited Institute for Research in Molecular Medicine (INFORMM), Health Campus, Universiti Sains Malaysia, Kubang Kerian Kelantan from June 6-12, 2009 to attend the discussion meeting on possible research collaboration.

Delivered a talk entitled "Shigellosis in Kolkata: Past, present and future" on June 7, 2009 at INFORMM.

Participated in the International symposium on "Fifty years of discovery of cholera toxin: A tribute to Dr S N De" held at Kolkata during October 25-27, 2009.

Attended First IBSC General Meeting at Institute of Molecular Medicine (IIM), Kolkata on November 13, 2009.

Conducted PhD viva examination at PGI. Chandigarh on 30.04.2009.

Acted as expert of the selection committee for the selection of Junior Bacteriologist at Union Public Service Commission, Dholpur House, New Delhi-110069 on 18.03.2010.

Dr. T. Ramamurthy

Attended the 12th Asian Conference on Diarrhoeal Diseases and Nutrition (ASCODD), Yogyakarta, Indonesia, May 25-27, 2009 and delivered an invited talk on "Antimicrobial resistance: Evolving problems and possible control measures".

Attended the 6th meeting of PulseNet Asia-Pacific Net work in Bangkok, Thailand and presented report on "PFGE characteristics of *Vibrio cholerae* O1 El Tor variant".

Participated in the discussion meeting on Designing of PFGE data base for PulseNet Asia Pacific Net work and WHO CHOLDInet at the Center of Disease Control (CDC), Atlanta, USA from January 20 to February 2, 2010 and gave a talk on "Glimpses on research activities of National Institute of Cholera and Enteric Diseases, Kolkata, India".

Dr. S. Dutta

Participated in the 13th International Conference on Emerging Infectious Diseases of the Pacific Rim: *Focus on Enteric Diseases* held at Kolkata April 6-9, 2009 and delivered a talk on April 8, "Evaluation of New-Generation Rapid Diagnostic Serologic Tests for Diagnosis of Acute Typhoid Fever".

Attended international symposium on "Fifty years of Discovery of Cholera Toxin: A Tribute to S. N. De, organized by NICED, held in Kolkata from Oct 25-27, 2009.

Attend 5th National Award Function of National Innovation Foundation (NIF), a body supported by DST, GOI on Nov 18-19, 2009 at IARI, PUSA, New Delhi.

Dr. Anup Palit

Participated in International conference in environment, occupational and life style concerns-trans disciplinary approach, (envoc Health 2009) ROHC-NIOH, Bangalore, September 16-19, 2009.

Chairman, Session on "Disease, Diagnosis & prevention" at above conference.

Presented paper entitled "Impact of climate change on diarrhoeal diseases with emphasis on cholera-introspection and mitigation pathways".

Participated in 3rd India Probiotic Symposium, New Delhi, November 21-22, 2009.

Invited and participated in The National Conference organised by Bharatiya Rajbhasa Parishad at Puri, Orissa, December 9-11, 2009.

Meeting and interaction with scientist of NIMR, New Delhi to formulate and finalize the joint collaborative proposals on "Retrospective study" and "Preparedness and response" modalities, for "The impact of climate change on vector borne and diarrhoeal diseases" prior to final submission to WHO for research grant, September 5-8, 2009 at New Delhi.

Member, Drinking Water sectional Committee, FAD25, Ministry of Consumer affairs, Food and Public distribution, GOI, and attended meeting held at Bureau of Indian Standards, New Delhi on January 28, 2010.

"First Meeting of the Regional Technical Advisory Group (RTAG) Prevention and Control of Acute Diarrhoea and Respiratory Infections" April 23-24, 2009, WHO-APW meeting

Informal consultation on research to assess communicable disease impact of climate change, Kolkata, India August 24-26, 2009, WHO-APW meeting

Regional consultation on cross-border collaboration in disease control, Kolkata, April 28-30, 2009, WHO APW meeting

Dr. B. L. Sarkar

Attended at the 13th international conference on emerging infectious diseases in the Pacific Rim held at Kolkata from April 6-9, 2009.

Attended at the fifty years of discovery of cholera toxin: a tribute to S. N. De held at Kolkata from October 25-27, 2009.

Presented paper entitled "Mission and vision of NICED with special emphasis on cholera bacteriophages" at the International symposium on Emerging trends in infectious diseases: TB & GI infections at IIT, Kharagpur from January 05-07, 2010.

An invited lecture entitled "Role of cholera bacteriophages in Kolkata environsa paradigm" delivered at the National conference on Diversity and prospects of microbial resources (MiDiCon 2010) held at NBU, Siliguri from February 26-28, 2010.

Attended and acted as a Chairperson of a session of Golden jubilee symposium on contemporary trends in plant and microbial sciences held at Burdwan from March 19-20, 2010.

Invited by Austrian National Reference Laboratory, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria during August 4-14, 2009 to collaborate with Austrian counterpart on cholera phages; invited for ongoing collaboration study to support the Austrian counterpart in an endeavour to isolate Vibrio phages from lake Neusiedl at Vienna, Austria.

Dr. R. K. Nandy

Attended symposium entitled "Fifty years discovery of cholera toxin: A tribute to SN De" organized jointly by NICED, IICB and Bose Institute, Kolkata, during October 25-27, 2009.

Attended symposium entitled "3rd India Probiotics Symposium: probiotic foods in health and disease" organized by NICED, Kolkata and ICMR, New Delhi, during November 21-22, 2009.

Talk entitled "Diarrheal diseases in India: an under recognized reality" presented in symposium entitled "Gastroenterology in India" organized by Indian Society for Gastroenterology, Kolkata, during December 9-13, 2009.

Attended workshop on ELISPOT organized by Cellular Technology Limited, at Cleveland, USA during August 17-21, 2009.

Attended a training course in the area of Clinical Immunology at International Vaccine Institute (IVI), Seoul, Korea; from November 2-6, 2009.

Dr. A. K. Mukhopadhyay

Oral Presentation at the 44th United States-Japan Conference on Cholera and Other Bacterial Enteric Infections at San Diego, USA during October 12 to 14, 2009.

Invited as an expert for assessing the current microbiological practices to isolate, identify and store *Vibrio* organisms at Pemba and Unguja, Tanzania during March 23 to 28, 2009.

Poster entitled "Analysis of Type Three Secretion System (TTSS) in clinical *Vibrio cholerae* O139 strains that do not harbor *ctx*" In Fifty Years of Discovery of Cholera Toxin: A Tribute to SN De; held in Kolkata; Period October 25-27, 2009. Organized jointly by IICB, Bose Institute and NICED, Kolkata.

Poster entitled "Usefulness of Crystal VC rapid dipstick tests in a cholera outbreak". In Fifty Years of Discovery of Cholera Toxin: A Tribute to SN De; held in Kolkata; Period October 25-27, 2009. Organized jointly by IICB, Bose Institute and NICED, Kolkata.

Conducted/participated in 13th International Conference on Emerging Infectious Diseases in the Pacific Rim - Focused on Enteric Diseases at Kolkata" held in Kolkata during April 6-9, 2009" funded by NIAID and NIH, USA.

Worked as organizing member for "Fifty Years of Discovery of Cholera Toxin: A Tribute to SN De" held in Kolkata; Period October 25-27, 2009; Funded jointly by ICMR, CSIR, Bose Institute, DBT and DST, Govt. of India.

Dr. S. Basu

Parijat Das, A.K. Singh, Subhashree Roy, Sudipta Dasgupta, Yosifumi Takeda, Sulagna Basu. Commensalism and Pathogenicity in E.coli isolates from neonates in a tertiary care hospital. 44th United States-Japan Conference on

Cholera and Other Bacterial Enteric Infections at San Diego, USA on October 12-14, 2009.

Parijat Das, A.K. Singh, Subhashree Roy Sudipta Dasgupta, Yosifumi Takeda, Sulagna Basu. *E.coli* and *Klebsiella pneumoniae* colonization in the gastrointestinal tract of neonates in a tertiary care hospital, U.S.-Japan Cooperative Medical Science Program (CMSP) 13th International Conference on Emerging Infectious Diseases in the Pacific Rim (Kolkata, India: April 6-9, 2009).

Subhasree Roy, A.K. Singh, Parijat Das, Sudipta Dasgupta, Yosifumi Takeda, Sulagna Basu. Fecal carriage of extended spectrum ß lactamase (ESBL)- producing Enterobacteriaceae in a neonatal intensive care unit, U.S.-Japan Cooperative Medical Science Program (CMSP) 13th International Conference on Emerging Infectious Diseases in the Pacific Rim (Kolkata, India: April 6-9, 2009).

Dr. H. Koley

Nivedita Roy, Soumik Barman, Amit Ghosh, Amit Pal, Krishnendu Chakraborty, Santa Sabuj Das, Dhira Rani Saha, and Hemanta Koley. Immunogenicity and protective efficacy of *Vibrio cholerae* Outer Membrane Vesicles (OMVs) in Rabbit Model. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, at Kolkata, India on April 6-9, 2009.

Soumik Barman, Ranjit Kumar, Nivedita Roy, Dhira Rani Saha, Hemanta Koley. The efficacy and immunogenicity of a Live Transconjugant Hybrid (LTSH) strain of *Shigella dysenteriae* type 1 in Guinea pig Model. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, at Kolkata, India on April 6-9, 2009.

A Raychaudhury, P Mukherjee, Hemanta Koley, Nivedita Ray Soumik Barman,and Ak Mukherjee. Analy sis of type III secretory system (TTSS) in clinical *V. cholerae* O139 strains that do not harbor *ctx*. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, at Kolkata, India on April 6-9, 2009.

S. Ghosh, K Chakraborty, Hemanta Koley and S. Das Identification of novel Virulence factor of Salmonella typhi. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, at Kolkata, India on April 6-9, 2009.

T. Takashasi, Hemanta Koley, T. Ramamurthy and Y Takeda. Characterization of a hemolysin produced by *Vibro fluvialis.* 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, at Kolkata, India on April 6-9, 2009.

Soumik Barman, Suryatapa Das, Nivedita Roy, Dhira Rani Saha, T. Ramamurthy, M.K. Chakrabarti, G.B. Nair, Y. Takeda and Hemanta Koley. Homologous Protection of Infection by Heat-killed *Shigella* Strains in Guinea pig Model. International joint forum on Infectious Diseases 2009, September 16-17, 2009. Siam City Hotel, Bangkok, Thailand.

Nivedita Roy, Suryatapa Das, Soumik Barman, Hemanta Koley. Anti-colonization and Anti-diarrheal Effects of Natural Plant Products on *V. cholerae* in Animal Models. International Symposium on Fifty Years of Discovery of Cholera Toxin Hyatt Regency Kolkata during October 25-27, 2009.

Soumik Barman, Sohini Chatterjee, Suryatapa Das, Nivedita Roy, Swapan Kumar Niyogi, Ranajit Kumar, T. Ramamurthy, Hemanta Koley. *Shigella flexneri* 3a Encodes Drug Resistance Due to Involvement of 6.3 kb and 47 kb plasmids International Symposium on Fifty Years of Discovery of Cholera Toxin Hyatt Regency Kolkata during October 25-27, 2009.

Workshop on laboratory Biosafety and Biosecurity Nov 17-19, 2009 Indian Veterinary Research Institute, Anand Nagar, Bhopal, India.

Workshop on Committee for the purpose of control and supervision of experimental on animals, Jadavpur, Kolkata.

PhD DEGREE AWARDED

Saswati Sinha was awarded the degree by Jadavpur University for her thesis "Phenotypic and Genotypic Characterisation of *Haemophilus influenzae* Isolated from Healthy and Sick Children" under the supervision of Dr. S. K. Niyogi.

K. Rajendran was awarded the degree by Jadavpur University for his thesis "Novel statistical applications for the inference of spatial and temporal distribution of cholera in and around Calcutta, India." under the supervision of Dr. T. Ramamurthy.

Mayukh Das was awarded the degree by Jadavpur University for his thesis "Molecular analysis of *V. cholerae* bacteriophages: cloning and sequencing of phage DNA" under the supervision of Dr. B. L. Sarkar.

Tushar Subhra Bhowmick was awarded the degree by Jadavpur University for her thesis "Studies on phage typing of *V. cholerae* and comparison with other molecular typing system" under the supervision of Dr. B. L. Sarkar.

Souvik Chatterjee was awarded the degree by Jadavpur University for his thesis "Molecular characterization of diarrhoeagenic *Vibrio cholerae* strains with emphasis on their toxigenic trait" under the supervision of Dr. R. K. Nandy.

Amit Ray Choudhuri was awarded the degree by Jadavpur University for his thesis "Comparative genetic analysis and molecular characterization of the newly emerged *Vibrio cholerae* O1 Inaba strains isolated from patients with diarrhoea in Kolkata, India" under the supervision of Dr. A. K. Mukhopadhyay

Nivedita Ray was awarded the degree by Jadavpur University for her thesis "Studies on adhesion colonization and patho-physiological activity of *Vibrio cholerae* during course of gastrointestinal infection in animal model" under the supervision of Dr. H. Koley.

BIOCHEMISTRY

The focus of the Division of Biochemistry lies in elucidating the molecular mechanism of host-pathogen interactions in diarrheal diseases. Therefore first 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 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, take up the 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. As this is a frontier area of biomedical research, we ventured into this area after developing our infrastructure. Our research interests are 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 Officer Tapan Roy, Laboratory Technician
Senior Research Fellow	:	Abhisek Ghosal Avishek Ghosh
Junior Research Fellows	:	Sreerupa Ganguly Moumita Mondal
Research Assistant	:	Subrata Sabui

1. *Vibrio cholerae* cytolysin/hemolysin (VCC): Structure- function relationship of a pore-forming toxin (PFT) with multiple biological functions

Principal Investigator : Kalyan K. Banerjee

Co-Principal Investigator : Nabendu Chatterjee

Vibrio cholerae cytolysin/hemolysin (VCC) is a pore-forming toxin (PFT) that causes lysis and death of a wide spectrum of eukaryotic cells by destroying selective permeability of the plasma membrane lipid bilayer. Earlier, we characterized the 65 kDa toxin as a multi-domain protein with discrete regions involved in self-assembly of the monomer to a â-barrel heptamer and in carbohydrate-dependent interactions with target membrane glycoproteins. Proteolytic deletion of the C-terminus â-prism lectin domain caused inactivation of the hemolytic activity without impairing membrane-binding and oligomerization activities. Elucidation of the three-dimensional structures of the membrane-inserted oligomers formed by the fully active 65 kDa toxin and its 50 kDa truncated variant by cryo-electron microscopy revealed that in contrast to the 65 kDa oligomer which had 7-fold symmetry, the 50 kDa oligomer was asymmetric. Presumably, the distorted morphology of the oligomer generated by the truncated variant interferes with its correct insertion into the lipid bilayer preventing formation of a functional channel. Biochemical studies of the interaction of the 65 and 50 kDa toxin variants with erythrocyte stroma suggested that VCC might be intrinsically incapable of moving into the lipid bilayer core due to its low amphipathicity. However, it interacts strongly with the erythrocyte integral membrane glycoprotein, glycophorin through the C-terminus â-prism lectin domain. This interaction seems to drive translocation of the 65 kDa toxin to the membrane bilayer core causing formation of a functional diffusion channel.

2. Molecular mechanism of enterotoxigenic *Escherichia coli* adherence in the intestine: host- pathogen relationship: Molecular characterization of enterotoxigenic *Escherichia coli* colonization factors.

Principal Investigator : Nabendu Chatterjee

Co-Principal Investigator : T. Ramamurthy

Enterotoxigenic *Escherichia coli* (ETEC) are an important cause of diarrheal disease in humans, affecting children and adults. ETEC strains have over 20 distinct, human-specific ETEC adhesins or colonization factor antigens (CFAs). The overall goal is to develop a simple and specific method for detection of different CFAs for typing ETEC and identify the most prevalent CFAs and characterize them.

We have developed a multiplex PCR-based method to detect common CFAs for quicker analysis. We found most ETEC strains were expressing CS6 (78%). Sequence analysis of both the CS6 structural genes (*cssA* and *cssB*) revealed that there was presence of point mutations. CssA was found to have three alleles and CssB had two, leading to AIBI, AIIBII, AIIIBI, AIBII and AIIIBII CS6 subtypes. The point mutations in the different alleles suggested there were changes in amino acids, which ultimately reflected in the partial alteration of the secondary structure of both subunits as determined by computational analysis. Cellular binding studies in Caco-2 cells with representative ETEC strains suggested that CS6 with AI or AIII allelic subtypes showed higher binding capacity than AII, whereas BI showed stronger binding than BII. The AII and BII alleles were mostly detected in normal persons, where AI or AIII and BI were predominant in strains isolated from patients.

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

Principal Investigator : Nabendu Chatterjee

Co-Principal Investigator : Kalyan Banerjee

Vibrio cholerae O1, a cause of epidemic diarrheal disease, normally resides in marine ecosystems in association

with 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.

V. cholerae binds to chitin and uses it 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.

We have recently shown that GbpA plays a role in intestinal adherence of *V. cholerae* by binding to intestinal mucin. We are in the process of defining the exact region needed for mucin binding. Our results also show that cellular factors were able to increase GbpA expression. On the other hand, GbpA was able to activate secretion of IL-8 from the host cells.

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. The recombinant, refolded ChiA2 showed almost similar enzymatic activity as of wild type ChiA2. The ChiA2 was also tested for its mucinase-like activity with a K_m of 7.8 mg/ml. Further characterization of ChiA2 is in progress.

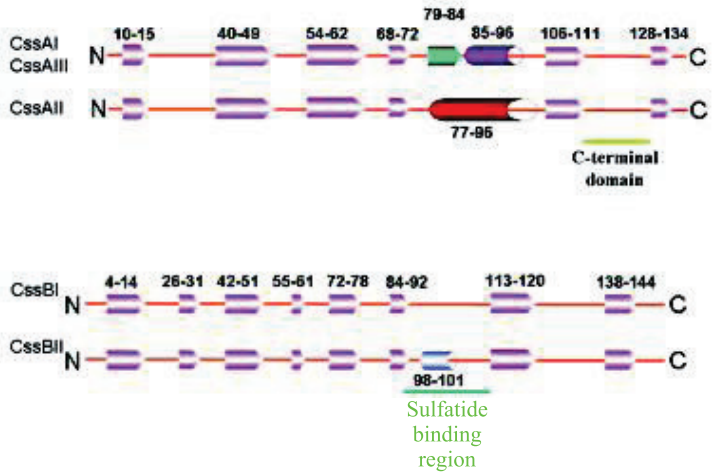


Fig. 1: Secondary structure of CssA subunit (Top) and CssB subunit (Bottom) as predicated theoretically from amino acid sequence. The barrel and arrow denote the α-helix and β-sheet respectively. The numerical number on the top of each barrel and arrow indicates the respective amino acid position of α-helix and β-sheet

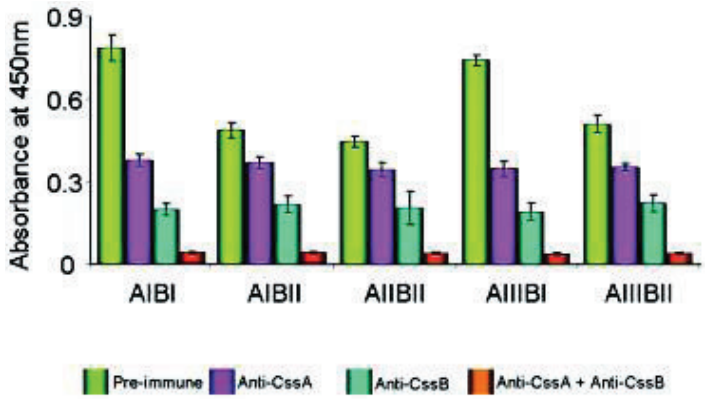


Fig. 2: Relative binding of representative CS6-allelic subtypes expressing ETEC (~5×10^6 CFU) to Caco-2 cells as determined by ELISA.

PRESENTATIONS AND VISITS

Dr. K. K. Banerjee

13th International Conference on Emerging Infectious Diseases in the Pacific Rim held at Kolkata, India during April 6-9, 2009.

International Symposium on Fifty Years of Discovery of Cholera Toxin: A tribute to S. N. De held at Kolkata, India during October 25-27, 2009.

Title of the talk: *Vibrio cholerae* hemolysin: Voyage of a pore-forming toxin from water to the hydrocarbon core of the membrane-lipid bilayer.

Dr. N. S. Chatterjee

13th International Conference on Emerging Infectious Diseases in the Pacific Rim held at Kolkata, India during April 6-9, 2009.

Poster Title: Chitin-binding protein GbpA of *Vibrio cholerae* and its role in the intestine.

Poster Title: Colonization factor CS6 of enterotoxigenic *Escherichia coli* has phenotypic and genotypic variations in patients of Kolkata, India.

44th US-Japan Cooperative Science Program, Cholera and Other Enteric Diseases held at San Diego, California, USA during October 12-14, 2009.

Title of the talk: Analysis of genetic diversity in the colonization factor CS6 of enterotoxigenic *Escherichia coli* of Kolkata, India.

International Symposium on Fifty Years of Discovery of Cholera Toxin: A tribute to S. N. De held at Kolkata, India during October 25-27, 2009.

International Symposium on Emerging Trends in infectious Diseases: TB & GI Infections held at Indian Institute of Technology-Kharagpur, India during January 5-7, 2010.

Title of the talk: A Tale of a colonization factor of enterotoxigenic *Escherichia coli.*

PhD DEGREE AWARDED

Budhaditya Mazumdar was awarded the degree by Calcutta University for his thesis "Structure-Function Analysis of *Vibrio cholerae* Hemolysin, a Pore-forming Toxin with Dimorphic Existence as a Water-soluble Monomer and an Amphipathic Oligomer" under the supervision of Dr. K. K. Banerjee.

Rudra Bhowmick was awarded the degree by Jadavpur University for his thesis "Molecular characterization of chitin-binding protein and chitinase from *Vibrio cholerae*" under the supervision of Dr. N. S. Chatterjee.

Abhishek Ghosal was awarded the degree by Jadavpur University for his thesis "Molecular characterization of a colonization factor antigen of enterotoxigenic *Escherichia coli*" under the supervision of Dr. N. S. Chatterjee.

CLINICAL MEDICINE

The Division of Clinical Medicine is conducting two studies on hospital based surveillance of diarrhoeal diseases. One surveillance project is conducted at Infectious Diseases Hospital where every 5th hospitalized patient of all age groups is surveyed on 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 are enrolled. One of the scientists is also involved in basic research 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 have also conducted research projects funded by external funding agencies. It was observed 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, with good safety profile and were well tolerated when administered to healthy Indian infants.

Scientists are trying 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 evaluating the role of probiotics for the better management of rotavirus associated diarrhea in children. An extramural grant has been received to study the regulation of antimicrobial peptide expression in the intestinal epithelial cells.

Scientists were involved in investigation of epidemics of diarrhoeal diseases and unknown fever. They were also involved in human resource development by providing training to the service providers like doctors and Para medical staff.

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

1. Outpatient based surveillance of diarrhoeal diseases at Dr. B. C. Roy Memorial Hospital for Children, Kolkata.

Utpala Mitra

Objectives of this study are to establish a systematic surveillance of diarrhoeal diseases and to identify the enteropathogens among the surveyed children who are attending Out Patients Department (Diarrhoea Treatment and Training Unit, run by NICED) at Dr. B.C. Roy Memorial Hospital for Children, Kolkata. This OPD based surveillance on diahhroeal diseases in children was initiated in January 2010. This project has been undertaken to determine the etiological identity of these diarrhoeal episodes which may lead to better management of these patients and develop useful strategies for prevention in future.

The systematic surveillance enrolls every 5th patient of first 5 days of the week who are attending OPD with the history of diarrhea. The clinical set up has been standardized 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 2253 diarrhoea patients visited the OPD of which 415 (18.4%) children were enrolled in the systematic surveillance system during January to April, 2010. Of these children 61.4% were male and 38.6% were female. Almost all the children (99%) were below 5 years of age. Seventy three percent of these children were from Hindu families and 27% from Muslim families. Average monthly income of majority of these families (82.4%) was Rs. 2500 to 5000. 59% children were from surrounding urban areas, 38% from rural areas and 2.7% from urban slums. Fig.1 shows the types of diarrhoea of enrolled children. Fifty nine percent children had the history of loose stools (unformed soft stools), 22.2% had watery diarrhea, 14.9% children had mucoid diarrhea and 3.9% had frank bloody diarrhea (dysentery). Around 20% enrolled children had the history of fever and cough. Fig.2 shows the dehydration status of the enrolled children. Majority (93.3%) of children showed 'No dehydration' and 6.7% children had “Some” dehydration at the time of enrollment as classified by WHO guidelines. Different enteropathogens detected from stool samples of the children (n=408) are depicted in Table-1. Though 415 children were enrolled, stool samples or rectal swabs were taken from 408 children as the parents of 7 children refused to provide samples. Major sole bacterial pathogens were *Campylobacter jejuni* (7.4%) and EAEC (5.9%). Major viral pathogen was rotavirus (21.5%).

2. A hospital based clinical study on efficacy of single dose Azithromycin and standard dose of Norfloxacin in the treatment of cholera in adult

M. K. Bhattacharya

The main objectives were to assess the efficacy and susceptibility of Azithromycin and Norfloxacin in the treatment of Cholera.and to find out suitable alternative drugs in the treatment of multidrug resistant *Vibrio cholerae* infection.

Cholera still ranks high in the etiology of diarrhoeal diseases in Kolkata. Acute watery diarrhoea caused by *Vibrio cholerae* is an important cause of hospitalization at the Infectious Diseases and Beliaghata General [ID & BG] Hospital in Kolkata. Nowadays the mortality rate of Cholera has been dramatically decreased due to appropriate dehydration management and proper antibiotic therapy. In different recent studies it has been shown that most of the drugs usually used in cholera treatment now-a days is more or less resistant to causative agent of the disease. Recently, few studies showed that NORFLOXACIN and AZITHROMYCIN are both very sensitive to the said organism. A very recent outbreak investigation of cholera conducted by a clinical team from National Institute of Cholera and Enteric Diseases in Purba Midnapur showed 100% sensitivity towards Norfloxacin and Azithromycin. Further clinical trials are necessary to established the efficacy, sensitivity, resistance pattern,

patients' tolerance, any adverse effect etc towards both the drugs .So careful administration of these effective drugs will afford protection against cholera and enable us to combat any emergency during outbreak situations of cholera.

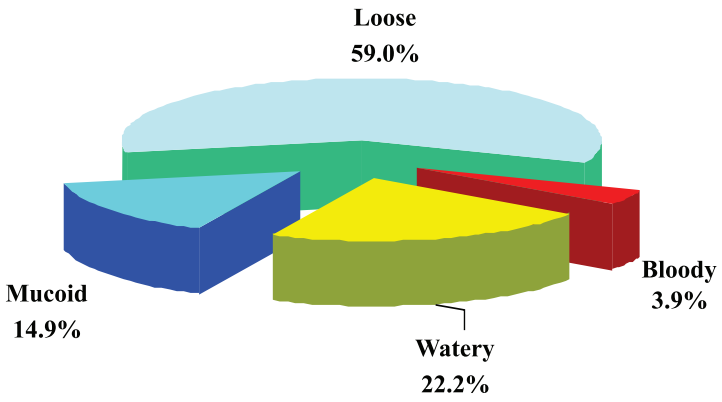


Fig. 1: Type of diarrhoea of enrolled children (n=415)

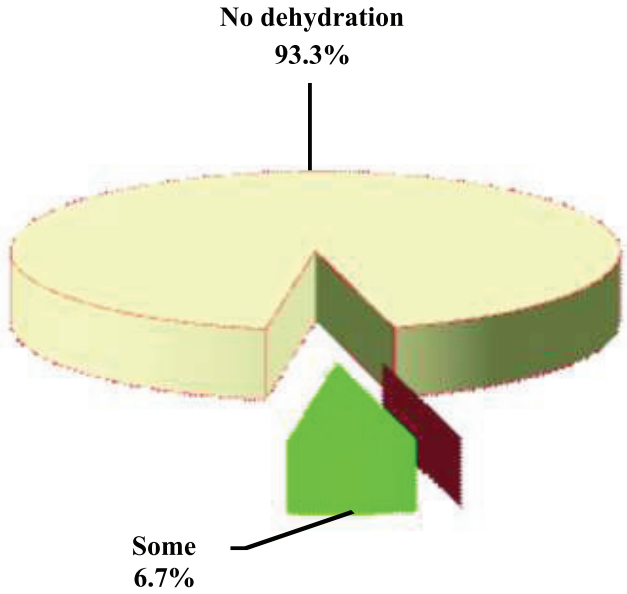


Fig.2 : Dehydration status of enrolled children (n=415)

Table-1: Aetiology of diarrhoea surveillance among children in 2010

Pathogen	All Age Group	
	Total (%)	Sole (%)
Bacteria		
Vibrio cholerae O1	8 (1.4)	3 (0.5)
Vibrio cholerae O139	0	0
V. cholerae Non O1 Non O139	5 (0.8)	2 (0.3)
V. Parahaemolyticus	0	0
Vibrio fluvialis	4 (0.7)	1 (0.2)
Aeromonas spp.	1 (0.2)	1 (0.2)
Campylobacter jejuni	81 (13.7)	42 (7.1)
C. coli	4 (0.7)	1 (0.2)
Shigellae	29 (4.9)	20 (3.4)
Salmonella	5 (0.8)	2 (0.3)
EPEC	40 (6.8)	15 (2.5)
ETEC group	44 (7.4)	20 (3.4)
EAEC	79 (13.4)	28 (4.7)
Virus		
Rotavirus	122 (47.5)	44 (17.1)
Adenovirus	66 (25.7)	10 (3.9)
Norovirus G1	3 (1.2)	0
Norovirus G2	17 (6.6)	8 (3.1)
Sapovirus	7 (2.7)	1 (0.4)
Astrovirus	3 (1.2)	0
Parasite		
Blastocystis hominis	1 (0.4)	0
Entamaeba histolytica	4 (1.8)	1 (0.4)
Giardia lamblia	42 (18.7)	9 (4)
Cryptosporidium spp.	10 (4.4)	1 (0.4)
Mixed Pathogen		155 (26.2)
No pathogen		227 (38.4)
Total		591 (100)

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

S. S. Das

Toll-like receptors (TLRs) are cell surface or intracellular receptors that recognize a wide variety of pathogen-associated molecular patterns (PAMPs). They not only form an integral component of innate immune system but also define the subsequent development of the adaptive immune responses. Bacterial flagellin is a major proinflammatory PAMP that functions as TLR5 ligand. Several critical amino acid residues over the NH2- and COOH terminal domains of flagellin were shown to determine the ligand-receptor affinity and the consequent activation of the NF-κB signaling pathways. A recent report suggests activation of TLR5 at a different cellular compartment by a non-conserved domain of flagellin and raises the possibility of considerable variation in intracellular signaling induced by different flagellins. However, a detail mechanistic study to address this issue has never been undertaken. We have explored how the flagellins of *Vibrio cholerae* (FlaE subunit) and *Salmonella typhimurium* (FliC) differentially regulate TLR5 downstream signaling pathways and gene expression despite having similar amino acid residues over the conserved domains and equal binding affinity for TLR5 (**Fig 1a**, TLR5 pull-down with biotinylated flagellins). Studies with synthetic inhibitors revealed that FlaE regulates IL-8 expression mainly through ERK MAPK- dependent pathway, while FliC predominantly utilizes P38 MAPK and NF-kB (Fig 1b). Direct activation of ERK MAPK and NF-kB signaling pathways was investigated by western blot analysis, which clearly suggests differential activation by the two flagellins (Fig 1c). EMSA was performed to investigate differential recruitment of transcription factors. While FlaE activates AP1, FliC regulates NF-kB in a P38 MAPK-dependent manner (Fig 1d). Luciferase reporter assay showed that FlaE and FliC predominantly activates AP1- and NFkB-dependent transcription, respectively (Fig 1e and 1f). We are currently focused on deciphering the complexities of TLR5 signaling induced by flagellins of diverse origins.

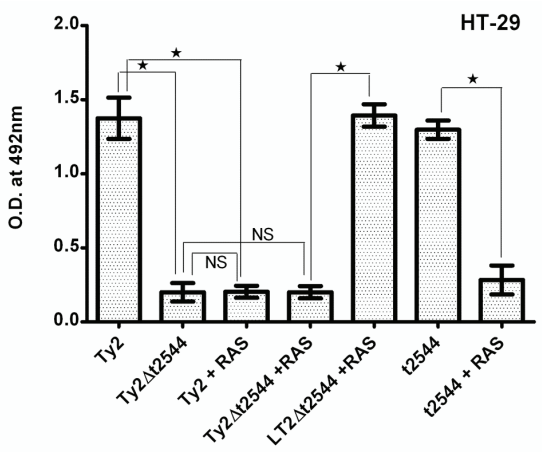


Fig. 1

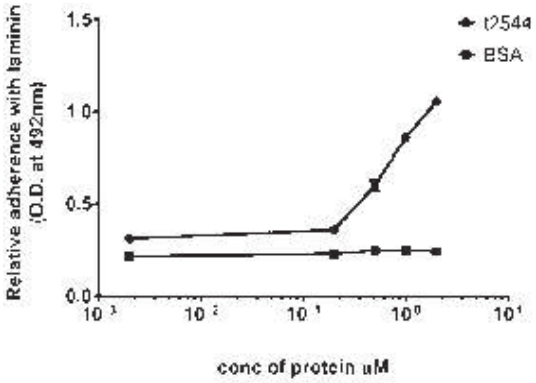


Fig. 1

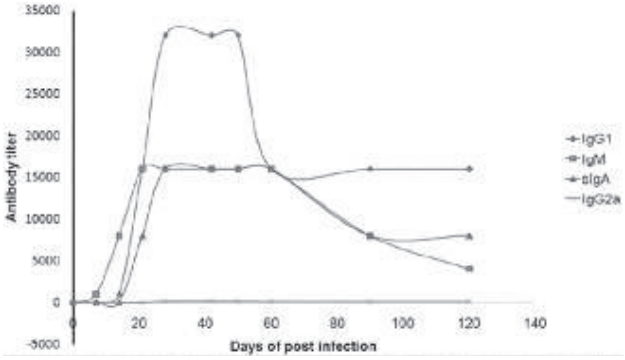


Fig. 2

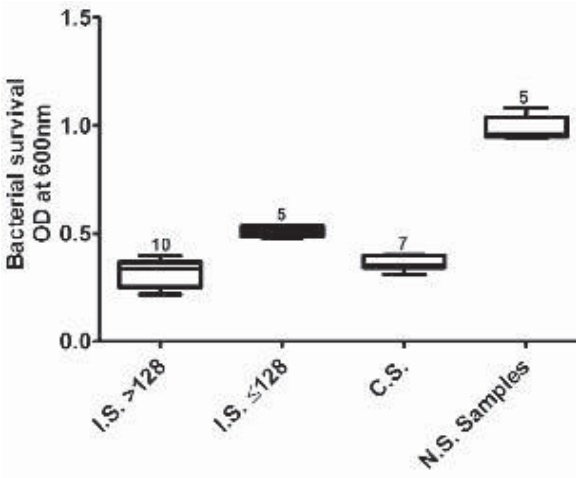


Fig. 3

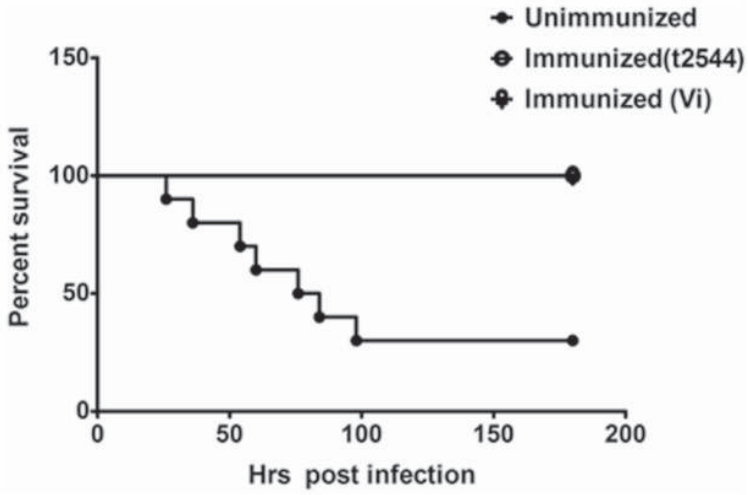
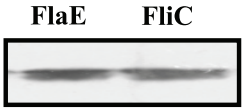


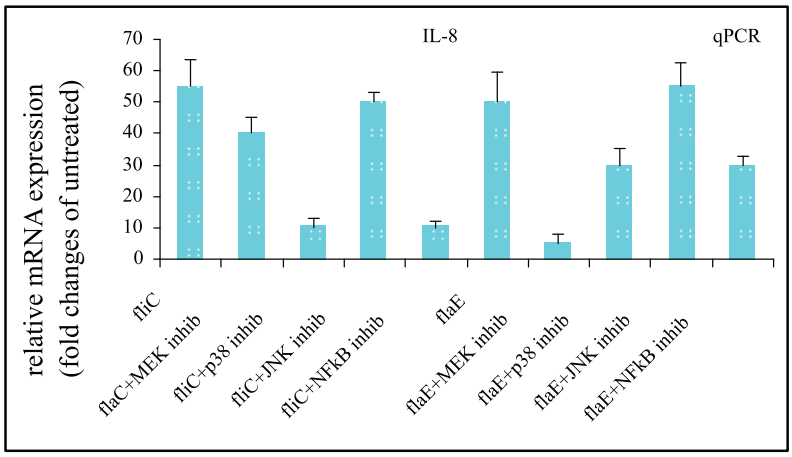
Fig. 4

A.

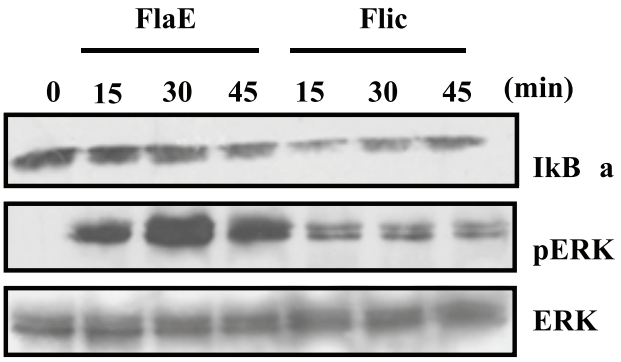


IP: streptavidin
WB:TLR5

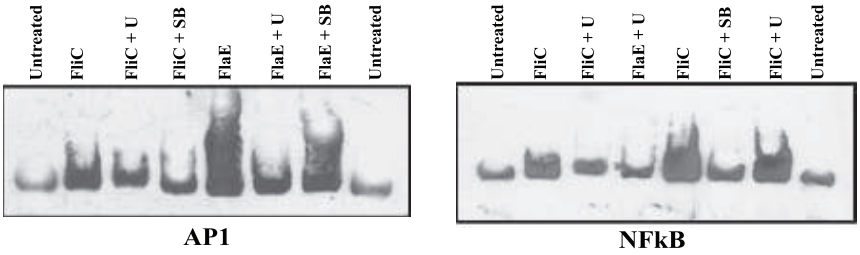
B.

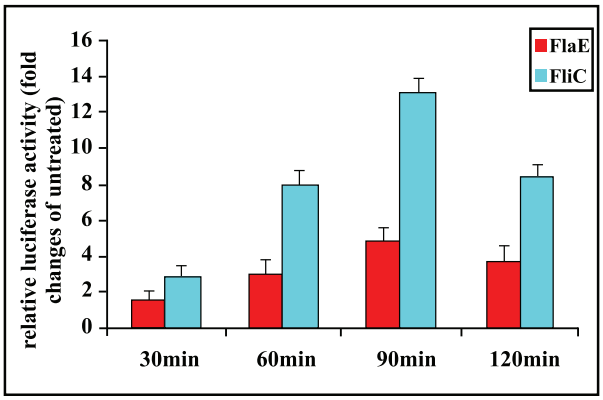


C.

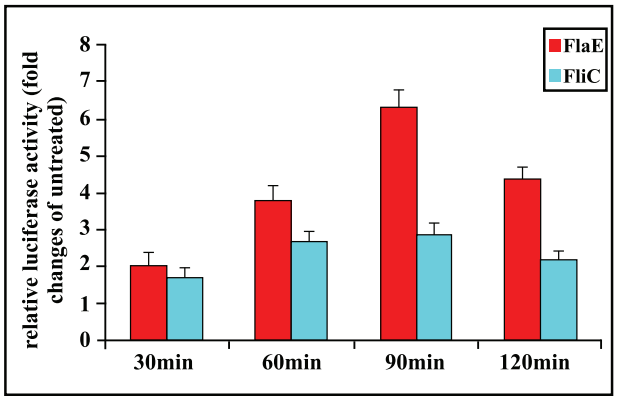


D.



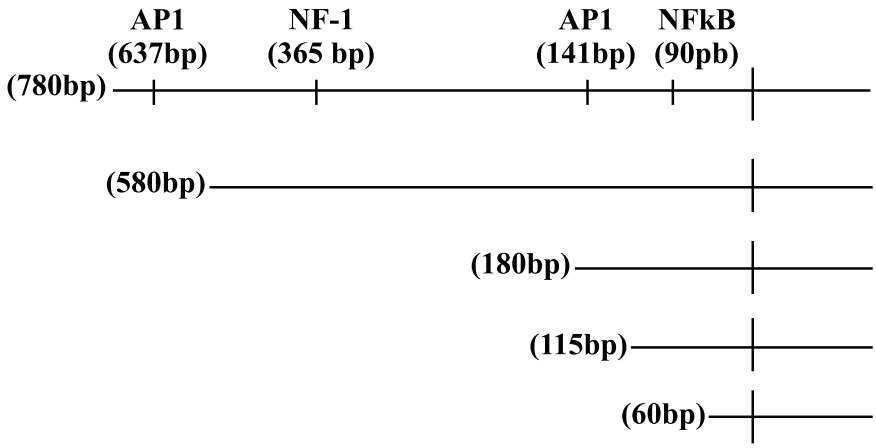


NFkB-Luc

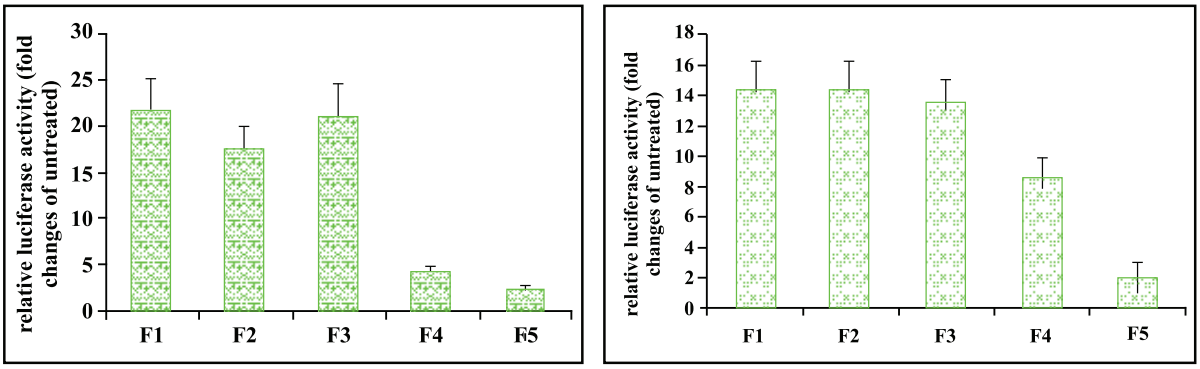


AP1-Luc

E.



F.



4. A study on the identification of novel virulence factors of *Salmonella typhi* and their role in pathogenesis and immune response.

S. S. Das

More than half of all *S. typhi* genes still remain un-annotated. While pathogenesis is incompletely understood, treatment is complicated by the emergence of drug-resistance and limited effectiveness of the current vaccines. In a search to find novel virulence proteins, we employed computational approaches and identified several putative proteins including adhesion /invasion molecules, mediated genes, transposons, integrase and IS elements. Further studies showed that a 27 kDa outer membrane protein (t2544) plays a major role in bacterial adhesion to the host through high-affinity binding to extracellular matrix component laminin. Role of t2544 in host cell adhesion is underscored by significantly reduced binding to colon epithelial cells HT-29 by the t2544 mutant bacteria as well as inhibition of binding of the wild type strain and the recombinant protein by pre-incubation with rabbit antiserum (RAS) (Fig 1A & 1B). t2544 is required for the pathogenesis of *S.* typhi as revealed by reduced systemic invasion of mice by the mutant bacteria that results in markedly increased LD_{50} compared to the wild type. t2544 is also strongly immunogenic and prolonged high titres of serum IgG and intestinal secretory IgA against t2544 were detected in the immunized mice (Fig 2). Seroconversion with high titres of bactericidal antibody was also found in naturally-infected individuals (Fig 3). Finally, mice pre-immunized with recombinant t2544 were protected against subsequent bacterial challenge (Fig 4). The present study describes a novel laminin-binding adhesin of *S. typhi* that is required for bacterial pathogenesis. Rapid seroconversion with the appearance of protective antibodies in naturally infected humans raises the hope of using this protein as a subunit or conjugate vaccine against *Salmonellosis*.

AWARDS AND HONOURS

Dr. S. S. Das

Received Travel Award from Okayama University, Japan to attend the 44th Joint Panel Meeting of the US-Japan Cooperative Medical Science Program on Cholera and Other Bacterial Enteric Infections, held from October 12-14, 2009 at San Diego, California, USA.

Selected external examiner for MSc. Part II, Department of Biotechnology, Jadavpur University.

Selected external faculty, MSc. Part II, Department of Microbiology, University of Calcutta.

PRESENTATIONS AND VISITS

U. Mitra

13th international conference on Emerging Infectious diseases of the Pacific Rim: Focus on Enteric diseases held at Grand Hotel, Kolkata during April 6-9, 2009.

Seminar on "Environment Day" organized by Indian Science congress Association, Kolkata chapter held at NICED, Kolkata on June 5, 2009.

International symposium on "50 years of discovery of cholera toxin: A tribute to SN De" held at Kolkata during October 25 - 27, 2009.

3rd Probiotics symposium organized by NICED & ICMR at New Delhi during November 21-22, 2009.

"Workshop on Nutrition" organized by Nestle Nutrition Institute, South Asia Region held at Taj Bengal Hotel , Kolkata on February 17, 2010.

M.K. Bhattacharya

Attended "Policymakers meeting on the introduction of cholera vaccination using new generation oral vaccines in India" at Hotel Taj Mahal, New Delhi on April 10, 2009.

Attended "3rd India Probiotics Symposium "Probiotic Foods in Health & Disease" held in New Delhi from November 21-22, 2009.

Attended "13th International Conference on Emerging Infectious Diseases in the Pacific Rim - Focus on Enteric Diseases at Kolkata" held in Kolkata during April 6-9, 2009.

Attended International Symposium on protective nutrients February 23-24, 2010 at New Delhi, India.

Dr. S. S. Das

Research paper entitled "Ligands differentially regulate TLR5 signaling and target gene expression in an affinity-dependent and independent manner" presented at the 44th Joint Panel Meeting of the US-Japan Cooperative Medical Science Program on Cholera and Other Bacterial Enteric Infections, held from October 12-14, 2009 at San Diego, California, USA.

"Identification of a novel virulence factor of *Salmonella typhi*" presented at the 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, held from April 6-9, 2009 in Kolkata, India.

"cAMP signal transduction pathway regulates human cathelicidin expression" presented at the 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, held from April 6-9, 2009 in Kolkata, India.

DATA MANAGEMENT

The Division of Data management primarily focuses on good data management practices to produce reliable, complete and accurate data from the various research projects of this Institute. Hospital based diarrhoeal diseases surveillance at Infectious Disease Hospital (IDH), Kolkata is an ongoing project to identify various 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.

K. Rajendran

The objectives of the study is to compare the climatic characteristics such as temperature, relative humidity (RH) and rainfall with observed infection of *Vibrio cholera* causing diarrhoea in the Infectious Diseases Hospital, Kolkata and to assess long term changes to develop Time series model and Mathematical Statistical Models. In climatic factors, the difference of RH and temperature [i.e., morning (max)-evening (min)] were used in the analysis. This procedure was relevant to identify the actual causative factors instead of mean factors. The mean factors purposefully have been averted to avoid the influence of high variation in the series.

Seasonal Auto integrative Moving Average (SARIMA)

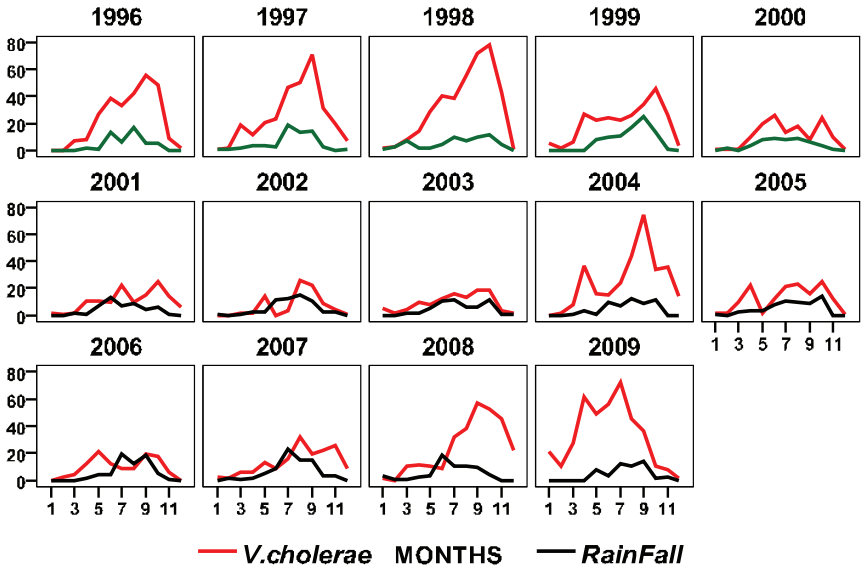
Time series model: Seasonal Auto Integrative Moving Average (SARIMA) has created candidate model of month-wise *V. cholerae* infection with predictor variables of RH, temperature and rainfall were in SARIMA (1, 0, 0) (0, 1, 1) along with seasonal difference to stabilize model. The periodicity, seasonality and pattern of *V. cholerae* infection were investigated for future prevention. Heavy rainfall indirectly stimulated the *V. cholerae* infection. High RH favours *V. cholerae* infection, that was linearly related whereas high temperature (mean) does not favour the *V. cholerae* infection.

Generalized Linear Model (GLM)

V. cholerae infection with rainfall

Daily *V. cholerae* (inclusive of O1, O139 and non-O1, non-O139 serogroups) positive cases and RH, temperature and rainfall were used for GLM and to ensure the variability of relative humidity in relation to *V. cholerae* infection, the data were divided into rainfall or no rainfall with *V. cholerae* infection.

The evening high RH (p=0.05) significantly favoured *V. cholerae* infection while RH and temperature were 86% and 29°C, respectively with rainfall around 10mm. The zero difference RH co-incidence with least variation of day temperature (<=5°C) favoured *V. cholerae* infection while RH (p=0.04) and temperature (p=0.04) were 90% and 28°C respectively with high rainfall (22 mm). The RH variation (p>0.05) was 10% in day co-incidence with

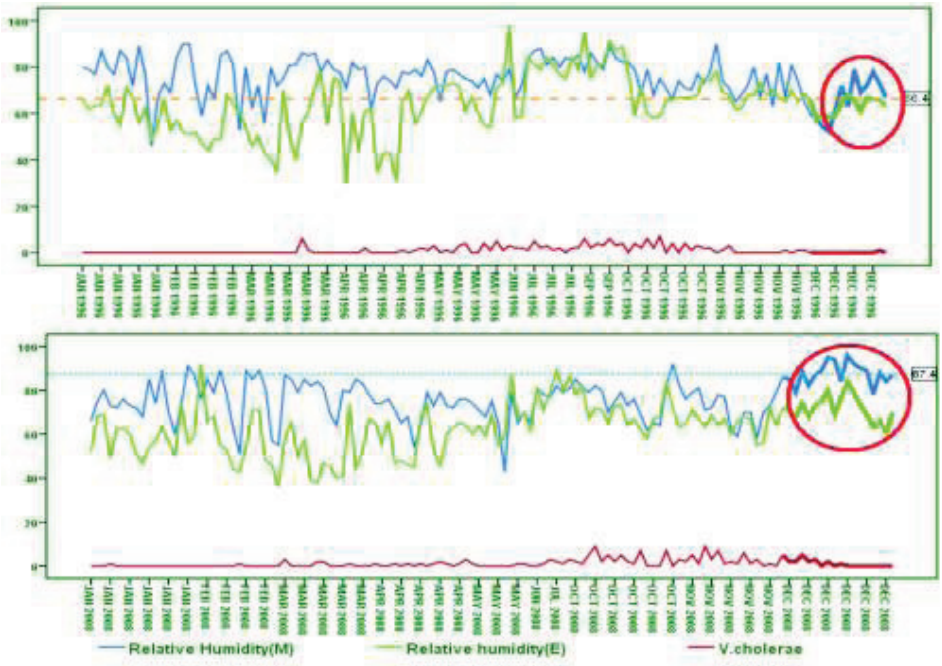


Relational impact Rainfall associated *V. cholerae* infection at Kolkata

temperature variation >5-10°C (p=0.02) favoured *V. cholerae* infection while RH and temperature were 84% and 29°C respectively with rainfall 13 mm. The monsoon (June-September) season significantly favoured *V. cholerae* (p<0.001) infection with high RH (86%), moderate temperature (29°C) and minimum average days rainfall (13 mm). Moreover, the post monsoon (October- November) season also significantly supported *V. cholerae* infection (p<0.001) with favourable temperature, RH and rainfall (27°C, 86% and 13mm, respectively).

V. cholerae infection with no rainfall

The evening high RH favoured (p<0.001) *V. cholerae* infection when RH and temperature were 79% and 29°C, respectively. Zero difference relative humidity (p<0.001) co-incidence with moderate variation of day temperature (>5°C-10°C) that favoured *V. cholerae* infection when the RH and temperature were 76% and 29°C, respectively. RH variation to 10% in day time favoured (p<0.001) *V. cholerae* infection even when the temperature was 27°C. The monsoon season seems to be highly favourable for *V. cholerae* infection (p<0.001) that coincided with day time high humidity (79%) and high temperature (31°C). In addition, the post monsoon season also showed significant association (p<0.001) with 27°C temperature and 75% RH. None of the other factors had significant association with the categorized RH, temperature and seasons in both categories.



Depicting 21% RH rise between 1996 and 2008 on unusual observed *V. cholerae* infection with no rainfall events

Unusual rise of V. cholerae infection in December 2008

The mean days RH (morning) has drastically increased to 21% in December 2008 (87.44±5.03) compared to December 1996 (66.43±8.5) while the temperature remained constant. Previously, it was predicted that winter would be warmer than normal owing to the La Niña event in 2007-2008, which was the strongest since 1988-1989. This may be attributable to the sudden increase of RH as well as the *V. cholerae* infection.

Identified ARIMA (1,0,0) (0,1,1) Model depicts climatic impact for Rainfall, Temperature and Relative Humidity of the *V. cholerae* infection at IDH, Kolkata.

Model parameters	Estimate	|t| - values	p-value
Rainfall: Non Seasonal Lags AR(1)	0.638	9.724	<0.001**
Seasonal Lags MA(1)	0.809	9.721	<0.001**
I(1)	Seasonal Difference		
Temperature: Non Seasonal Lags AR(1)	0.638	9.724	<0.001**
Seasonal Lags MA(1)	0.809	9.721	<0.001**
I(1)	Seasonal Difference		
Relative Humidity: Non Seasonal Lags AR(1)	0.645	9.909	<0.001**
Seasonal Lags MA(1)	0.792	9.721	<0.001**
I(1)	Seasonal Difference		
Relative Humidity	0.638	2.627	0.010*

*moderately significant, ** highly significant

PRESENTATIONS AND VISITS

B. Manna

Participated in 13th International Conference on Emerging Infectious Diseases in the Pacific Rim - Focus on Enteric Diseases at Kolkata" held in Kolkata during April 6-9, 2009.

Attended the Investigators Meeting for the project titled Diarrhoeal Disease Infants and Young Children in developing Countries (The Global Enteric Multi Centric Studies)in Kolkata from September 14-16, 2009.

Attended Vaccine Global Congress and displayed poster on "Burden of Dengue fever in eastern Kolkata, India: Data from a prospective study" in Singapore from October 3-7, 2009.

Participated in 3rd Probiotic Symposium in New Delhi from October 22-23, 2009.

Participated in the international symposium on 50years of discovery of Cholera Toxin: A Tribute to SN De in Kolkata from October 25-27, 2009.

Acted as resource person in "Epidemiology and Vaccinology Workshop for Representatives of the Democratic People's Republic of Korea" in January 2010 and presented three lectures on Basic concepts of Biostatistics.

Attended training course on Good Clinical Practices. March 2, 2010 in Kolkata, India organized by Shantha Biotech and National Institute of Cholera and Enteric Diseases.

K. Rajendran

K. Rajendran, A. Sumi, M. K. Bhattacharya, B. Manna, N. Kobayashi, G. B. Nair and T. Ramamurthy. Spectral analysis of Climate, EI Niño and solar effects on the prevalence of Cholera in Kolkata, India. The 13th International Conference on Emerging Infectious Diseases of the Pacific Rim: Focus on Enteric Diseases. April 6-9, Kolkata, India.

K. Rajendran, A. Sumi, M. K. Bhattachariya, B. Manna, D. Sur, N. Kobayashi, and T. Ramamurthy. Influence of relative humidity in *Vibrio cholerae* infection: A Time series model. an International symposium on "fifty years of

discovery of cholera Toxin: A tribute to SN De at Kolkata during October 2009.

Attended Workshop on" NIH/NIAID research resources" held on April 5, 2009 at NICED, Kolkata.

Attended GIS training program on "Introduction to Arc View 9.3" held during 23-24 April, 2009 at ESRI, Kolkata India.

Attended Advance training programme on "Cyber law, Information Security and Software Quality assurance for Scientists and Technologists" Sponsored by DST, Government of India, held during July 6-10, 2009 at Indian Institute of Public Administration, New Delhi, India.

Attended Short-Term training programme on "Soft Computing (Sofcom '09)" held during July 27-31, 2008 at center for soft computing research, *INDIAN STATISTICAL INSTITUTE, India.*

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 studied using cryoelectron microscopy and single-particle analysis. The 3-D structure of hemolysin oligomer, a pore-forming toxin of *Vibrio cholerae,* has already been studied. 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 is 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. 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 microscopy. 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 Officer
		S. Kumar, Sr. Laboratory Assistant
		B. R. Mallick, Lab Attendant
Senior Research Fellow	:	Somnath Dutta

1. Pathogenetic implications of *H. pylori strains in* gastroduodenal diseases and gastric cancer

D R Saha

In a preliminary study, we examined the influence of *cag* PAI on gastric infection and MMP-9 production in mice and cultured cells. Two unrelated mouse adapted *H. pylori* strains were used SS1 and AM1 (Indian strain). SS1 is often used as the standard mouse adapted strain for experimental infection (The Sydney strain). AM1 that lacks the *cag* PAI was used to study the *cag* PAI importance in inflammation. Both strains of *H. pylori* were grown on brain-heart infusion agar (BHI; Difco) supplemented with 7% sheep blood, 0.4% isovitalex and the antibiotic amphotericin B (8µg/ml), trimethoprim (5µg/ml) and vancomycin (8µg/ml). Nalidixic acid (10µg/ml), polymixin B (10µg/ml) and bacitracin (200µg/ml) were added to this medium when culturing *H. pylori* from mouse stomachs. The plates were incubated at 37ºC under 5% O_2, 10% CO_2 and 85% N_2. In all experiments, overnight grown cultures on BHI agar plates were used.

Groups of C57BL/6 mice were inoculated separately with *H. pylori* strains AM1 *cag* and SS1 cag^+. Gastric tissues were histologically examined and bacterial colonization was scored by quantitative culture. Mice infected with either cag^+, or *cag* *H. pylori* strains showed inflammatory changes in gastric mucosa and elevated MMP3 production.

PRESENTATIONS AND VISITS

A. N. Ghosh

Attended "13th International Conference on Emerging Infectious Diseases in the Pacific Rim" from April 6-9, 2009. sponsored by: National Institute of Cholera & Enteric Diseases, Kolkata, India, National Institutes of Health (NIH), USA, National Institute of Allergy and Infectious Diseases (NIAID), USA.

Attended "International Symposium on Fifty Years of Discovery of cholera toxin: A Tribute to S.N. De" organized by National Institute of Cholera and Enteric Diseases, Indian Institute of Chemical Biology and Bose Institute, Kolkata, India during October 25-27, 2009.

D. R. Saha

Attended the US-Japan CMSP: 13th International Conference on Emerging Infectious Diseases in the Pacific Rim-focus on Eneteric Diseases held in Kolkata, India from April 6-9, 2009 and presented a poster on 'Magnitude of active gastritis among *H. pylori* infected symptomatic and asymptomatic subjects from Kolkata population'

Attended a 3 day international symposium on 'Fifty years of Discovery of Cholera toxin': A tribute to S N De - October 25-27, 2009 at Salt Lake City, Kolkata, India.

Attended the 58th Annual conference of Indian association of Pathologists and Microbiologists from December 18-21, 2009 at Science City, Kolkata, India.

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 diarrheal disease morbidity and mortality. The Division is engaged in collaborative projects with international research institutes like International Vaccine Institute Seoul, Korea and other research Institutions like Johns' Hopkins, University of Maryland, USA. The division has successfully completed large scale vaccine trials.

Epidemiologists of the division are also frequently called for outbreak investigation of diarrhea, unknown fever, H5N1 surveillance and helping 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

1. Surveillance for Dengue Fever in Eastern Kolkata

Principal Investigator : Sekhar Chakrabarti

Co-Principal Investigator : Dipika Sur

Co-Investigator : Suman Kanungo, Byomkesh Manna, Shyamalendu Chatterjee, Provash Sadhukhan, Shanta Dutta in Collaboration with International Vaccine Institute, Seoul, Korea

Objectives:

To determine the incidence and burden attributable to dengue along with the epidemiologic, clinical and virologic characteristics.

To assess the characteristics of severe dengue fever through health care facility based enhanced sentinel surveillance for febrile illness.

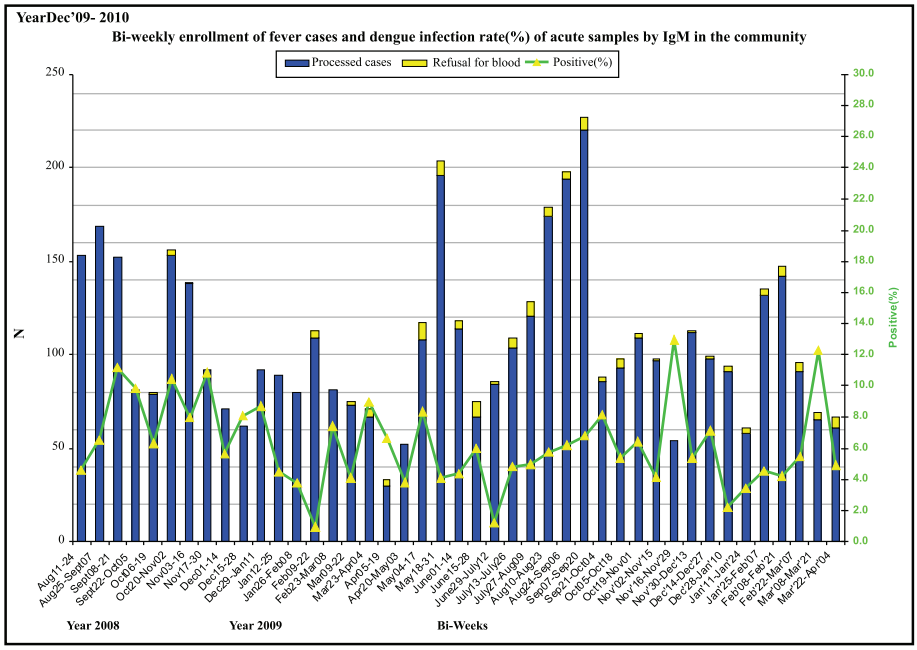
Results:

During 20 months of surveillance, a total of 4633 patients presented at the field outposts with 0-7 days of febrile illness and among them 4515 (97.4%) samples were collected. Overall, dengue detection rate by IgM ELISA among acute fever cases was 6.2%. It was higher in the age group of 0-15 years. The overall crude dengue rate was 2.6/100/year but the highest incidence (7/100/year) was observed in 0-5 years age group of children. So far, DEN-1 is the prevailing strain.

The percentages of RT-PCR are DEN 1-46.2, DEN 2-29.1, DEN 3-0.7, DEN 4-24

Conclusion:

This data suggests 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. This is one of the pioneer studies in determining the population based incidence of dengue in our country.



2. Diarrheal Disease in Infants and Young Children in Developing Countries

Principal Investigator : Dipika Sur

Co-Investigator : T. Ramamurthy, B. Manna & S. Kanungo
in collaboration with University of Maryland

Objective:

This project is aimed to estimate the population-based burden, etiology and etiological burden of enteric pathogens, among children less than five years, suffering from severe diarrhoea and consequences of severe diarrhea among these children.

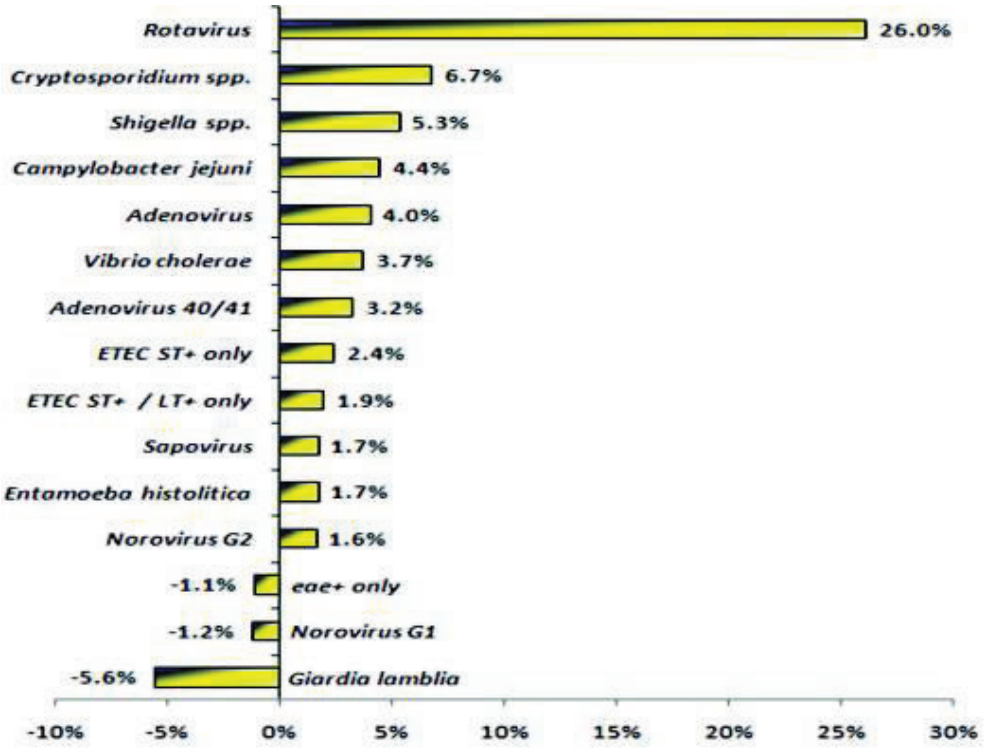
Activities:

The study site was selected in the slum areas within Wards 14, 31, 34, 58 and 59 of Kolkata Municipal Corporation (KMC) in eastern Kolkata. Currently, a total of 1,95,994 population including approximately 13,517 children are under Demographic Surveillance System (DSS) at 4 months interval for collection of demographic events. A Case-Control study was conducted with cases (from the study sites only) being enrolled from Two Sentinel Health Centres (Infectious Diseases Hospital and Dr B.C. Roy Memorial Hospital for Children) and also two SHC's at community level, controls matched for age and sex from the neighborhood of the case.

Results:

Since the beginning of the study, (Dec'07-Mar'10), a total of 4,413 births were detected and 201 deaths were reported from Demographic Surveillance System. A total of 1187 cases and 1475 controls of children aged below five years were enrolled with 516 cases in 0-11 month, 441 cases from 12-23 month and 230 from 24-59 months of age.

ERI of causative organism for diarrhea



ERI: Excess Risk of Infection

3. **Safety and immunogenicity of a Killed oral cholera vaccine among infants 10 weeks to less than 12 months of age when given concomitantly with EPI vaccines**

Dipika Sur

This phase-II clinical trial is an individually randomized, double-blinded, placebo-controlled trial in 300 healthy infants aged 10 weeks to 12 months allocated to receive either a bivalent killed oral cholera vaccine or a placebo. The primary objectives are (a) to determine the safety of the two-dose killed oral cholera vaccine among infants, and (b) to determine immune responses to one and two doses of killed oral cholera vaccine among infants, when given concomitantly with EPI vaccines.

This trial has been registered with the WHO and the Clinical Trials Registry, India. The study instruments, including the CRFs and consent forms are ready, most of the supplies, including the EPI vaccines have been procured, and the study staff have been hired and trained according to guidelines and set time schedule. However, due to need for some minor protocol modification (changed strategy for infant recruitment), we have not initiated the actual study activities, which is expected to begin sometime in July, 2010.

4. **A randomized controlled trial (Phase-II/III) of the live recombinant oral cholera vaccine (VA1.4) in eastern Kolkata**

D. Sur

Main target is to see the vaccine protective efficacy through a large scale Phase III field trial in cholera endemic areas. For that population enumeration is targeted as well, setting up of community based surveillance is planned in preparation for Phase III cholera vaccination.

Rapid assessment of 1,30, 000 population completed.

Census completed.

Surveillance initiated as per plan.

5. **Factors responsible for HIV transmission in married couples: a step towards intervention development.**

Samiran Panda

Art and testimonial: A Unique Community Based Approach to Reducing HIV/AIDS Stigma in West Bengal

The intramural project of NICED (ICMR) on HIV transmission in married heterosexual couples in West Bengal is currently in its final stage of recruitment of the participants. The project is being implemented in collaboration with the community based organization named 'Society for Positive Atmosphere and Related Support to HIV/AIDS' (SPARSHA) and the department of 'Dermatology, Venereology and Leprosy' of the RG Kar Medical College and Hospital, Kolkata. Clinical specimens are being collected following examination of the consenting participants who are either in concordant or discordant relationship for HIV. The laboratory tests conducted at NICED on the clinical samples following their transportation in appropriate condition include various aspects such as genital infections, serology for relevant co-infections and detection of vaginal anti-microbial peptides. The participants requiring treatment are being provided medication free of charge from the RG Kar Medical College and

Hospital. The data generated through this project is presently being computerized and the final analysis is pending. Without the collaborating partners, it would not have been possible for the project to reach its present stage. Field workers of this project, some of whom live with HIV, deserve a special mention as well for its successful development and implementation.

The experimental project supported by WBSARDM on HIV stigma reduction in the villages of West Bengal was of 18-months duration. Non-government organizations- SPARSHA and 'Make Art / Stop AIDS' (MA/SA) jointly developed this intervention where local performing artists worked hand-in-hand with community health workers some of whom lived with HIV. While training, and day-to-day implementation was overseen by SPARSHA, NICED evaluated impact of this project that ended in December 2009. Although the analysis is yet to be completed in full, preliminary findings are quite encouraging. Clearly 'people living with HIV' (PLH) and their family members witnessed positive changes in their lives as a result of this intervention. Another noteworthy finding of this project was drafting of anti-discriminatory declaration by schools in the intervention arm. Moreover 'stigma reduction committee' formed with active participation of PLH and community influencers in the intervention villages took up HIV-stigma related issues in the neighboring villages as well underlining an innovative scale-up potential of the intervention. Quantitative data analysis (facilitated by Biostatistics Department of CMC, Vellore) capturing positive attitudinal change in family-heads towards PLH in intervention clusters has been accepted for poster presentation in the international conference on AIDS to be held in Vienna during July, 2010.

6. Oncogenic HPV among female population with and without HIV infection in West Bengal, India

Kamalesh Sarkar

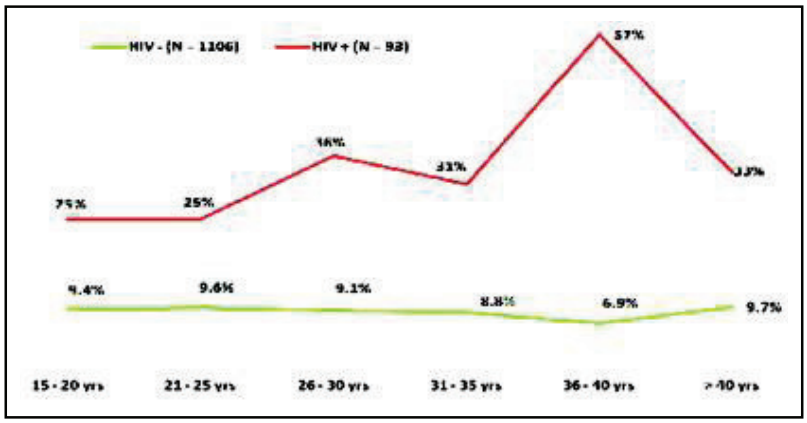
A total of 93 HIV positive women and 1106 HIV negative women participated in this study as cases and controls respectively. Mean age of cases was 29 years and that of controls was 30 years. The mean age of marriage for cases was 18 years and mean age of marriage for controls was 16 years. Mean duration of sexual life for HIV positive cases was 10 years and mean duration of sexual life for HIV negative cases was 13 years. Among HIV positive study population, prevalence of HPV was found to be 56% (n=52), prevalence of oncogenic HPV was 46.2% (n=43) and prevalence of HPV 16 and/or 18 was 32.2% (n= 30). Among HIV negative study population prevalence of HPV 16 and/or 18 was 9.1% (n=101) [OR = 4.7; CI: 2.8 7.9]. Thus, HIV positive females were almost 5 times more at risk of acquiring HPV 16 and/or18 co-infection than HIV negative females. Figure shows age-wise distribution of oncogenic HPV (indicated by HPV genotype-16 and/or 18) among cases and controls.

It shows that prevalence of HPV 16 and 18 is almost constant (approx. 9%) across different age groups in controls. But, in cases, prevalence of HPV 16 and/or 18 shows a rising trend till 40 years after which prevalence decreases. Prevalence of HPV 16 and/or 18 is higher among cases in each age group compared to controls. HPV co-infection was found to be significantly higher among cases than controls in both <=30 years and > 30 years indicted by Odds Ratio [(OR=4.1; CI: 2.1 7.6) & (OR=6.4; CI: 2.6 15.7)]

The duration of sexual life and age adjusted Odds Ratios show that, HIV positive females have a significantly higher risk of harboring co-infection with HPV 16 and/or 18 than HIV negetive females as indicated by Odds Ratio & confidence intervals as shown in the Table.

Oncogenic genotype-18 was the predominant pathogen (19%) followed by that of type-16 (16%) in HIV infected study subjects. Strikingly, prevalence of HPV 18 among the controls was found to be very low (n=10; 0.9%). Among other genotypes, HPV 52, 58 and 39 were observed in a sizable portion of cases. So, it is really alarming to observe

that there are other circulating oncogenic genotypes available in varying frequencies in the community. About 17% (n=15) of the cases were infected by multiple oncogenic strains of HPV of which 8 were infected with dual genotypes, 6 were infected with triple genotypes and only 1 was infected with 4 genotypes. HPV 16 and/or 18 were present in 13 out of 15 multiple genotype infected cases.



Age-wise distribution of oncogenic HPV among cases (HIV+ve) and controls (HIV-ve)

Duration of sexual exposure and Age adjusted Odds Ratios for cases and controls

	HPV+ve	HPV-ve	OR (CI)
Sexual exposure duration < =5 yr and Age < = 30 yrs			
Cases	7	18	3.3 (1.1 – 9.5)
Controls	24	203	
Sexual exposure duration > 5 yr and Age < = 30 yrs			
Cases	12	27	4.6 (2.0 – 10.3)
Controls	40	411	
Sexual exposure duration > 5 yr and Age > 30 yrs			
Cases	10	19	5.5 (2.2 – 13.7)
Controls	37	389	

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

Dr. Alok Kumar Deb

The primary objectives are to determine (a) overall incidence of diarrheal illnesses, (b) drug sensitivity pattern of the major pathogens, (c) risk factors for diarrhea and dehydration, and (d) cause-specific mortality and proportionate mortality due to diarrhea. Children of consenting parents, aged 0-4 years of either gender residing within the Langalberia PHC area of South 24-Parganas district are eligible to participate. To reach the objectives, the study consists of (a) surveillance for diarrhoeal diseases (b) case-control study to determine risk / preventive factors, and (c) verbal autopsies.

Initially, we developed the study instruments and local community volunteers were recruited and trained for this study. We started baseline data collection for the households and children on July 01, 2009, which were completed on February 28, 2010. In 6275 families, there were 26645 people (13726 male and 12919 female), including 1453 children (753 male and 700 female) up to 4 years of age. The approximate annual birth rate was 13.6 per 1000 population. 71.6% of the families were Hindu and 21.5% Muslim; the average family size was 4.2 and 58% of the families had average monthly income below Rs. 3000. Further analyses are being carried out and other study components (e.g. specimen collection and case-control study, as well as verbal autopsy) are expected to follow soon.

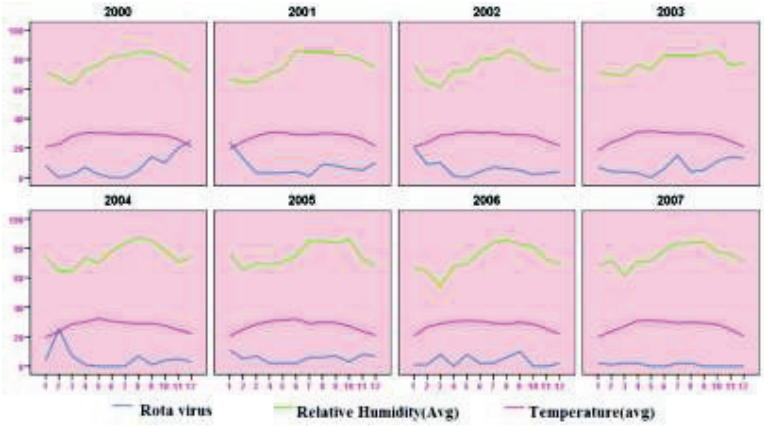
8. Impact of climate change on burden of Rota viral dairrhoea: a hospital based retrospective study September 2009- November 2009

Principal Investigator : Suman Kanungo

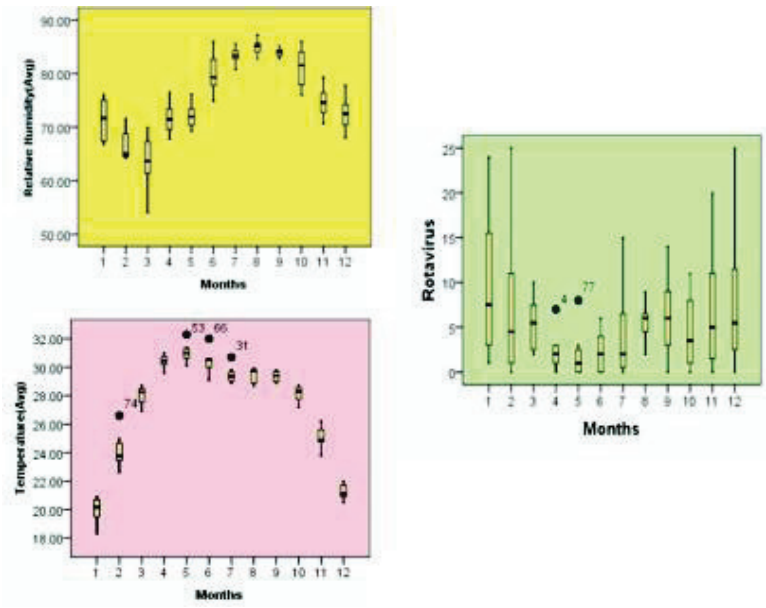
Co-Investigator : Dipika Sur, Byomkesh Manna, K. Rajendran and G. Balakrish Nair

The study was carried out to find out the relationship between variation in climatic conditions and rotavirus dairrhoea.

Seasonal pattern in Rota virus infection



Explorative Data Analysis of rotavirus infection



In this study, data collected over eight years (January 2000 December 2007) from Dr. B.C.Roy Memorial Hospital for Children was studied. Month wise data were structured and checked to exclude missing information and was plotted in a sequential curve.

This was designated as pilot study for a very short duration conducted only in three months. Nevertheless, this pilot study has brought out some important findings. The exploratory and time series analysis of the data showed that rotavirus infection was directly related to relative humidity. The winter months in Kolkata are around November to February, where we found the maximum incidence of rotaviral infections. The optimum temperature of 20°C and relative humidity of 70% may favour rotavirus infection during winter season, as there is high prevalence of rotavirus infection, during this period.

However other non climatic factors like sanitation and hygiene, water sanitation interplay with these climatic factors for the rotaviral infections in children. The data on non climatic factors were sparse and could not be analyzed to see any confounding effect on the burden.

9. A cluster randomized, placebo-controlled trial to measure the protection conferred by a single dose of the reformulated killed whole cell oral cholera vaccine in Kolkata, India.

Principal Investigator : Suman Kanungo

Co-Investigator : Dipika Sur, Byomkesh Manna, Swapan Kumar Niyogi

The primary objective was to evaluate the protective efficacy of a single dose regimen of an oral, bivalent killed whole cell cholera vaccine given to healthy, non-pregnant residents aged one year and over in Kolkata, India, against culture-proven *V. cholerae* O1 diarrhea detected in all treatment settings serving the population over 12 months of follow-up.

Design:

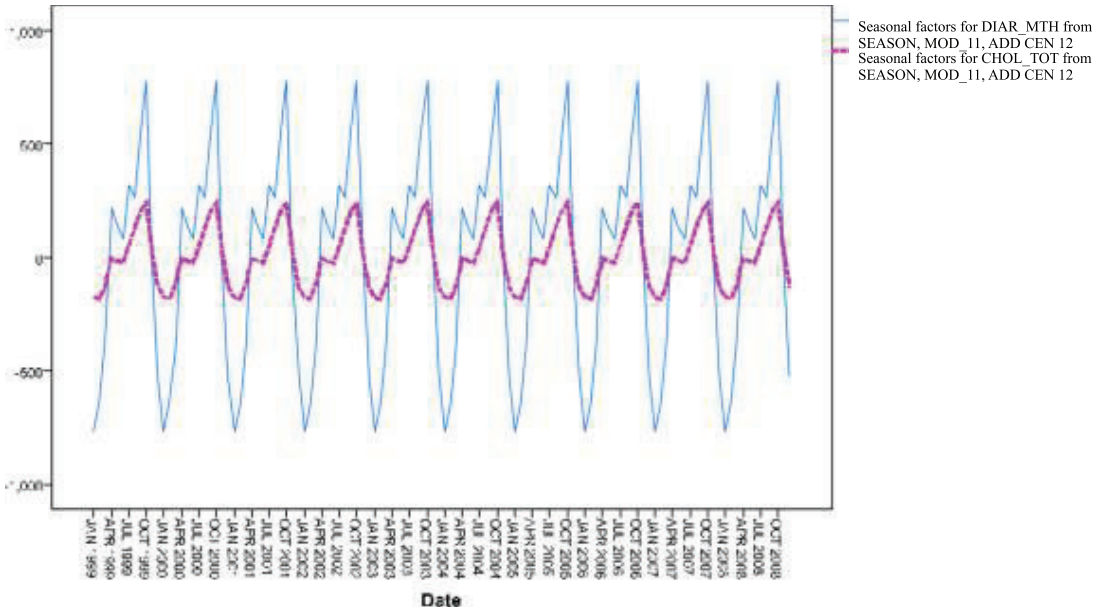
Placebo-controlled trial randomized by household of residents to one of two equal-sized groups:

A single dose regimen of oral, bivalent killed whole cell cholera vaccine given with water.

A single dose regime of oral placebo (killed *Escherichia* coli K12 strain whole cells) given with water.

Subjects:

Non-pregnant subjects aged 12 months and older who are permanent residents of Kolkata.



Specific exclusions: too ill to leave bed on the day of dosing

Sample size:

A geographic population of 84,452 is required for the study.

The study will be performed in select impoverished urban areas of the Kolkata Municipal Corporation where there is no ongoing cholera vaccine trial. Prior to the start of the study, census of the study area will be performed to enumerate all de jure residents in the area. Subsequent census updates will be performed annually using PDAs.

The study has been approved by the Scientific Advisory Committee of NICED and Institutional Ethics Committee. The study is planned to commence with census in urban slum in Aug 2010.

AWARDS AND HONOURS

D. Sur

National Academy of Medical Sciences Member (MNAMS)

National Academy of Sciences, India Fellow (FNASc)

"Diarrhoeal and Enteric Vaccines Advisory Committee" (DEVAC) of WHO - Member

K. Sarkar

Received an ICMR award known as 'Dr. M. K. Seshadri Award' for significant contribution in practice of community medicine in the field of HIV/AIDS on September 18, 2009.

PRESENTATIONS AND VISITS

D. Sur

Attended 13th International Conference on Emerging Infectious Diseases of the Pacific Rim: Focus on Enteric Diseases and presented "Field trial of a Reformulated Bivalent Killed Whole Cell Oral Cholera Vaccine in India Interim Analysis Results of a Cluster- Randomized Placebo-Controlled Trial" April 6-9, 2009 at Kolkata.

Participated in the Integrated Control of Acute Diarrhoea and Respiratory Infections Meeting of the Regional Technical Advisory Group, WHO April 23-24, 2009, Kolkata, India.

Participated in the international symposium "50 Years of Discovery of Cholera Toxin: A Tribute to SN De" in Kolkata from October 25-27, 2009.

Participated in 3rd Probiotic Symposium in New Delhi from October 22-23, 2009.

Chaired a session during the meeting on 'Focus on Neglected Tropical Infectious Diseases: Integrating Low Cost Vaccine into Global Cholera Control' at Annecy, France April 14-17, 2009.

Attended "Meeting of the cholera ad hoc working group for SAGE 2009" at WHO, Geneva June 29-30, 20 09.

Presented on "Cholera Vaccine Trials: an update" at WHO "Diarrhoeal and Enteric Vaccines Advisory Committee (DEVAC) meeting" at Malaga, Spain September 7-8, 2009.

Presented paper on "Effectiveness of Typhoid Immunization in an urban informal settlement in Kolkata" at 58th Annual Meeting of American Society of Tropical Medicine and Hygiene held at Washington DC, USA from November 18-22, 2009.

Presented on "Climate Variability and Diarrhoeal Diseases with Emphasis on Cholera" at Indo US Workshop on Climate Change and Health. August 31 - September 2, 2009, at Goa.

Faculty for training workshop as "Epidemiology and Vaccinology Workshop for Representatives of the Democratic People's Republic of Korea" organized by the International Vaccine Institute (IVI) and National Institute for Hygiene and Epidemiology (NIHE) in Hanoi, Vietnam from January 16- 31, 2010.

Samiran Panda

Attended 'South Asia Regional Knowledge Forum' organized by SARDM partnership of World Bank and Bill and Melinda Gate's Foundation's Avahan program and co-hosted by the National AIDS Control Organization (NACO) at New Delhi, India from March 15-16, 2010.

Attended the workshop entitled 'Evaluating the impact of development programs: turning promises into evidence' at Kathmandu, Nepal during February 22-26, 2010 upon invitation of the World Bank, Washington, USA.

Kamalesh Sarkar

Participated as a faculty at Asia Oceania Genital Infection & Neoplasia (AOGIN) Conference held at Hyatt Regency Hotel, Kolkata, during April 25-26, 2009.

Worked as Temporary Advisor, WHO-SEARO, New Delhi, and organized WHO Southeast Asian Regional Workshop on Cross-border Collaboration in Disease Control, held at Hotel ITC Sonar Bangla, Kolkata, April 28-30, 2009. Also presented our work on cross-border transmission of HIV through drug & sex-trafficking in India.

Alok Kumar Deb

Attended the International Symposium on "Fifty Years of Discovery of Cholera Toxin: A Tribute to S.N. De" organized by NICED, IICB and Bose Institute, Kolkata from October 25-27, 2009.

Acted as Resource Person in the "Informal Consultation on Research to Assess Communicable Disease Impact of Climate Change", held in Kolkata during August 24-26, 2009, organized by WHO-SEARO.

Meeting with NIMR scientists to finalize the WHO generic proposals on "Retrospective study" and "Preparedness and response" (regarding Climate change and Diarrhea / Cholera) during September 6-8, 2009, held at NIMR, New Delhi.

Good Clinical Practice (GCP) Training held by IVI, Korea and Shantha Biotechnics, Hyderabad at National Institute of Cholera and Enteric Diseases, Kolkata, India on March 2, 2010.

Attended and chaired a session in the Post-Surveillance meeting organized by NACO Regional Institute (East) in Kolkata during March 17-19, 2010.

S. Kanungo

Participated in the 13th International Conference on Emerging Infectious Diseases of the Pacific Rim: Focus on Enteric Diseases and presented a poster on "Immune responses following one versus two doses of the reformulated killed whole cell oral cholera vaccine in Eastern Kolkata, India"

Participated in the Integrated Control of Acute Diarrhoea and Respiratory Infections Meeting of the Regional Technical Advisory Group, WHO April 23-24, 2009, Kolkata, India.

Presented data in the 5th PDVI Field site consortium meeting in Asilomer, California, USA from June 3-5, 2009.

Attended the Informal Consultation on Research to Assess Communicable Disease Impact of Climate Change Kolkata, August 24-26, 2009 in Kolkata and presented a study protocol on "Impact of climate change on diarrhoeal diseases with emphasis on cholera, a prospective study"

Participated in the international symposium on Fifty Years of Discovery of Cholera Toxin: A Tribute to SN De

Participated in 3rd Probiotic Symposium in New Delhi from October 22-23, 2009.

Participated in the workshop "Clinical Trial: Design, Analyses, Interpretation and Reporting" at Biostatistics Resource and Training Centre, Christian Medical College, Vellore, India from July 13-17, 2009.

Attended the Joint Indo-U.S. Workshop on Climate Change and Health in Goa, India August 30 September 2, 2009.

Attended the Training workshop as faculty "Epidemiology and Vaccinology Workshop for Representatives of the Democratic People's Republic of Korea" organized by the International Vaccine Institute (IVI) and the National Institute for Hygiene and Epidemiology (NIHE) in Hanoi, Vietnam from January 16-31, 2010.

Attended training course on Good Clinical Practices, in Kolkata, India, on Mar 2, 2010 organized by Shantha Biotech and National Institute of Cholera and Enteric Diseases.

PhD DEGREE AWARDED

Baishali Bal was awarded the degree by West Bengal University of Health Sciences for her thesis "A study to understand the epidemiology of non-tobacco substance use, sexual abuse and HIV/AIDS in street children of North Kolkata city" under the supervision of Dr. K. Sarkar.

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, Laboratory Assistant
Research Scientist	:	Ratna Biswas
Junior Research Fellows	:	Krittika Sasmal Subhadeep Mukherjee

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

Tapas Biswas

Porin expanded T cells by inducing cell division and protecting from apoptosis through direct costimulation by TLR2. The TLR2 costimulation revealed increase of CD25 expression on CD3-stimulated T cells followed by IL-2 release. Inhibition of ERK1/2 activation and NF-β translocation impeded porin-induced proliferation of CD3-stimulated T cells, indicating a direct role of ERK and NF-β in proliferation of the cells. Study on the role of NF-β and ERK on proliferation and survival of activated $CD4^+$ T cells portrayed pivotal role of ERK as evidenced from drop in survival of the activated T cells and Bcl-X_L in presence of ERK inhibition. Release of TNF- and IFN- on porin treatment and their absence in presence of anti-TLR2 Ab confirmed the TLR2 dependence and Th1 bias of the effector functions of activated $CD4^+$ T cells. Next, flow cytometric and immunoblot analysis revealed suppression of TLR2 and MyD88 expression, respectively, in the cells transfected with TLR2 and MyD88 siRNA confirming the importance of the two molecules as key players in porin-mediated response of $CD4^+$ T cells. The expression of the chemokines MIP-1 and MIP-1 and their receptor unequivocally indicated the activation of T cells. This data indicate that in tune with TCR stimulation, porin induces TLR2 costimulation that initiates downstream signaling for both T cell survival and proliferation, and expression of specific cytokines potentiating the property of porin in priming the adaptive immune response.

PRESENTATIONS AND VISITS

T. Biswas

Participated in the International Symposium: Fifty years of Discovery of Cholera Toxin: A Tribute to S. N. De, October 25-27, 2009 at Kolkata.

PhD DEGREE AWARDED

Pallavi Banerjee was awarded the degree by Jadavpur University for her thesis “Maturation of Dendritic Cell by *Shigella* Porin Incorporated Liposome for T Cell Differentiation and Response” under the supervision of Dr. T. Biswas.

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., it also 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 the transcriptomic and proteomic level. A surveillance of enteric parasites from stool samples collected from different hospitals are regularly done in this lab to understand 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 from 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
		Avik Kunar Mukherjee
Junior Research Fellow	:	Dibyendu Raj
		Sumallya Karmakar
Research Assistant	:	Punam Chowdhury
		Koushik Das

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

Sandipan Ganguly

In the present study, our goal was to find out the ribonucleoproteins involved in binding with snRNAs for RNPP formation which is considered to be the major step in ribosome biogenesis. Three major proteins namely Fibrillarin, GAR1 and F protein and two snRNAs namely snRNA J and H were identified and cloned.

Fibrillarin is one of the major protein in RNPP complex formation and it forms the complex with its N terminal Glycine Arginine rich domain. GARI is involved in binding with H/ACA box during snRNPP formation. Small RNA putative protein F binds with both Box C/D and H/ACA motif of snRNAs. All these proteins were purified after successful recombinant expression in *E.coli* system. Antibody has been raised for these proteins and localization study of these proteins indicated their nuclear localization as well as formation of multiprotein-RNA complex. Bioinformatic study followed by mutation of protein binding motifs of the two snRNAs following GEMSA, has clearly suggested that some of the important protein binding sites in snRNA are different in primitive eukaryote *Giardia* than other fully evolved and developed eukaryotes.

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

Sandipan Ganguly

Giardia lamblia, a microaerophilic intestinal parasite, causes epidemic and endemic diarrhoeal illness called giardiasis in developed and developing countries. Giardia has to tolerate 60μM of O_2 concentration within the gut where it resides, but this amitochondriate lacks cytochrome and other conventional respiratory oxidases, responsible for reactive oxygen species detoxification. The research project was envisaged with the main objective to know how Giardia survives and establishes its pathogenesis in human body using its own characteristic oxidative stress management pathway.

A full genomic DNA library has already been prepared to date and a DNA array has also been constructed. The DNA Chip has been hybridized with control and stressed cDNAs labelled with Cy3 and Cy5 dyes. From the scanning analysis, the genes which are differentially regulated have been identified and sequenced. The list of genes with more than 10 fold differential regulation and corresponding Gene IDs is shown in Table 1.

Names of the genes	Gene_ID
Metabolic enzyme coding genes	
NADH Oxidase	GL50803_9719
NADH Ferrredoxin Oxidoreductase	GL50803_17151
Pyruvate Ferredoxin Oxidoreductase	GL50803_114609
Thioredoxin Reductase	GL50803_9827
Nitroreductase	GL50803_15307
Arginine deiminase	GL50803_112103
Malate dehydrogenase	GL50803_3331
Alcohol dehydrogenase	GL50803_13350
Phosphatase and kinase coding genes	
CAM Kinase	GL50803_16034
Serine threonine protein phosphatase	Gl50803_21498

Transcriptional/translational and cell divisional protein coding genes	
Small subunit rRNA	GL50803_r0019
Large subunit rRNA	GL50803_r0013
TAR RNA loop binding protein	GL50803_32741
Nuclear LIM interactor interacting factor-I	GL50803_14905
TMP 55	GL50803_137641
Protein 21.1	GL50803_13590
FtsJ cell division protein	GL50803_16993
Spindle pole protein	GL50803_8512
Structural proteins coding genes	
Beta Giardin	GL50803_4812
Dynein light chain	GL50803_7578
Some other important protein coding genes	
Hsp70B2 cytosolic form	GL50803_88765
Hsp90 alpha	GL50803_98054
Cysteine rich variant specific protein	GL50803_113297
Sodium-hydrogen exchanger III	GL50803_102647
Cathepsin B precursor	GL50803_17516
Hypothetical protein coding genes	
Hypothetical protein	GL50803_17453
,,	GL50803_41258
,,	GL50803_9752
,,	GL50803_11772
,,	GL50803_15039
,,	GL50803_6464
,,	GL50803_16980
,,	GL50803_13274
,,	GL50803_3421
,,	GL50803_113722
,,	GL50803_8509

AWARDS AND HONOURS

S. Ganguly

Top Reviewer Honour (2009-2010) from Parasitology International journal published by Elsevier.

AIR interviews as a resource person on many popular scientific subjects on enteric diseases.

Resource person & technical expert in Fluorescence Correlation Spectroscopy and Fluorescence Lifetime Imaging in Saha Institute of Nuclear Physics, Kolkata, India.

Resource person for confocal microscopy technical committee at RMRIMS Patna, ICMR.

Reviewer of different peer reviewed International Journals *viz*. Parasitology International, Epidemiology and Infection, Chemotherapy, Indian Journal of Gastroenterology etc.

PRESENTATIONS AND VISITS

Sandipan Ganguly

Participation and presentation of three papers in International symposium on emerging infectious diseases US Japan meet, Kolkata, April 2009.

Participation and paper presentation in International Joint Forum on Infectious Diseases, September 16-17, 2009, Bangkok, Thailand.

Participation as a resource person in 58th Annual Conference of Pathologists and Microbiologists (APCON) 2009, Dec 2009, Kolkata, India.

Organizing head and resource person for the Workshop during 58th Annual Conference of Pathologists and Microbiologists (APCON), December, 2009, Kolkata.

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 in depth study of these signaling mechanisms.

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 course of our studies, two forms of Hemagglutinin Protease (HAP), viz. a mature 45-kDa and another processed 35-kDa form has been purified from a nonO1, nonO139 strain 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 homologous challenge which may lead to develop a simple, practical and effective vaccine against shigellosis.

The 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
Junior Research Fellows	:	Poulomee Karmakar

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

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 kD protein is capable of providing significant protection in rabbits against the challenge with virulent *Shigella flexneri* 2a. Therefore, in the present study an effort has been made to purify and characterize the 34 kD OMP of *S. flexneri* 2a and study its immune response. We have already purified the 34 kD OMP. Further, characterization of the purified 34 kD MOMP was done. The circular dichroism study revealed that MOMP has predominantly α-helix content. The purified protein exists as a monomer of 30.5 kD molecular weight and has a stokes radius of 24.6 Å (Fig.1). It was found that the antibody raised in BALB/c mice immunized with purified MOMP of *S. flexneri* 2a reacted strongly with whole cell preparation of *S. flexneri* 2a in an immunoblot assay. The immunofluorescence microscopy and antibody absorption studies revealed that the murine antiserum epitope was surface exposed on an intact bacterium. The cross-reactivity and conservation of MOMP antigen among *Shigella* spp. was also confirmed by immunoblot analysis. The purified antigen also up-regulated the production of nitric oxide, granulocyte colony stimulating factor and IL-12p70 by the peritoneal macrophage of BALB/c mice. Further studies are going on to identify the macrophage cell surface receptor and the in-depth mechanism of macrophage activation in response to the protein.

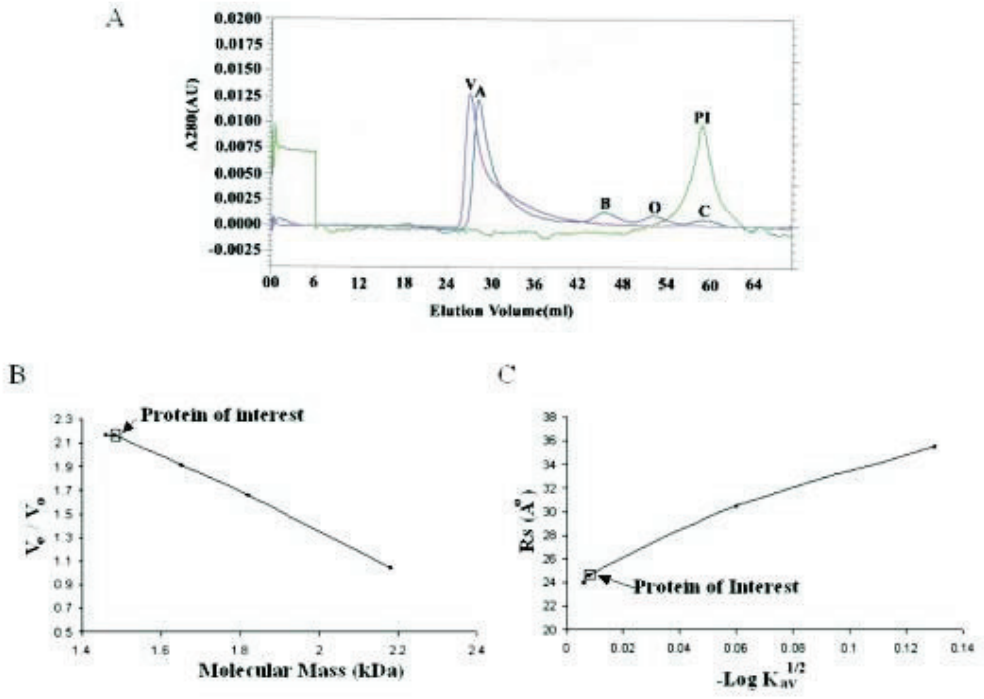


Fig 1. A: Analytical gel filtration chromatography. Conditions are described in the experimental section. Typical elution profiles (A_{280}) are shown with the positions of calibration markers: A, alcohol dehydrogenase; B, bovine serum albumin; O, ovalbumin; C, carbonic anhydrase; PI, purified 34 kDa OMP. The void volume is indicated by V. AU, absorbance units. B: Linear fit of the relative elution volume versus the logarithm of molecular weight of standard proteins. The arrow indicates the molecular weight of the purified MOMP is 30.5 0.07. C: Linear fit of known Stokes radii as a function of the measured partition Coefficient (K_{av}). The arrow shows the partition coefficient of purified MOMP, corresponding to 24.6 0.06.

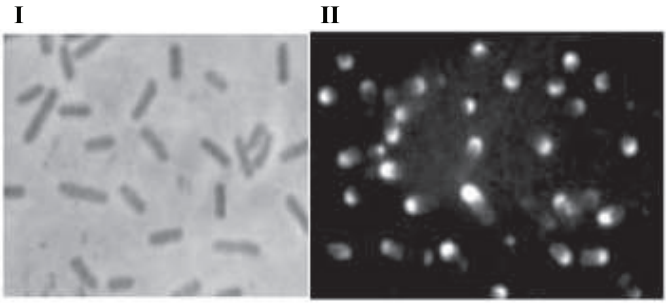


Fig. 2: Immunofluorescence microscopy of *Shigella flexneri 2a*. Bacteria harvested from mid-log cultures were treated with anti MOMP antibody (diluted 1:100 in PBS) and FITC-conjugated secondary antibody. The phase-contrast image is on the left-hand Side (I) and the IF image is on the right-hand side (II).

2. Studies on proteases of *Vibrio cholerae*

Amit Pal

Two well-characterized proteases secreted by *Vibrio cholerae* O1 strains are hemagglutinin protease (HAP) and *V. cholerae* protease (PrtV). The *hapA* and *prtV* knock out mutant, *V. cholerae* O1 strain CHA6.8?*prtV,* still retains residual protease activity. We partially purified the residual protease secreted by strain CHA6.8?*prtV* from culture supernatant by anion-exchange chromatography. The major protein band in native PAGE was identified by MS peptide mapping and sequence analysis showed homology with a 59-kDa trypsin-like serine protease encoded by VC1649. The protease activity was partially inhibited by 25 mM PMSF and 10 mM EDTA and completely inhibited by EDTA and PMSF together. Partially purified serine protease from CHA6.8?*prtV* induced hemorrhagic fluid accumulation in an RIL assay (FA=1.2 +/-0.2, n=3) and histopathological studies on rabbit ileum showed complete destruction of the villus structure with hemorrhage in all layers of the mucosa. Protease incubated with PMSF and EDTA induced a significantly reduced FA ratio (FA=0.3 +/-0.05, n=3) with almost complete normal villus structure. Histopathological studies of rabbit ileum treated with crude protein from CHA6.8?*prtV*?VC1649 strain induced minimal presence of RBC accumulation. We report for the first time the presence of a novel 59-kD serine protease in a *V. cholerae* O1 strain.

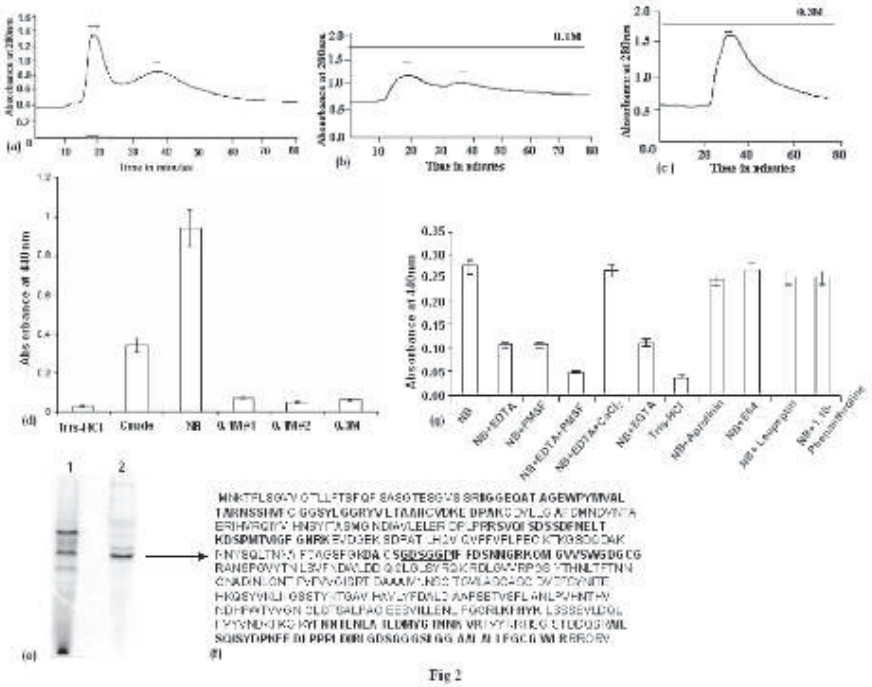


Fig 2

Partial purification and identification of protease: Chromatographic profile of ammonium sulphate precipitated crude proteins from culture supernatants of CHA6.8?*prtV* strain loaded onto an anion exchange column (DE-52). **(a)** Proteins eluted in the non-binding fraction (NB), **(b)** proteins eluted with 0.1 M NaCl, **(c)** proteins eluted with 0.3 M NaCl, +/- shows presence or absence of protease activity, **(d)** azocasein assay with pooled samples (30 µg) NB, 0.1 M#1, 0.1 M #2, 0.3 M and crude proteins. **(e)** Native PAGE profile (lane 1) of crude proteins of CHA6.8?*prtV* strain and (lane 2) of partially purified protease (NB) from DE-52 column. The marked protein band was analyzed by MS/MS sequencing and the peptides highlighted showed homology with a 59-kDa trypsin-like serine protease encoded by VC1649. **(f)** The underlined **GDSGGP** are the amino acid sequences around the serine residue present in trypsin-like serine proteases. **(g)** Protease inhibition test of NB fraction (5 µg) with protease inhibitors 10 mM EDTA, 25 mM PMSF, 25 mM PMSF and 10 mM EDTA, 10 mM EDTA and 20 mM $CaCl_2$, 10 mM EGTA, 1 µg/ml aprotinin, 28 mM E64, 1 µg/ml leupeptin and 10 mM 1,10-phenanthroline incubated for 30 mins at 37°C. Residual protease activity was assayed by azocasein assay. Twenty-five mM Tris-HCl was used as a negative control. The values shown are the means with standard deviations from three experiments

Fig. 4

Rabbit ileal loop assay: RIL response of partially purified protease (50 µg, NB) showing significant hemorrhagic fluid accumulation (FA ratio 1.2 +/-0.2 n=3) and its effect after inhibition with 25 mM PMSF and 10 mM EDTA (NB+PMSF+EDTA) shows significant decrease in fluid accumulation (FA ratio 0.3 +/-0.05 n=3). Twenty five mM Tris-HCl with 25 mM PMSF+ 10 mM EDTA was used as a negative control (FA ratio=0.12 +/-0.002, n=3).

AWARDS AND HONOURS

Manoj Chakraborti

Nominated as Secretary /Convener. Medical Science Section, West Bengal Academy of Science and Technology, 2009-2011.

Elected as Council member, Indian Science Congress Association, 2009-2010.

Elected as General Secretary (Membership Affairs), Indian Science Congress Association, 2010-2013.

Elected as Vice-President The Physiological Society of India, 2010-2014.

Awarded the Platinum Jubilee oration of 2009 of the Physiological Society of India, International Conference on Integrative Physiology: Modern perspective and Platinum Jubilee celebration of the Physiological Society of India held at Science City Convention Centre, Kolkata, November 12-14, 2009.

Member of the Editorial Board of Indian Journal of Physiology and Allied Sciences, Asian Journal of Experimental Sciences and Al Ameen Journal of Medical Sciences.

PRESENTATIONS AND VISITS

M. K. Chakraborti

M. K. Chakrabarti. "Current trends of diarrheal disease research". Invited lecture delivered at the Orientation Programme for College and University Teachers, UGC Academic Staff College, University of Calcutta on July 10, 2009.

M. K. Chakrabarti. "Vaccines against enteric diseases". Invited lecture delivered at AICTE Sponsored QIP Refresher course for College and University Teachers, Academic Staff College, Jadavpur University, Calcutta on August 3, 2009.

D. Pore, N. Mahtata and M. K. Chakrabarti. An outer membrane protein of *Shigella flexneri* 2a: Potential Candidate vaccine against shigellosis at International conference on Fifty Years of Cholera toxin: A tribute to Dr. S.N. De on October 25-27, 2009 in Kolkata.

N. Mahtata, D. Pore and M. K. Chakrabarti Translocation of protein kinase C-α in human colonic carcinoma cell line requires an organized actin cytoskeleton at International conference on Fifty Years of Cholera toxin: A tribute to Dr. S.N. De on October 25-27, 2009 in Kolkata.

M. K. Chakrabarti. "Water quality and disease burden" Key note address delivered at the World Plumbing Day organized by Plumbing Association of India at Kolkata on March 11, 2010.

M. K. Chakrabarti. "Vaccines Against Diarrhoeal Diseases" Invited lecture delivered at UGC Refresher course for College and University Teachers, Academic Staff College, Department of Bioinformatics, Vidyasagar University, Midnapore on March 15, 2010.

M. K. Chakrabarti. "Present status of vaccine against shigellosis" Key note address delivered at the conference on Challenges in Research on Modern Biology in 2020 at V.P. College of Science, Bangalore University on March 29, 2010.

Amit Pal

Participated in the National Conference of Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) held on January 15, 2010 at India Habitat Centre, Lodhi Road, New Delhi.

Attended the International Conference on "Emerging Infectious Diseases of the Pacific Rim: Focus on Enteric Diseases" held from April 6-9, 2009 at Kolkata, India.

PhD DEGREE AWARDED

Pinki Chowdhury was awarded the degree by Jadavpur University for her thesis "Molecular cloning of calcium sensing receptor (CaSR) from a human colon carcinoma cell line (COLO-205) and its involvement in the mechanism of action of different bacterial virulence factors" under the supervision of Dr. M. K. Chakrabarti.

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 rotaviruses, noroviruses, sapoviruses, astroviruses, adeno viruses and picobirna viruses during the diarrhoeal outbreaks in the state or country. In addition, epidemiological and molecular characterization of HIV strains among 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 its 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
Research Scientist/Pool Officer	:	Rakhi Dey
Young Woman Scientist	:	Mehuli Sarkar
Senior Research Fellows	:	Anurodh S. Agrawal Dipanjan Dutta Ranajay Mullick Roni Sarkar SM Nataraju Madhu Sudan Pativada Anupam Mukherjee Parikshit Bagchi Shiladitya Chattopadhyay
Junior Research Fellows	:	Nilanjana Biswas Umesh Chandra Halder Rahul Kumar

1. Genotyping of HIV-1 strains using Multiregion Hybridization Assay (MHA) among Injecting Drug Users in Manipur.

Investigator : Sekhar Chakrabarti

Manipur, one of the states in the northeastern region of India bordering Myanmar, is very close to the 'Golden Triangle' (composed of northern Thailand, eastern Myanmar and western Laos). This region is the world's leading opium- and heroin-producing area. Reported cases of HIV-infected individuals are quite high in Manipur (11% HIV positivity among women in antenatal clinics and 15% among patients in sexually transmitted diseases clinics) due to international drug trafficking and sharing of contaminated needles and syringes. As reported earlier, the number of injecting drug users (IDUs) estimated all over the world is 13.2 million (0.3% of the estimated 4 billion adult population), the majority of which (10.3 million) live in developing countries. The presence of dual-subtype infection, intersubtype recombination between subtype C and Thai B has been reported in Manipur using a heteroduplex mobility assay (HMA) based on *gag* (p24-p7) and *env* (C2-V3) regions. Blood samples were collected from IDUs in Manipur after obtaining their informed consent. HIV-1 testing was done by ELISA (Immunogenetics, Belgium) followed by the TRIDOT assay according to the policy of the country's National AIDS Control Program.

Previously genotyping studies were carried out through HMA. As HMA is restricted to two genomic regions only, the real scenario of genetic diversity of HIV-1 cannot be ascertained. A multiregion hybridization assay (MHA) has been developed based on real-time PCR using subtypespecific TaqMan probes within different regions of the HIV-1 genome. This technology is a powerful tool in tracking new circulating recombinant HIV-1 strains.

In MHAbce v.2, multiple short fragments were amplified throughout the HIV-1 genome (Fig. 1) to assess the hybridization of clade-specific fluorescent probes in realtime PCR. The viral RNA was extracted from plasma using a viral RNA mini kit. The primers and probes (Applied Biosystems, USA) were used with respect to subtype B, C and AE . The genomic regions within *p17* , protease *(pro)*, reverse transcriptase *(rt)*, integrase *(int)*, first exon of tat *(tat)*, C3 in gp120 (gp120), and *gp41/nef* were amplified in separate first-round PCR using cDNA synthesized by reverse transcription-PCR from viral RNA. The second round real-time PCR was performed with TaqMan 2 x Universal PCR Master Mix including an aliquot of each amplicon from first-round PCR products distributed in separate sets of second-round PCRs with subtype-specific probes for (p17, pro, rt, int, tat, gp120, gp41 and nef) gene fragments. During the amplification, emission of fluorescence started due to the breakage of linkage between

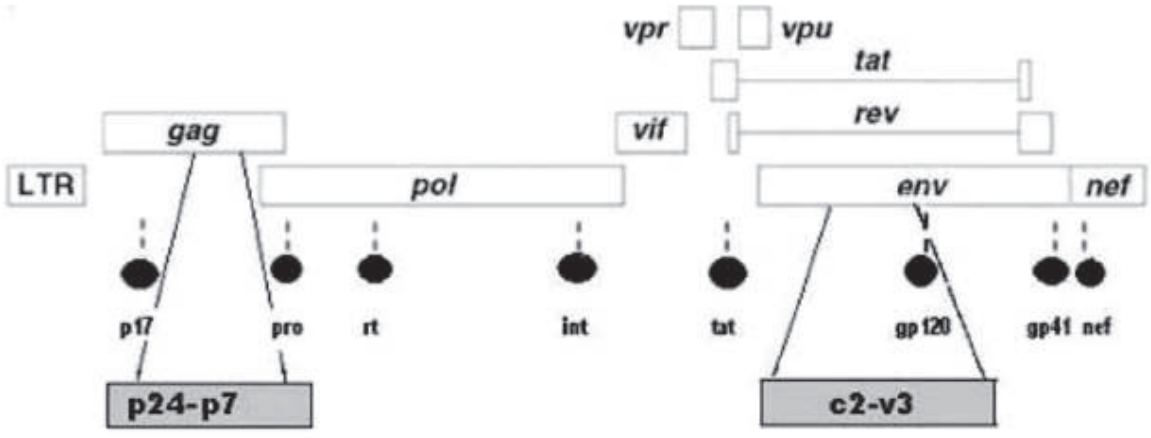


Fig. 1

reporter and quencher. The fluorescence was measured and analyzed using SDS v2.1 software (Applied Biosystems). The second-round real-time PCR amplification was performed in a 96-well ABI PRISM 7900HT sequence detection system. The results of MHA showed the presence of multigenomic variants and dual probe reactivity (Fig. 2). Our data provides evidence for the possibility of new emerging strains of HIV-1 along with dual infection among the IDU population from Manipur. This is the first time implementation of MHAbce (version 2; v.2) among the IDU population from Manipur (India) to characterize HIV-1 strains with improved scope and clarity is being reported. Thus a continuous monitoring of HIV-1 strains in this part of the country as well as characterization of emerging new recombinant forms is warranted, which might help in designing appropriate candidate HIV-1 vaccine.

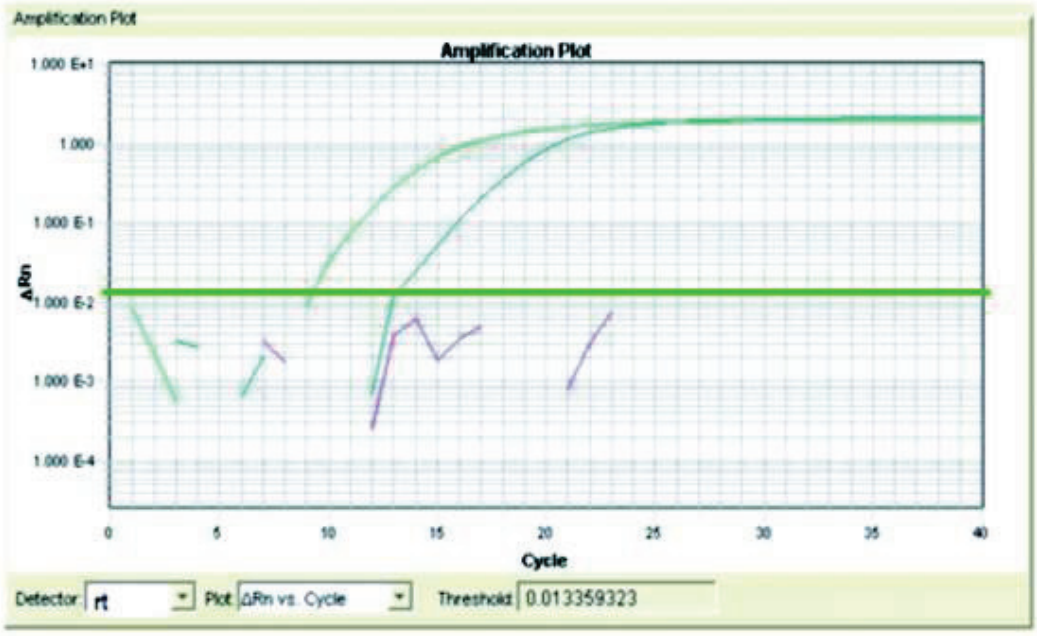


Fig. 2

2. Detection and molecular characterization of rotaviruses

Triveni Krishnan

Two human Group B Rotavirus strains were detected from diarrhoeic cases (a child and an adult) and characterized by reverse transcription, amplification by polymerase chain reaction and sequencing of the eleven segments of genomic double stranded RNA. The various genes of both Kolkata strains were closely related [more than 95%] to human Group B rotavirus strains from Myanmar and Bangladesh. Although human Group B rotavirus remains largely associated with adult diarrhoea, it is apparent from the present analysis that human Group B rotavirus which had infected the child in Kolkata was closely related to the strain infecting adults.

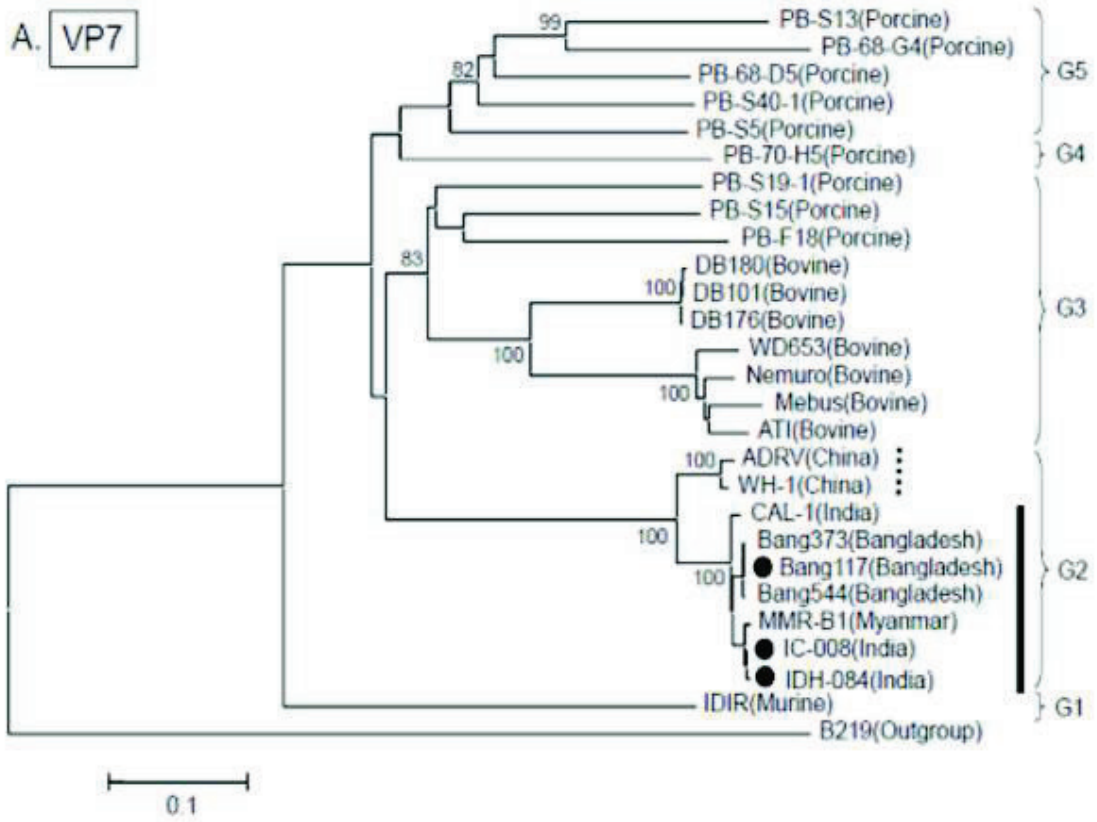
The VP1 gene of diarrhoea case RU172 exhibited higher genetic relatedness to Wa-like human G12 Group A Rotaviruses than porcine strains. VP2, VP3 and NSP2 genes clustered separately from the Wa-like human (including G12) and porcine clusters, while the VP6, NSP1 and NSP3, NSP4 and NSP5 genes clustered with porcine and porcine-like human strains. These observations suggest that the porcine G12 strain might have originated from porcine-human reassortment events, or alternatively the Wa-like human and porcine G12 strains might have originated from a common ancestor and then gradually evolved through genetic drift and shift in course of time.

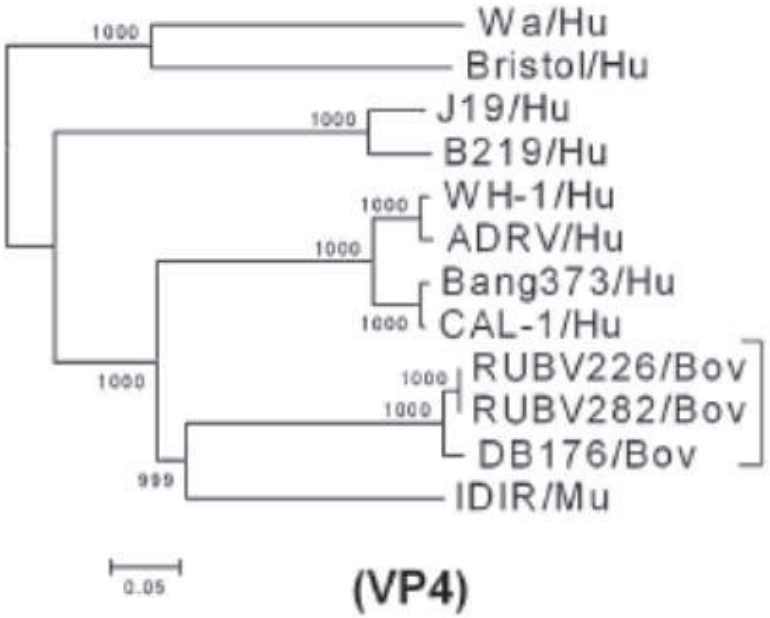
The complete nucleotide sequence of VP1, VP2, VP4, VP6, NSP1 and NSP2 encoding genes of three bovine Group B rotaviruses strains detected from Kolkata exhibited high genetic diversity with cognate genes of human, murine and ovine Group B Rotaviruses. Interestingly, as with Group A rotaviruses, the bovine VP1, VP2, VP6 and NSP2 genes appeared to be more conserved than the VP4 and NSP1 genes among strains of different species. These findings have provided important insights into the genetic makeup and diversity of bovine Group B Rotaviruses and also identified a novel VP4 genotype.

3. Detection and molecular characterization of human caliciviruses and astroviruses

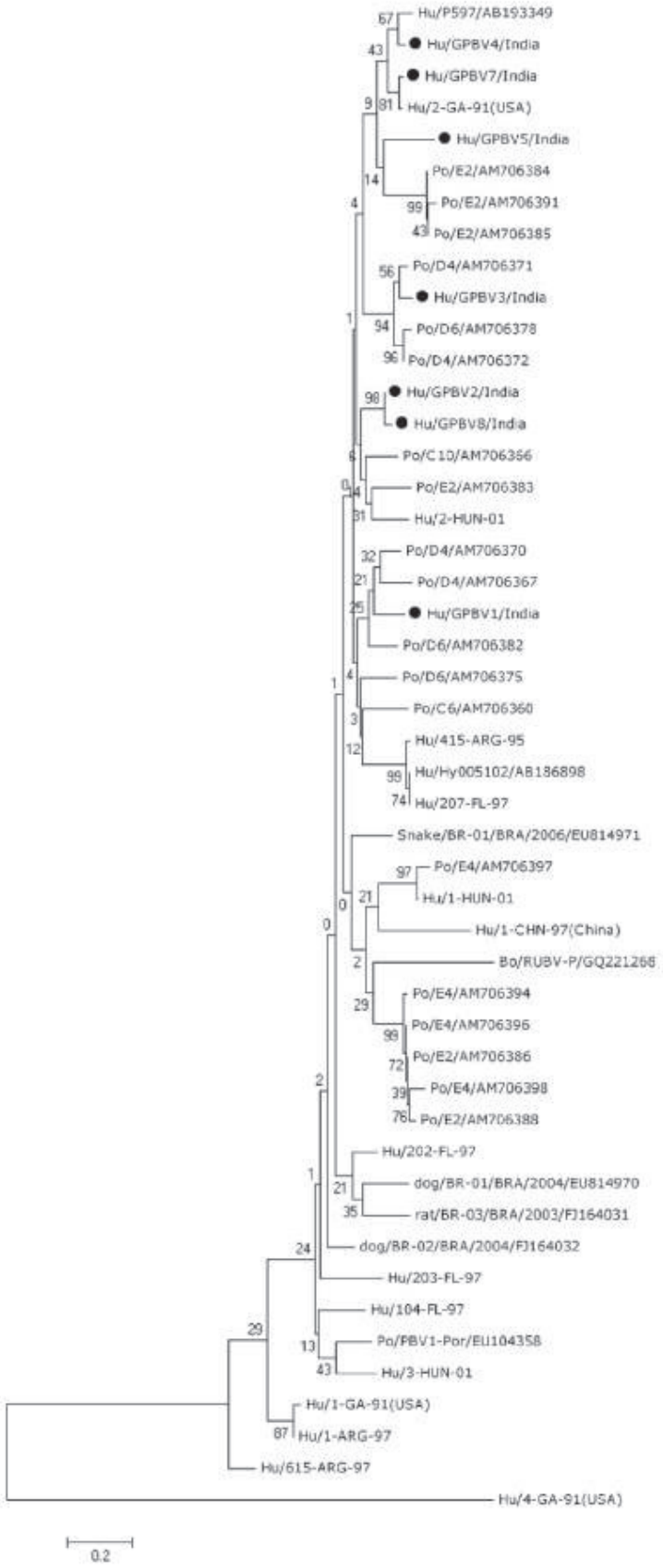
Triveni Krishnan

HuCVs were detected by reverse transcription-polymerase chain reaction (RT-PCR) of the partial RNA dependent RNA polymerase gene (RdRp) and capsid gene and confirmed by sequencing. The sequences were analyzed and the recombination point was detected. 22 cases were HuCV positive with 21 NoVs; 12 NoV cases (54.5%) were GII.4; 6 cases showed 99% identity with the new variant Japanese strain Hu/NoV/GII.4/OC07138/JP; 3 novel NoVGII inter-genotype recombinant strains V1628/IND, V1656/IND and V1737/IND were also detected. The RdRp region of V1628 showed 96% identity with Pont de Roide 673/FRN whereas capsid region resembled GII.7/Osaka F140/JPN strain (98%); the strain V1656 showed 98% identity with RdRp region of GII.4/Monastir 375/TUN but capsid region resembled GII.8/Leverkusen 267/DE (91%); the strain V1737 showed 88% identity with RdRp of GII.5/Minato 6/N1/6/JPN whereas capsid region resembled the GII.12/Gifu 96/JPN (93%). During characterization of Caliciviruses two strains of NoV GII.b and one strain of each NoV GI.1/V1622/06/IND, GI.3/V1707/07/IND,GII.3/V1668/IND, GII.16/V1729/IND, Sapovirus GII.1/V1716/IND were also detected. The emergence of new variant of GII.4/2007, three novel NoV GII inter-genotype recombinant strains and various other NoVs, indicated the remarkable genetic diversity of the HuCVs as diarrhoeagenic viruses circulating in Kolkata, India.


A.
VP7
PB-S13(Porcine)
PB-68-G4(Porcine)
PB-68-D5(Porcine)
PB-S40-1(Porcine)
PB-S5(Porcine)
PB-70-H5(Porcine)
PB-S19-1(Porcine)
PB-S15(Porcine)
PB-F18(Porcine)
DB180(Bovine)
DB101(Bovine)
DB176(Bovine)
WD653(Bovine)
Nemuro(Bovine)
Mebus(Bovine)
ATI(Bovine)
ADRV(China)
WH-1(China)
CAL-1(India)
Bang373(Bangladesh)
Bang117(Bangladesh)
Bang544(Bangladesh)
MMR-B1(Myanmar)
IC-008(India)
IDH-084(India)
IDIR(Murine)
B219(Outgroup)
G5
G4
G3
G2
G1
99
82
83
100
100
100
100
100
100
0.1


Wa/Hu
Bristol/Hu
J19/Hu
B219/Hu
WH-1/Hu
ADRV/Hu
Bang373/Hu
CAL-1/Hu
RUBV226/Bov
RUBV282/Bov
DB176/Bov
IDIR/Mu
1000
1000
1000
1000
1000
1000
1000
1000
999
0.05

(VP4)


Hu/P597/AB193349
Hu/GPBV4/India
Hu/GPBV7/India
Hu/2-GA-91(USA)
Hu/GPBV5/India
Po/E2/AM706384
Po/E2/AM706391
Po/E2/AM706385
Po/D4/AM706371
Hu/GPBV3/India
Po/D6/AM706378
Po/D4/AM706372
Hu/GPBV2/India
Hu/GPBV8/India
Po/C10/AM706356
Po/E2/AM706383
Hu/2-HUN-01
Po/D4/AM706370
Po/D4/AM706367
Hu/GPBV1/India
Po/D6/AM706382
Po/D6/AM706375
Po/C6/AM706360
Hu/415-ARG-95
Hu/Hy005102/AB186898
Hu/207-FL-97
Snake/BR-01/BRA/2006/EU814971
Po/E4/AM706397
Hu/1-HUN-01
Hu/1-CHN-97(China)
Bo/RUBV-P/GQ221268
Po/E4/AM706394
Po/E4/AM706396
Po/E2/AM706386
Po/E4/AM706398
Po/E2/AM706388
Hu/202-FL-97
dog/BR-01/BRA/2004/EU814970
rat/BR-03/BRA/2003/FJ164031
dog/BR-02/BRA/2004/FJ164032
Hu/203-FL-97
Hu/104-FL-97
Po/PBV1-Por/EU104358
Hu/3-HUN-01
Hu/1-GA-91(USA)
Hu/1-ARG-97
Hu/615-ARG-97
Hu/4-GA-91(USA)
0.2

3. Multisite monitoring of Influenza Virus strains in India, Phase I

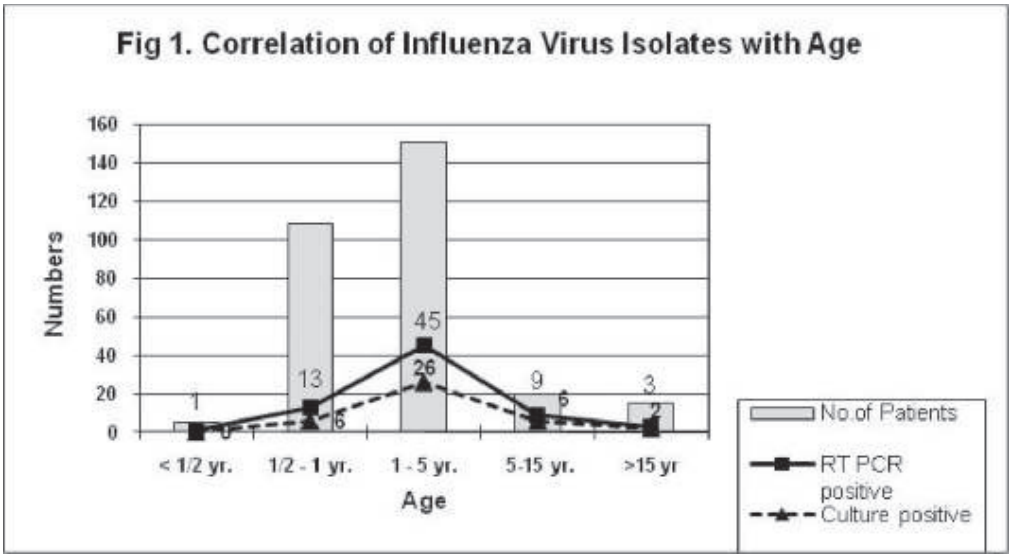
Mamta Chawla-Sarkar

In course of active influenza surveillance, total of 503 patients were enrolled during the study period. Out of 503 cases, 75 (14%) were positive for Influenza A or B type. In comparison only 45 samples were culture positive (8%). Predominant strains were H3N2 as well as H1N1. Maximum number of isolates were obtained during May-June correlating with rains (Fig. 1). In addition to seasonal flu, outbreaks due to pandemic H1N1 were also addressed. A total of 2106 samples were screened until 31st Dec 2009. Of which 155 were positive for novel H1N1 and 98 were positive for seasonal flu (H3N2/H1N1). Compared to high frequency of seasonal influenza in children, pH1N1 infection was predominant in 18-36 yrs age group (Fig2).

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

Mamta Chawla-Sarkar

Analysis of Rotavirus-Host interactions: 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 activate PI3K/Akt and NFκB pathways compared to the isogenic NSP1 wild type strain A5-13 and resulted in early induction of apoptosis (Fig. 3). In virus infected cells NSP1 coimmunoprecipitated with p85-subunit of PI3K (phosphoinositide 3-kinase) confirming direct interaction between cellular and virus protein (Fig. 4). Furthermore PI3K/Akt inhibitors attenuated rotavirus growth significantly confirming importance of cellular proteins during rotavirus infection.



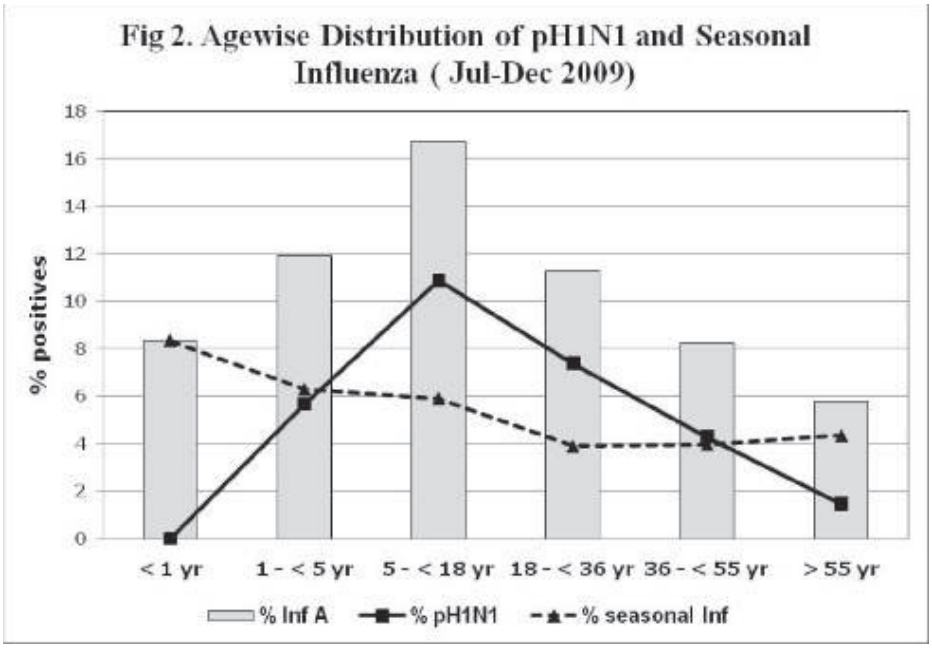


Fig 2. Agewise Distribution of pH1N1 and Seasonal Influenza (Jul-Dec 2009)
% positives
18
16
14
12
10
8
6
4
2
0
< 1 yr
1 - < 5 yr
5 - < 18 yr
18 - < 36 yr
36 - < 55 yr
> 55 yr
% Inf A
% pH1N1
% seasonal Inf

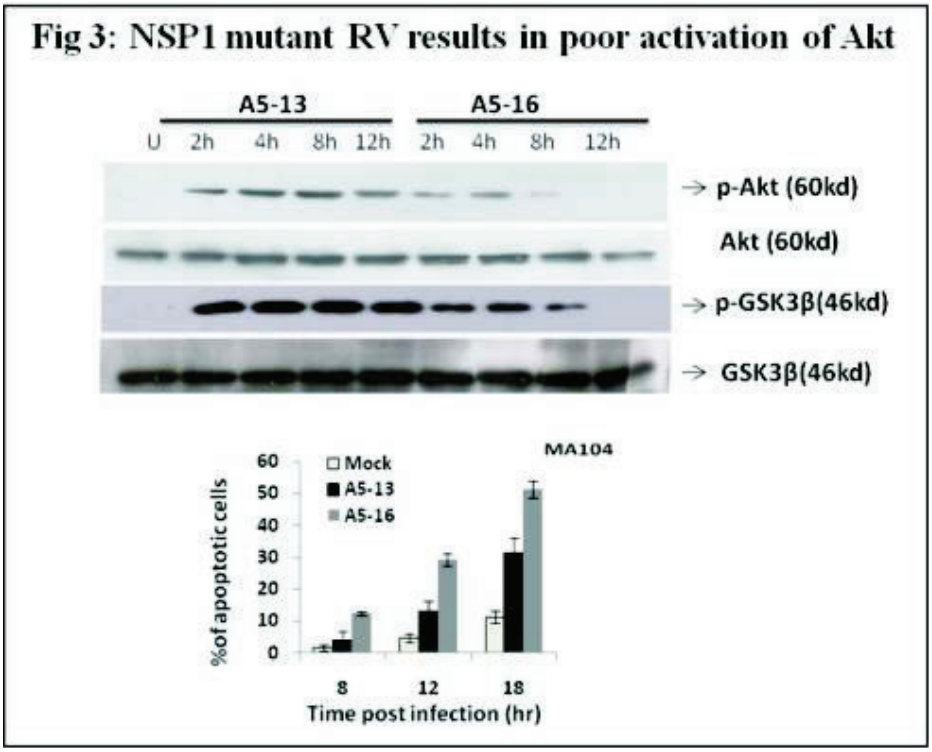
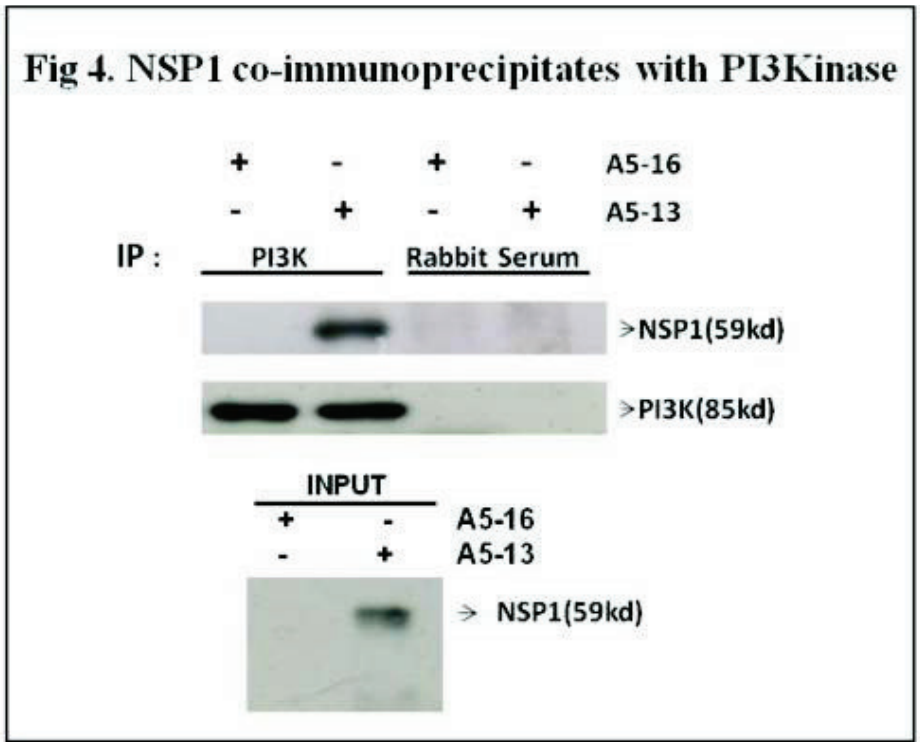


Fig 3: NSP1 mutant RV results in poor activation of Akt
A5-13
A5-16
U
2h
4h
8h
12h
2h
4h
8h
12h
→ p-Akt (60kd)
Akt (60kd)
→ p-GSK3β(46kd)
→ GSK3β(46kd)
MA104
Mock
A5-13
A5-16
%of apoptotic cells
60
50
40
30
20
10
0
8
12
18
Time post infection (hr)



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

B.Ganesh

The genus, Picobirnavirus (PBV), Spanish '*pico*' = 'small', 'birna' for '*bipartite RNA*' genome, belongs to the family *Picobirnaviridae* under the proposed order *Diplornavirales*. PBV infections have been reported from different animal species and humans with or without diarrhoea.

23 Picobirnavirus positive faecal specimens were detected by Polyacrylamide gel electrophoresis (PAGE) and silver staining from a set of 1112 faecal specimens collected from an urban slum community in Kolkata. The picobirnaviruses showed either large profile (n=22) or small profile (n=1). 13/23 positives were amplified by RT-PCR as 201bp amplicon with genogroup I primers [PicoB25(+) and PicoB43(-) specific for RNA dependent RNA polymerase (RdRp) gene fragment encoded by genomic segment 2] and seven amplicons were sequenced [GPBV1-5, 7-8].

Sequence analyses showed that four PBV strains [GPBV-1-3,8] resembled porcine PBV strains (D4, D6, C10) reported in 2008 from Hungary and two PBV strains [GPBV-4,7] resembled human PBV strains (P597 Kolkata and 2-GA-91 USA) with the maximum nucleotide (nt) identity ranging from 78% to 92%. One strain GPBV5 clustered with human PBVs and porcine PBVs that were reported from Hungary, Venezuela and Argentina showing close homology to human-like PBVs. The detection of porcine-like Picobirnaviruses in diarrhoeic children aged <5 years, is suggestive of zoonotic transmission. Therefore, the close monitoring of their global spread as well as in-depth molecular characterization is essential for better understanding of emerging PBV strains. (Figure)

AWARDS AND HONOURS

S. Chakrabarti

Guest faculty of West Bengal University of Health Sciences.

Course taken: Recombinant DNA technology for M. Sc (Biotechnology)

Member, Board of Studies; M. Sc (Biotechnology); West Bengal University of Health Sciences.

Member, Ph D Committee; West Bengal University of Health Sciences.

Guest Faculty of Vidyasagar University; Department of Physiology and Community health.

Member, Project Review Committee on HIV/AIDS, ICMR, New Delhi

Member, Expert Committee on H1N1; Department of Health & Family Welfare, Govt. of West Bengal.

Chairman, Technical & Purchase Committee, National Institute of Biomedical Genomics.

Member, National HIV Drug Resistance Committee, National AIDS Control Organization, Govt. of India.

T. Krishnan

Assigned as associate editor through the peer review process for three research papers submitted to the journal BMC Infectious Diseases in October 2009, and twice in January 2010.

Assigned as reviewer of manuscript prior to publication for several journals viz. Archives of Virology [17, May 2009]; Journal of General Virology [1, June 2009]; International Journal of STD and AIDS [30, June 2009]; Archives of Virology [30, June 2009]; Virus Research [29, July 2009]; Archives of Virology [30, August 2009]; American Journal of Tropical Medicine and Hygiene [3, October 2009]; Infection Genetics and Evolution [3, February 2010]; Current Bioactive Compounds [12, February 2010]; Archives of Virology [27 February 2010]

Served as external examiner for M.Sc Botany (Part I) Grand Viva Paper XI Theory Exam, 2009 in Bethune College, University of Kolkata.

Served as paper setter/ external examiner for M. Sc (second semester) Theory Paper VII (Group B) Virology Exam, 2009 of Vidyasagar University, Midnapore, West Bengal.

Served as paper setter/ Third examiner for M. Sc (Part I) Botany Theory Paper IB Microbiology Exam, 2009 of Bethune College, University of Kolkata.

Assigned to examine PhD thesis submitted to All India Institute of Medical Sciences, New Delhi on 10, March 2010.

Invited to contribute a chapter entitled 'Picobirnavirus' in the book entitled Molecular detection of Human Viral Pathogens edited by Dr Don Liu and published by Taylor and Francis Group

Invited to participate and present the research paper in the International Joint Forum on Infectious Diseases 2009 held at Siam City Hotel, Bangkok, Thailand from 16-17, September 2009.

PRESENTATIONS AND VISITS

S. Chakrabarti

Attended Bi-regional workshop on prevention and surveillance of HIV drug resustance Bangkok, Thailand, May 20-22, 2009 as nominated by Govt. of India.

Resource person in the Academic Staff College, Calcutta University.

Lecture delivered: HIV/AIDS: Indian Scenario, July 14, 2009.

Participated at the joint inter country workshop on Nipah, Hendra and Ebola-Reston viruses, Research and Public Health, October 13-16, 2009 Maroochydore, Brisbane, Australia.

Lecture delivered: NIPAH virus outbreak in West Bengal, India.

Resource person at the workshop on H1N1 infection at Swasthya Bhavan, Govt of West Bengal; March 3-5, 2010.

Lecture delivered: Laboratory diagnosis for Novel H1N1 infection.

Delivered A K Chandra memorial lecture at Department of Botany, University of Calcutta. March 25, 2010.

Lecture delivered: Molecular Characterization of HIV-1 strains circulating in Eastern and North eastern parts of India.

T. Krishnan

Mukti Kant Nayak, Nataraju SM, Debarati Chatterjee, Balasubramanian Ganesh, Ganesh C. Sahoo, Rittwika Bhattacharya, Jan Vinje, Nobumichi Kobayashi, Phalguni Dutta, Utpala Mitra, and Triveni Krishnan. Detection of new variant of Norovirus GII.4 and intergenotype recombinant strains of NVGII among children in Kolkata, India. Paper presented (Poster) & Abstract Published in the US-Japan Co-operative Medical Science Programme (CMSP): 13th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim – Focus on Enteric Diseases during April 6-9, 2009 at Kolkata.

Nataraju SM, Mukti Kant Nayak, B Ganesh, Debarati Chatterjee, Rashmi Arora, Jan Vinje and Triveni Krishnan. Noroviruses causing acute watery diarrhoea among children in Kolkata. Paper presented (Poster) & Abstract Published in the US-Japan Co-operative Medical Science Programme (CMSP): 13th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim – Focus on Enteric Diseases during April 6-9, 2009 at Kolkata.

Shovan Das, Chowdhury S, Nataraju SM, B Ganesh, Ghosh M, Bhattacharya MK, Rashmi Arora and Triveni Krishnan. Age group is a key factor for effective vaccination against rotavirus diarrhoea among children in Kolkata, India. Paper presented (Poster) & Abstract Published in the US-Japan Co-operative Medical Science Programme (CMSP): 13th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim – Focus on Enteric Diseases during April 6-9, 2009 at Kolkata.

Madhusudhan Pativada, Debarati Chatterjee, Mukti Kant Nayak, B.Ganesh, Nataraju S.M, Nobumichi Kobayashi and Triveni Krishnan. Higher prevalence of rotavirus G and P untypables – probable indication of evolution in genotypes. Paper presented (Poster) & Abstract Published in the US-Japan Co-operative Medical Science Programme (CMSP): 13th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim – Focus on Enteric Diseases during April 6-9, 2009 at Kolkata.

Triveni Krishnan, Rittwika Bhattacharya, Ganesh C Sahoo, Mukti Kant Nayak, Nataraju SM, Debarati Chatterjee, Balasubramanian Ganesh and S.K. Bhattacharya. Rare genetic diversity of astrovirus strain from Kolkata, India. Paper presented (Poster) & Abstract Published in the US-Japan Co-operative Medical Science Programme (CMSP): 13th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim – Focus on Enteric Diseases during April 6-9, 2009 at Kolkata.

Triveni Krishnan. Delivered a talk entitled Novel strains of gastroenteritis viruses among children in Kolkata on June 12, 2009 under Society of Biological Chemists [India] Kolkata Chapter, at Bose Institute, Centenary Campus, Kolkata.

Triveni Krishnan, Madhusudhan Pativada, Nataraju Seegekote Mariyappa, Mukti Kant Nayak, Balasubramanian Ganesh, Krishnan Rajendran, Debarati Chatterjee, Nobumichi Kobayashi, Masahi Ishino and Shigeo Nagashima. Recombinant Norovirus strains and other etiological agents of viral diarrhoea in Kolkata, India. Presented at International Joint Forum on Infectious Diseases 2009 held at Siam City Hotel, Bangkok, Thailand from September 16-17, 2009.

Participated in the International Symposium of "Fifty years of Discovery of Cholera Toxin: A tribute to S.N. De" organized by NICED, IICB and Bose Institute during October 25-27, 2009 at Kolkata.

MadhuSudhan Pativada, Nataraju S.M, B.Ganesh, Triveni Krishnan and Nobumichi Kobayashi. Higher prevalence of Rotavirus G and P untypables in Kolkata and usage of multiplex PCR method for genotyping-probable indication of evolution in genotypes. Paper presented (Poster) & Abstract Published in the ICGEB-IUBMB Workshop: Human RNA viruses held at New Delhi during February 10-12, 2010. (Received Travel Grant to participate in the workshop).

Mamta Chawla-Sarkar

P. Bagchi, D. Dutta, S. Chattopadhyay, A. Mukherjee, N. Kobayashi and M. Chawla-Sarkar. Rotavirus Nonstructural Protein-1 Facilitates Virus Growth by Delaying Premature Apoptosis in Infected Cells. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, India, April 6-9, 2009.

Dutta, P. Bagchi, S. Chattopadhyay, A. Mukherjee, N. Kobayashi and M. Chawla-Sarkar. Host Cellular Proteins: Key Determinants of rotavirus infection and pathogenesis. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, India, April 6-9, 2009.

Mukherjee, S. Chattopadhyay, D. Dutta, S. Ghosh, T.N. Naik, N. Kobayashi and M. Chawla-Sarkar. Genomic diversity of human group A rotaviruses: Increasing evidence of complex zoonotic transmission events. A. Mukherjee, S. Chattopadhyay. 13th International Conference on Emerging Infectious Diseases in the Pacific Rim, India, April 6-9, 2009.

P. Bagchi, D. Dutta, N. Kobayashi and M. Chawla-Sarkar. Evasion of cellular responses: multifunctional role of rotavirus nonstructural protein-1. International Joint Forum on Infectious Diseases, Bangkok, Sep 16-17, 2009. (Oral Presentation).

S. Agrawal, M. Sarkar and M. Chawla-Sarkar. Comparative evaluation of multiplex real-time PCR and conventional RT-PCR during a two year surveillance study for influenza and respiratory syncytial viruses among children in Kolkata, India. 12th Annual ESCV meeting Istanbul, Turkey, Sept. 27-30, 2009.

Dutta, P. Bagchi, N. Kobayashi, K. Taniguchi, M. Chawla-Sarkar. Molecular chaperone Hsp90 positively regulates rotavirus infection. Domestic Congress of Virology, Japan, Oct 25-27th 2009. (Oral Presentation)

P. Bagchi, D. Dutta, N. Kobayashi, K. Taniguchi, M. Chawla-Sarkar. Rotavirus NSP-1 suppresses virus-induced cellular apoptosis to facilitate viral growth during early stages of infection. Domestic Congress of Virology, Japan, Oct 25-27th 2009. (Oral Presentation)

P. Bagchi, D. Dutta, S. Chattopadhyay and M. Chawla-Sarkar. Evasion of host immune responses: modulation of cellular survival and apoptotic pathways by RNA viruses. XXXIII All India Cell Biology Conference *and* International Workshop on Cell Cycle Regulation. *Universityof* Hyderabad, Hyderabad – December 10-13 2009.

Surveillance and genomic characterization of novel influenza virus (swine H1N1) strains circulating in eastern India.. M. Sarkar, R. Dey, A. S. Agrawal, A. Mukherjee, S. Chakrabarti and M. Chawla-Sarkar. 97th Annual Meeting on Indian Science Congress, Thiruvananthapuram, India from Jaunary 3-7, 2010. (Oral Presentation)

Surveillance of rotavirus strains circulating in Manipur, north-eastern India: Increasing prevalence of zoonotic transmission. Anupam Mukherjee, Ng. Brajachand Singh and M. Chawla-Sarkar. 97th Annual Meeting of Indian Science Congress, Thiruvananthapuram, India from 3-7 January 3-7, 2010.

S. Agrawal, M. Sarkar, S. Ghosh, S Chakrabarty, M. Chawla-Sarkar. Surveillance and genetic characterization of circulating influenza-A virus strains in Kolkata 2005-2008. ICGEB-IUBMB Workshop on Human RNA Viruses, New Delhi, India, February 10-12, 2010. (Received Travel Grant to participate in the workshop).

S. Agrawal, S Chakrabarty & M. Chawla-Sarkar. A two year surveillance for influenza and RSV using Real-time PCR among children with acute respiratory infections in Kolkata reveals distinct seasonality of infection. 14th International Congress on Infectious Diseases, Miami, Florida, USA, March 9-12, 2010

Mukherjee and M. Chawla-Sarkar. Surveillance of rotavirus strains reveals evidence of emerging G12 and unusual human-animal reassortant strains in Manipur, North-eastern India. 14th International Congress on Infectious Diseases, Miami, Florida, USA, March 9-12, 2010.

B Ganesh

Balasubramanian Ganesh, Rittwika Bhattacharya, M.K.Nayak, Debarati Chatterjee, S.M.Nataraju, M.C.Sarkar, Shigeo Nagashima, Nobumichi Kobayashi, and Triveni Krishnan. Occurrence of Porcine Picobirnavirus in the diarrhoeagenic population in Kolkata: Evidence of Zoonoses?. Paper presented (Poster) & Abstract Published in the US-Japan Co-operative Medical Science Programme (CMSP): 13th International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim – Focus on Enteric Diseases during April 6-9, 2009 at Kolkata.

Attended a workshop on biological knowledge, discovery and bioinformatics (BKDBio) - A perspective from Disease Database on June 29-30, 2009 organized by Biomedical Informatics Center of ICMR, NICED, Kolkata.

Attended Career Development Workshop for Young Scientists of India at the Indian Institute of Chemical Biology (IICB) (CSIR) Kolkata on September 8, 2009.

Participated in the International Symposium of Fifty years of Discovery of Cholera Toxin: A tribute to S.N. De organized by NICED, IICB and Bose Institute during October 25-27, 2009 at Kolkata.

Balasubramanian Ganesh, Shovan Das, Seegikote Mariyappa Nataraju, Sourav Chowdhury, Mihir Bhattacharya, Mrinmoy Ghosh, Rashmi Arora, Umesh D Parashar, Jan Vinje, Nobumichi Kobayashi, Triveni Krishnan. Rotavirus infection: Clinical case studies in children below 5 years of age at Kolkata, Eastern India during January 2008 to June 2009. Paper presented (Poster) & Abstract Published in the ICGEB-IUBMB Workshop: Human RNA viruses held at New Delhi during February 10-12, 2010 (Received Travel Grant to participate in the workshop).

PhD DEGREE AWARDED

Mukti Kant Nayak was awarded the degree by Jadavpur University for his thesis “Molecular analysis of human caliciviruses” under the supervision of Dr. T. Krishnan.

SERVICES

SERVICES

A. Bacteriology Division

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.

B. Bioinformatics Center

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 which was attended by young scientists, PhD and postdoctoral students as well as medical students from within and outside the institute.

C. Public Health Laboratory Division

Scientist	:	Malay Kumar Saha, Scientist D
Staff	:	Chittaranjan Pal, Technical Officer
		Somesh Chandra Bhunia, Technical Officer
		Salil Kumar Sadhukhan, Senior Technical Assistant
		Pramita Bhaumik, Technical Assistant
		Chaitali Das, Sweeper

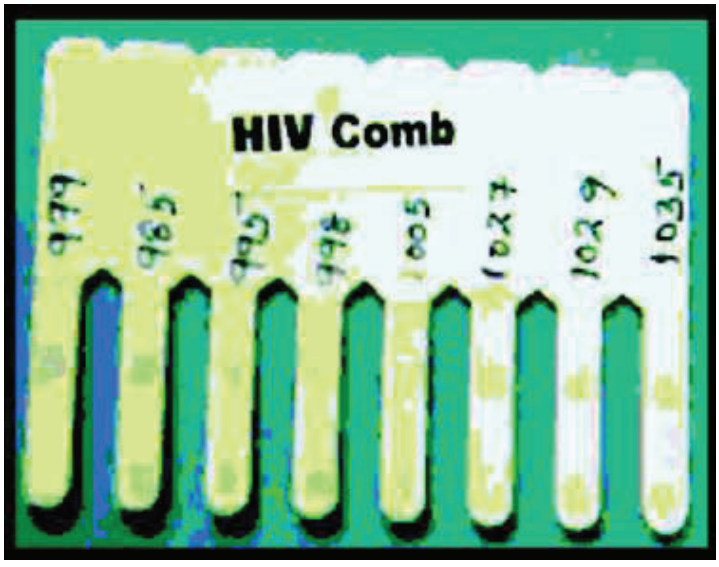
Services of Public Health Laboratory in NICED.

(A) Surveillance for HIV as one of the National Reference Laboratories

National AIDS Control Organization (NACO) Ministry of Health and Family Welfare, Government of India funds the HIV National Reference Laboratory (NACO-NRL) at National Institute of Cholera & Enteric Diseases since 1992. According to the NACO programme 5000+ ICTCs are supervised by 117 State Reference Laboratories which are under 13 National Reference Laboratories. An Apex Laboratory conducts EQAS for all the NRLs. The activities of NACO-NRL in NICED are as follows:

1. EQAS and Panel Sera preparation for SRL of different states of Eastern and North-Eastern India.
2. Confirmation of HIV testing results of the samples received from different SRLs.
3. Sentinel Surveillance for HIV infection.
4. Training for Doctors, Laboratory/Program Supervisors and Medical Laboratory Technologists 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.
5. HIV, HCV, HBV and RPR kit evaluation for UNOPS (United Nations Office for Project Service), NationalAIDS Control Organization, West Bengal State AIDS Prevention and Control Society and other National and State agencies.

Amongst the responsibilities allotted to the NACO-National Reference Laboratory at NICED, Referral Services is of utmost importance. NACO-NRL, NICED has been entrusted with the responsibility of verifying results for all discordant samples sent by State Reference Laboratories, several hospitals and sometimes handles requests for foreign nationals. 125 samples were screened for HIV Antibody by ELISA, Rapid and/or Confirmatory Test during 2009 as depicted in the following Table.



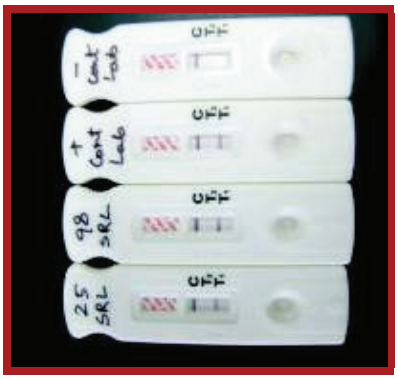
HIV Comb test performed as part of Referral Services

Table: Referral Service done for Eastern and North-Eastern states and institutions at NACO NRL, NICED, Kolkata

Source of Samples		No. Tested	No. of Positives
A.	**WEST BENGAL**		
	1. Command Hospital	59	58
	2. Patients from Hospitals	53	47
	3. Miscellaneous	04	02
	Sub Total	**116**	**107**
B.	**OTHER STATES**		
	1. Assam	02	Nil
	2. Meghalaya	02	02
	3. Orissa	01	Nil
	4. Sikkim	04	01
	Sub Total	**09**	**03**
	GRAND TOTAL	**125**	**110**

EQAS PROGRAMME OF NACO

External Quality Assessment is a programme in which a laboratory participates and receives a blinded, composite panel of samples from another laboratory conducting the EQA. An EQA compares the performance and results among different test sites, and indicates areas that require improvement in participating laboratories by identifying the loopholes in the process. The general objective is to provide early warning for systematic problems associated with kits or operations. Apart from all these gains, an EQA serves as evidence to quality testing.



Rapid Test for HIV Testing

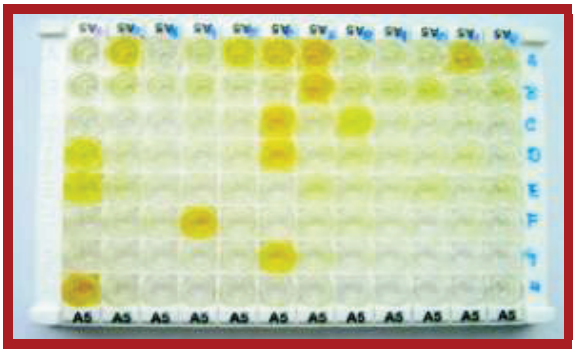
Proficiency Testing Panel

Quality Assurance at NACO-NRL, NICED has several dimensions, such as:

- EQAS: Quarterly Testing
- EQAS: Conducted by NRL for 5 States
- EQA: HIV Sentinel Surveillance'09
- EQAS Participation

EQAS: Quarterly Testing

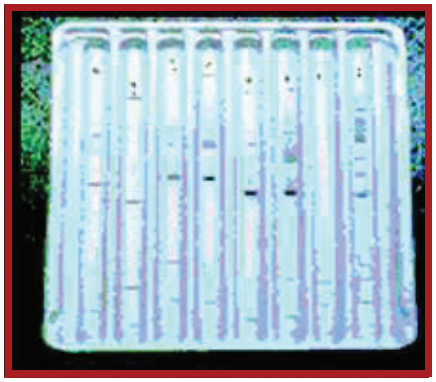
The primary role & responsibility of NRL is to supervise SRL for Quality Testing. NRL usually retests 20% positive or all positive (provided the sample number is low) samples and 5% of all negative samples received from SRLs (specimens are collected by ICTCs in the first 7 days of January, April, July and October & sent to the concerned SRLs).



HIV screening by ELISA

One of the primary duties of the NRL is to prepare a Proficiency Testing panel that is sent to each of the 11 SRLs pertaining to these five states, twice every year. In the year 2009, EQAS Programme was performed successfully for all the 11 State Reference Laboratories.

HIV Spot Test Cartidges (Left Negative test and Right Positive test)



HIV Testing by Western Blot

EQAS Participation

NACO-NRL, at NICED, Kolkata participates in the EQAS conducted by Apex Lab, National AIDS Research Institute (NARI), Pune. A Proficiency Testing Panel is sent from NARI, Pune two times a year to help identify areas of improvement in the laboratory. This year, in addition to the routine quality assessment, NICED also availed the opportunity to take part in an External Quality Assurance programme for Dried Blood Spot (DBS) testing with satisfactory performance.

Table: DBS-EQAS 2009

Sl.No	Panel	Period	Score
1.	DBS-EQAS Panel	June 2009	100%
2.	PT (Proficiency Testing) Panel	October 2009	100%

HIV SENTINEL SURVEILLANCE

Sentinel Surveillance was organized by NACO. During the period 01.01.09 to 31.12.09, 6985 DBS samples were received from Arunachal Pradesh, Manipur, Mizoram, Nagaland and Tripura. However only 6835 samples (97.9%) could be processed for HIV testing, as relevant supporting documents of some of the samples were found missing. Among these 582 samples were screened as HIV positive, amounting to 8.5% of the total samples tested.

Detection of HIV by Rapid Method

KIT EVALUATION

Diagnostic Assay Kits were evaluated for UNOPS (United Nations Office for Project Services), NACO, WBSAP&CS (West Bengal State AIDS Prevention & Control Society), ASACS (Assam State AIDS Control Society), SACS (Sikkim AIDS Control Society) and manufacturers.

The Table below summarizes different Rapid tests and ELISA kits for HIV, HBV and HCV detection that have been evaluated during the year 2009.

Kit-based testing for detection of Hepatitis B

Table: Kit Evaluation'09 at NACO NRL, NICED, Kolkata

Sl.No	Antibody Detected	No. of Kit Evaluated
1.	HIV ELISA Kits	10
2.	HIV Rapid Test Kits	07
3.	HBsAg ELISA Kits	08
4.	HBsAg Rapid Test Kits	04
5.	HCV ELISA Kits	11
6.	HCV Rapid Test Kits	04
	Total No. of Kits Evaluated	**44**

NACO-NRL support for SRLS

This year the three State Reference Laboratories visited were as follows:

1. SCB Medical College, Cuttack, Orissa
2. Assam Medical College, Dibrugarh, Assam
3. Silchar Medical College, Silchar, Assam

Meanwhile, training was imparted to 11 SRLs and NICED staff.

The year 2009 was very eventful for the NACO-National Reference Laboratory with active participation in several EQAS Programmes that were entrusted by several local and state hospitals for HIV testing. Apart from securing our position as a leading Reference Laboratory in the country, we look forward for accreditation of the laboratory by WHO and by NABL.

D. Animal House Section

Scientist : H. Koley, Scientist B

Staff : G.N. Patra, Technical Assistant
K. Biswas, Technical Assistant
K.C. Tudu, Laboratory Technician
S.R. Balmiki, Head Watchman
P. Turi, Head Sweeper
S. Hari, Laboratory Assistant
N.C. Mondal, Sweeper

E. Epidimic Investigations

Report of investigation of a diarrhoeal disease outbreak in Hyderabad by National Institute of Cholera and Enteric Diseases, Kolkata, team from May 9-12, 2009

A team of two epidemiologists (Dr Dipika Sur and Dr Suman Kanungo) and two microbiologists (Dr Ranjan Kumar Nandy and Dr Sulagna Basu) of NICED visited Hyderabad, Andhra Pradesh to conduct the investigation at the request of the superintendent of Gandhi Hospital, Hyderabad, where many cases were being admitted due to severe watery diarrhea associated with dehydration since one week. The team reached Hyderabad on May 9, 2009 and left Hyderabad on May 12, 2009 late night. The observations and the recommendation from the team are detailed below.

Clinical scenario in hospitals

The outbreak started from May 3, 2009 at Bholakpur. Patents start pouring in since May 3, 2009 mainly in two hospitals, Gandhi and Quarantine hospitals which are $2^{1}/_{2}$ km away from the affected area.

At Gandhi hospital total of 515 diarrhoea cases were admitted till May 3-11, 2009. There was only one death of a grade 3 malnourished child with diarrhea. All cases presented with acute watery diarrhea and were mostly admitted within 6-12 hours of initiation of symptoms. Average number of loose motions ranged from 6-20 in 24 hours. Patients were admitted in the paediatric, disaster and other special wards opened to combat the situation. IV fluids were instituted, average fluid administered was approximately 3-5 litres especially in paediatric cases. Severe vomiting was present in 60% of cases. About 55% presented with some dehydration and the rest with severe dehydration. The outbreak started with children only, followed by adult cases. On the whole, 70% cases were in the paediatric age group-the lowest age being 5 months, who was being exclusively breast-fed. By May 11, 2009 384 patients had been discharged. Treatment given was co-trimoxazole, intravenous fluids (Ringer Lactate), ORS and in case of vomiting, anti-emetic and antacids were given.

At Quarantine hospital, a 300 bedded hospital, $2^{1}/_{2}$ km. away hospital from the affected area Bholakpur, patients started pouring in, till May 11, 2009 173 cases of diarrhea were admitted. Out of the 173 patients 42 patients were tested for *V.cholerae* in their stool samples, 12 of them were positive for *V. cholerae* O1 Ogawa. The mean duration of stay in the hospital was 2.8 days, all were adults, mean age was 31 yrs ranging from 15 to 50 years. Children less than 5 years were not admitted Most of them had severe dehydration, presented with acute watery diarrhoea and vomiting, episodes of dairrhoea amounting to 10 per subject. The patients were treated with IV fluids, ORS, average 3.2 litres of fluid (Ringer Lactate Solutions) Doxycycline 300 mg stat and if required 100 mg two times a day for 5 more days, IV Ciprofloxacin and Metronidazole till discharge.

Community scenario

Bholakpur is an area situated about 2 km from Gandhi Hospital. It consists mainly of slum population of the minority community. Average family size is 6 and income Rs.2000-5000. Main occupation in the locality is skilled and unskilled laborers, small businesses. The total population is approx. 65,000. Water supply is through community taps from municipality the pipes having been laid 70-80 years back. Excreta disposal is mostly in latrines but stool of children are disposed indiscriminately. Most of the families have shared flushed latrines. The people live in premises, with an average of 4-5 families per premise. Rain water and sewage run through open drains lying in close proximity with the water pipes. In certain situations when water pressure is low, there is regular seepage from the open drains into the water pipes. Presently new pipelines are being installed in the area but the whole process is moving very slowly.

The community first faced the brunt of the outbreak on May 3, 2009. The daily number of cases increased to 600 by May 5, 2009. On May 7, 2009 the numbers went down to 500 and since then have been decreasing and was 150 cases on May 10, 2009. All these cases were referred to different hospitals like Gandhi Hospital, Fever Hospital, Nilofer and Osmania Hospital. Besides, around 68 cases have been treated in private hospitals. There have been 16 deaths reported from the area of which 10 were children below 5 years.

4 medical camps had been setup in the community manned by physicians and ancillary health staff. There was provision for IV fluids and ORS and drugs, namely ciprofloxacin, norfloxacin, tinidazole.

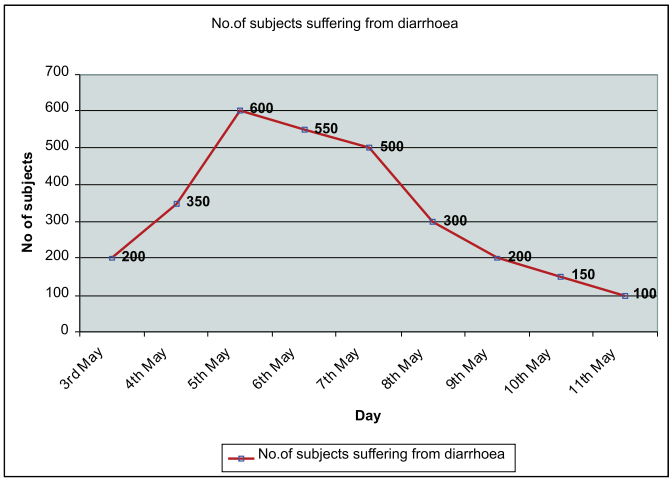


Fig. 1: Distribution of affected subjects over time

Hospitalization rate was 25.6%, attack rate was 4.5% and case fatality rate was 0.54%.

Laboratory diagnosis

Total number of diarrheal stool specimens analyzed by the NICED team was 24 and among these 17 showed growth on TCBS agar with a typical morphology of *Vibrio cholerae.* Isolated colonies were tested using TSI agar which showed a typical biochemical reaction that correlated to the presence of *V. cholerae.* Confirmation of *V.cholerae* O1 was done using polyvalent anti sera and these organisms were identified as ***Vibrio cholerae*** **El Tor O1 Ogawa**.

Results of antimicrobial sensitivity pattern of stool samples positive for *Vibrio cholerae*

Sensitive	Intermediate	Resistant
Ciprofloxacin	Tetracycline	Doxycycline
Norfolxacin	Ofloxacin	Co-trimoxazole

Prevention and control

From the situational analysis the following conclusions were drawn:

1. Immediate stoppage of present municipal water supply through community taps
2. Immediate action on an emergency basis to be taken to replace the old water pipes with new ones constructed as far away from open drains as possible to avoid contamination. Till safe water is instituted, future outbreaks cannot be ruled out.
3. Concrete lining of open drains for sewage flow; if possible set up sewage pipelines to avoid any chance of contamination with water pipelines.
4. Health education to the community members (especially mothers) regarding use of safe water, identification of danger signs and action to be taken, feeding practices, proper disposal of excreta, avoidance of food from outside. **All family members should wash their hands thoroughly after defecation, after cleaning a child who has defecated, after disposing of a child's stool, before preparing food, and before eating.**

5. House to house visits must be conducted to convey the messages on a one to one basis; this is more effective than pamphlets and miking.
6. Chlorination of tankers supplying water to the community presently.
7. Supply of chlorine tablets to the family (500 mg in 20litres of water).
8. Supply of ORS packets to the families with instructions for proper preparation and usage.
9. Supplementation with zinc for children already suffered from diarrhea-zinc suspension or tablets (10-20 mg) for two weeks.
10. Regular testing of water samples for coliform organisms.
11. Mobilisation of adequate manpower in the community including medical team to combat the disaster on war-footing.

Patient Management

1. Treatment plan given below in details (as per WHO guidelines) for
   - Fluids
   - Antibiotics
   - Feeding

Following antimicrobial susceptibility results it was recommended to administer Norfloxacin or Ciprofloxacin

Investigation of Avian flu outbreak at Hemtabad Block in Uttar Dinajpur District, as members of Central Rapid Response Team [RRT] from May 31, 2009 to June 10, 2009.

In response to the order No D320/1/2008 dated May 29, 2009 of Emergency Medical Relief (EMR),West Bengal a Central Rapid Response Team was deputed for investigation of Avian Influenza outbreak in Uttar Dinajpur District of West Bengal from May 31,2009. Dr. Shanta Dutta was deputed to visit the outbreak area from May 31, 2009 to June 5, 2009. On June 4, 2009 Dr. Triveni Krishnan relieved Dr. Shanta Dutta and joined Dr. Lina Bandhyopadhyay, Public Health Specialist AIIH&PH, Kolkata of the team, as directed by Director of National Institute of Cholera and Enteric Diseases and worked with the team till June 10, 2009.

After initial discussion with Dr. Bhaskar Bhattacharya, Joint Director, Swasthya Bhavan, Health Dept., West Bengal the team had reported to Dr. Bidhan Ch. Mishra, Dy. Chief Medical Officer of Health II, North Dinajpur district on May 31, 2009. They enquired about the ongoing outbreak of Avian Flu and worked out the detailed plan of action to visit some of the affected blocks during the active surveillance period of ten days. On June 1,2009 the members had visited the Chief Medical Officer of Health, Dr. P.S. Bhattacharya in his office and requested him to depute a trained physician from the State Government for the investigation. Demographic data of the district was collected from the office of the Chief Medical Officer of Health, Dr. Debabrata Roy, Chest Physician, Raiganj District Hospital was deputed to assist the Central Team on June 1, 2009 and no trained physician was deputed in his place thereafter. In absence of Dy. Chief Medical Officer of Health II, Dr. Amitava Mandal, Asst. Chief Medical Officer of Health, Raigunj Sadar, North Dinajpur actively coordinated the activities of central team.

The team had enquired about the ongoing outbreak of Avian Flu and worked out a plan to visit the affected blocks during the surveillance period. On enquiry it was observed that epicenter of the outbreak was the village called Kantor under Hemtabad Block, where unnatural death of birds was notified on May 29, 2009 in an organized farm and several backyard poultries, before the central team arrived.

On June 5, 2009, a meeting was held in the Jela Prashasanik Bhaban, Karanjora with Shri Pijus Kanti Dutta Additional District Magistrate(H) along with three members of ARD, ACMO(H) and Central RRT. The discussions included the activities completed with regard to surveillance in the 0-3km radius as well as 3-10km radius of the epicenter in Kantor. The Information Education Communication [IEC] status in the above mentioned area was reviewed; the Additional District Magistrate was appraised about imparting knowledge and training of the surveillance teams as well as other associated health personnel under the Blocks of Hemtabad, Raigunj and Kaliagunj respectively.

Approximately,22,541 birds had been culled and after several days, a few stray ones had appeared and these birds seemed to be normal and no fever or illness was detected around the neighbourhood or in the houses to which they belonged, in course of the active surveillance activities by the Central RRT.

Active Surveillance of fever had been established since 29 May 2009 within 0-3 km area around the epicenter on daily basis. Three blocks (Hemtabad, a part of Raiganj and Kaliyaganj block) were included within 3-10 km area around the epicenter. The State health personnel were actively engaged in fever surveillance along with deputed local link persons, trained by NGOs, ANM and health supervisors. They were regularly reporting their findings to the higher authorities for onward communication.

As per information provided by the health staff and members of ARD culling operation of active zone by animal husbandry department was started on May 29, 2009 and completed on May 31, 2009. Cullers were examined regularly by Medical Officers and provided with Tami Flu tablets under direct supervision. Cullers were isolated in one school building (Adarsh Vidyalaya in Hemtabad).

The Central RRT conducted sensitization /orientation /awareness programmes among different categories of health functionaries in the blocks of Hemtabad, Raigunj and Kaliagung respectively. Public Health Nurses, trained dais and Link men, NGOs etc in 0-3km and 3-10 km areas around the epicenter. The record of fever surveillance was being sent to the B.M.O.H. of respective blocks and finally compiled in the office of Dy C.M.O.H. II for further action.. The staff at various health centres were found to be aware of the fever surveillance, daily reporting system and need for urgent reporting of 'Acute Respiratory Infection' cases in close contact with the poultry, if there was any.

The Central RRT visited both the infected zone and surveillance zone daily and covered the following villages to interact with the residents of each area and to monitor the surveillance activities. It has been observed that the residents were aware about the disease but not the potential danger of the infection. Therefore they were sensitized on the issue and instructed to report as soon as possible to the health authorities for medical coverage. They were also informed that all children should wash their hands properly before eating their food and not to play with the domestic poultry population.

On June 3, 2009 an orientation programme was conducted on Avian Influenza at Hemtabad among surveillance workers viz. Dr Dibyendu Mandal [Block Medical Officer of Health] & other Medical Officers, Block Public Health Nurse, Block Sanitary Inspector and other functionaries.

On June 4, 2009 an orientation programme was conducted on Avian Influenza at Raigunj among surveillance workers viz Dr Gautam Mondal [Block Medical Officer of Health] & other Medical Officers, Block Public Health Nurse, Block Sanitary Inspector and other functionaries.

On June 7, 2009 an orientation programme was conducted on Avian Influenza at Kaliagunj among surveillance workers viz Dr M. Ghatak [Block Medical Officer of Health] & other Medical Officers, Block Public Health Nurse, Block Sanitary Inspector and other functionaries.

The Central RRT also interacted with the staff and Medical Officers of the Primary Health Centres in Hemtabad Block, Raigunj Block and Kaliagunj Blocks.

Hospital system/ Resource availability

Sufficient stocks of Tami Flu & PPE were maintained in the District Hospital, Hemtabad Block Public Health Centre. Demand for more stocks had been placed by Dy C.M.O.H. II and arrangements were made to procure those from adjacent districts (Malda). Initially sample collection kit was not available with the Pathology dept of District Hospital; subsequently one kit was procured on June 1, 2009, on urgent basis. Isolation wards were arranged in District Hospital and Hemtabad Block PHC. During the visit suggestions were made by the central team to fulfill the criteria of standard isolation ward. Only one ventilator was available at the district hospital, demand has been placed for three additional ventilators.

Sanitation of the affected area

On interacting with the health officials of Block PHC it was known that the birds were culled at nearby open space with all precautions and areas had been disinfected with lime and bleaching powder. The PPEs used by sanitizing workers were disinfected and destroyed. Disinfectant (bleaching material) was made available to all households in the nearby areas for spraying and surveillance indicated that necessary measures were being taken. The culling sites at the forest in Hemtabad [Baruibari] and Bhowgram adjacent to the cremation ground was visited by Dr Lina Bandhyopadhyay and the team. As the culling sites were not secured properly and no warning boards were in place, the CRRT advised that dumping site should be demarcated with proper fencing and clear warning board should be fixed properly for each dumpsite to avoid any sort of miscreant activities or tampering of the culled population.

Report of investigation of a diarrhoeal disease outbreak in Allepey, Kerala by National Institute of Cholera and Enteric Diseases, Kolkata, team from June 4-7, 2009

Dr Dipika Sur (epidemiologist) and Dr.T.Ramamurthy (microbiologist) of NICED visited Allepey, Kerala to conduct the investigation at the request of the Director, EMR, New Delhi, following reports of severe watery diarrhea cases associated with dehydration since May 13, 2009. The team reached Allepey on June 4, 2009 and made investigations in the hospitals, community and also had discussion with the Health authorities as well as the District Magistrate and Water Works officials.

The outbreak started from May 13, 2009 when the hospital authorities noted increasing number of diarrhoea cases being admitted as well as attending the out-patient departments. Reports also started coming in from the municipality as well as panchayat areas of increased number of diarrhoea cases.

Allepey district has 5 municipalities and 12 blocks. Of the 5 municipalities, Municipality of Allepey town, had 3 affected wards with approximately 1,00,000 population. There are two main hospitals in this municipality viz., the General Hospital and T D Medical College and Hospital. All serious cases were admitted in these two hospitals.

There was no data available from the municipal authorities regarding the diarrhoea cases occurring in the municipality area. Municipal health structure is very rudimentary with one doctor -Municipal Health Officer. There are some health workers who are basically involved in collection of information regarding health status in the community.

In the rural area, of the 12 blocks, 1 panchayat area of Champakullam block was affected. The panchayat area, named Kainakari with 24,000 populations, has 14 wards which were all affected, though wards 4, 10, 11 and 14 had maximum number of cases.

Piped water supply was available to 80% population in the municipality area. According to the municipal authorities, these pipes were very old and needed to be replaced. Others used water (including rural area) from open wells and personal tube wells. Water supply was very irregular and sometimes was not available for 2-3 days. There was acute scarcity of water in the affected area. People started using the highly polluted canal water for washing, bathing and even cooking. Further, 90% of the sources of drinking water had coliform count much higher than acceptable norms.

There is no sewerage system in Allepey. About 70% households in the municipal area have septic tanks others (including rural areas) connect pipelines to drain that empty out directly into the canal.

Clinical scenario in hospitals

A total of 300 diarrhoea cases had attended General hospital from May 13, 2009 to June 4, 2009 of which 84 cases had been admitted. All cases presented with acute watery diarrhea and were mostly admitted within 12-24 hours of initiation of symptoms. Some however had been admitted after 3-4 days of diarrhoea. Patients were admitted in the isolation wards which had been identified to combat the situation. IV fluids were instituted, average fluid administered were approximately 3-5 litres especially in paediatric cases. Severe vomiting was present in 60% of cases. About 50% presented with some dehydration and the rest with severe dehydration. About 50-60% patients complained of abdominal pain and fever. About 20% cases were in the paediatric age group. There was only one reported hospitalized death of a 68 year old woman whose stool sample had confirmed the presence *V.cholerae* O1 ElTor.

At T D Medical College and Hospitals (TDMC) a total of 94 cases of diarrhea had presented since 13th May of which 24 had been admitted, the lowest reported age being 7 months, whose stool was positive for *V.cholerae* O1 ElTor.

Treatment given in both hospitals was oral co-trimoxazole, IV Taxim, IV Amikacin, IV Metronidazole, intravenous fluids (Ringer Lactate or Normal Saline), ORS and in case of vomiting, anti-emetics. In some cases loperamide was also administered.

Of the 34 stool samples taken from hospitalized patients, 13 tested positive for *V.cholerae* O1 ElTor, Ogawa in the Microbiology department of TDMC. This information was confirmed by Dr Ramamurthy, microbiologist from NICED. Many of the samples were collected after administration of antibiotics.

Drug sensitivity Pattern

Sensitive	Resistant
Ciprofloxacin	Furazolidone
Norfloxacin	Co-trimoxazole
Ampicillin	Tetracycline
Gentamycin	
Chloramphenicol	
Erythromycin	

Community scenario

From May 13, 2009 171 diarrhoea cases had attended the OPD of the Primary Health Centre in Kainakari panchayat. The PHC is manned by one medical officer and his staff. They have to move from ward to ward for the surveillance by boat which is not easily available. The medical officer reported 2 deaths from his area but could not confirm that the deaths were due to diarrhoea.

The 14 wards covered by this PHC have 60 open wells which were supposed to be chlorinated at an interval of 2 weeks by health staff. Water collected from the PHC was tested for residual chlorine, but was found to be negative.

During the time of the investigation, water was being supplied in tankers by boat. Water tested from these tankers was strongly positive for residual chlorine.

Recommendations:

From the situational analysis the following conclusions have been drawn:

1. Increase supply of water both in quality and quantity.

a) Chlorination of all sources of drinking water. Residual chlorine especially at user end can be tested by chloroscope which is a very simple technique and can be easily conducted in field situations by the health workers.

b) More tube wells to be dug especially in areas where water is totally unavailable.

c) Since underground (underwater) pipes are supplying water to some of the water logged areas, this can be extended to other areas as well. The problem that might be faced is that in case of any leakage, it will be difficult to identify it under water.

d) Treatment plants for the canal water to be set up in remote areas, where supplying water from any other source is a difficult process.

e) Reverse Osmosis plants may be set up for every 2000-3000 population.

2. Sewage disposal

a) Sewage treatment before draining into canal water.

b) Installation of more septic tanks since the water table is very high, low cost techniques like two pit latrines will not be effective.

3. Disease surveillance

a) To be conducted regularly according to IDSP norms

b) To be extensively supervised and monitored

4. Co-ordination

a) There should be planned co-ordination between health staff and other community level workers by local panchayats to avoid duplication or missing data.

b) Co-ordination should also be present between the two hospitals e.g. the microbiology department of TDMC had conducted drug sensitivity but the results had not reached the concerned medical officers in either hospitals and they continued to administer drugs to which vibrios were resistant.

5. IEC

a) Health education to the community members (especially mothers) regarding use of safe water, identification of danger signs and action to be taken, feeding practices, proper disposal of excreta, avoidance of food from outside.

b) House to house visits must be conducted to convey the messages on a one to one basis. This should be accompanied by supply of chlorine tablets (500 mg in 20 litres of water) ORS packets with instructions for proper preparation and usage and supplementation with zinc for children (10-20 mg) for two weeks.

Commonly recommended drugs for cholera are as follows:

Drug name	Dose for Children	Dose for adults
Tetracycline	25-50 mg/kg/day in 4 divided doses	500 mg 4 times a day 3-5 days
Doxycycline	2.5 mg/kg/day	300 mg as single dose
Ciprofloxacin	10 -15 mg/kg/day in 2 divided doses	500mg-2 times a day for 3 days
Norfloxacin	10mg/kg/day in two divided doses	400 mg 2 times a day 3 days
Azithromycin	5mg/kg/day followed by 2.5 mg /kg/day for 2 days	500 mg single dose for 3 days

Following antimicrobial susceptibility results it was recommended to administer Norfloxacin or Ciprofloxacin with specific dosages.

Antimicrobial therapy

All cases of suspected cholera with severe dehydration should receive an oral antimicrobial known to be effective against strains of *Vibrio cholerae* in the area. This reduces the total volume of stool passed, causes diarrhoea to stop within 48 hours, and shortens the period of faecal excretion of *V. cholerae*. The first dose should be given as soon as vomiting stops, which is usually 4-6 hours after starting rehydration therapy.

Drugs recommended during the investigation

Since the present strain of *V.cholerae* was resistant to tetracycline, doxycycline and co-trimoxazole, the recommended drugs were norfloxacin and ciprofloxacin. IV Taxim, IV Amikacin should not be the first line of drug. IV Metronidazole has no place in the treatment and loperamide is totally contraindicated in diarrhoea of any aetiology.

Report of investigation of a diarrhoeal disease outbreak during Post AILA cyclone in North 24 Parganas by National Institute of Cholera and Enteric Diseases, Kolkata, team from June 12-13, 2009.

A team of two epidemiologists (Dr Dipika Sur and Dr Suman Kanungo) clinician (Dr. Mihir Bhattacharya) and microbiologist (Dr Ranjan Kumar Nandy) of NICED visited Sunderban area of North 24 Parganas to conduct the investigation for diarrhoeal outbreak at the request Jt. Director, PH &CD, Department of Health and Family Welfare, Govt. of West Bengal where many cases were being admitted due to acute watery diarrhea associated with dehydration since May 27, 2009 following the AILA cyclone which hit Sunderban area on May 25, 2009. The team reached Hashnabad on June 12, 2009 and left from Sandeshkhali II on June 13, 2009 evening. The observations and the recommendation from the team are detailed below.

Background information:

Sunderban area is the biggest delta region in West Bengal, criss-crossed by rivers, around islands and estuaries draining into the Bay of Bengal. It was seen from the data provided by the health officials that the worst affected blocks were Hingalganj and Sandeshkhali II.

The team approached Hingalganj block from Hasnabad along with Deputy CMOH II, North 24 Parganas and visited the control room at Dulduli PHC from where the logistics and deployment of manpower are taking place. Situation analysis was done there. The team then visited two medical camps, one in Dulduli and one in Jogeshgunj on June 12, 2009.

The salient features were:

The affected population at Hingalgunj: 1,22,700, Sandeshkhali II: 1,51,534. All the 9 GPs in Hingalgunj and 6 out of 8 GPs in Sandeshkhali II were badly affected. Several medical camps were opened since June 5, 2009. In Hingalgunj there were 30 medical camps in operation, in Sandeskhali II there were 32 medical camps in operation.

5 rectal swabs were collected from freshly admitted cases in Dulduli medical camp. On enquiry it appeared that daily about 25-30 cases were seen in the OPD of which some were admitted. A total of 115 cases had been admitted from June 6-11, 2009 highest numbers being on June 9, 2009 but cases did not stop coming even after that. An NGO had opened a medical camp in the area from May 30, 2009 till June 5, 2009. From their report, it was observed that 153 OPD cases had attended the camp of which 52 had been admitted.

The general situation in the camp was horrifying.

The camp had been opened in a school building. The patients were been laid on narrow benches (meant for seating of school children) and IV fluids and medicines were being administered. Every room was overcrowded.

There was no electricity, no provision for potable water and food supply was limited. The lone medical officer and his three staff were extremely overworked. They did not have any separate place to sleep. They had to sleep in the same room as the patients. Since embankments had been broken in the nearby areas, water gushed into the courtyard during high tide in the river- making the place totally uninhabitable. The high tide water seeped into a low-lying tube well and contaminated the tube well water which was the only source of water for the near-by population.

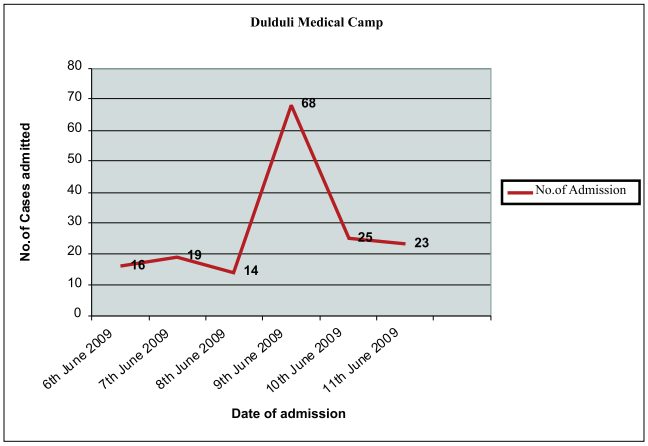


Fig. 1: Diarrhoea cases admitted in Dulduli Medical Camp (data available from June 6, 2009 onwards)

In Jogeshgunj medical camp an average of 20 diarrhoea cases attended the camp daily, of whom 153 patients had been admitted between June 6-11, 2009. The graph shows daily distribution of admitted cases. 4 rectal swabs were collected from fresh cases.

The overall situation was worse here. This camp had also been established in a school building. The admitted patients were kept in one big hall all lying on the floor. The patients with their IV drips were placed within one foot of each other. Here again the situation was totally chaotic with the medical officer and his staff trying to manage several patients at the same time. They complained that they were getting exhausted and would not be able to carry on for very long. From local people it was learnt that water seepage also occurs in the courtyard of the school during high tide.

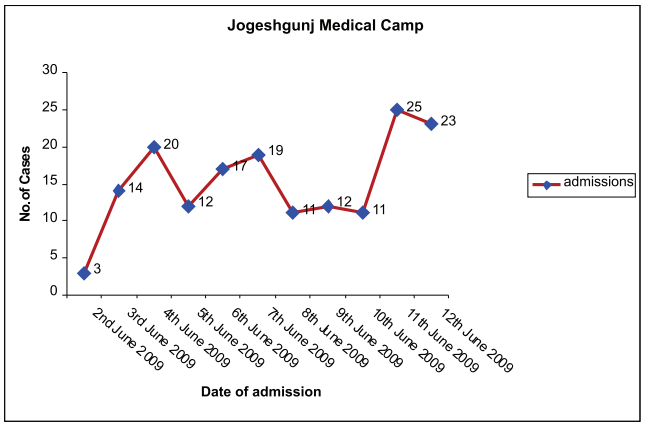


Fig. 2: Diarrhoea cases admitted in Jogeshgunj Medical Camp (data available from June 2, 2009 onwards)

Sandeshkhali II block has 8 GPs areas all of which are islands. The worst affected was Khulna GP which has a rural hospital. 143 cases had been admitted since May 27, 2009. 5 rectal swabs were collected from freshly admitted cases. The graph shows the daily admission of cases.

From the information gathered locally, the rural hospital had knee deep water for 4 days since the AILA disaster on May 25, 2009. The medical officers and other staff had to shift to the roof of the hospital for safety- as it is a single-storied building. In spite of that they treated patients standing in the water.

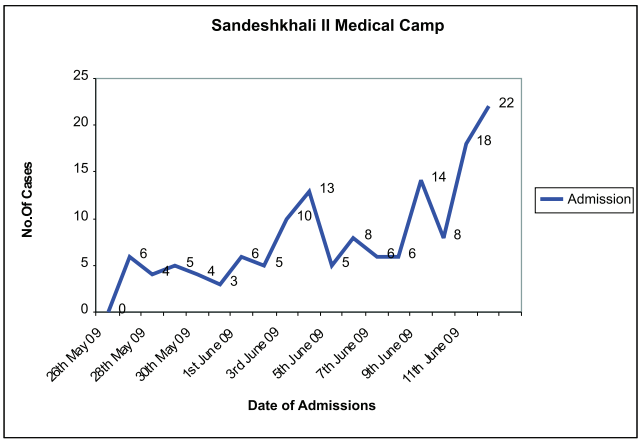
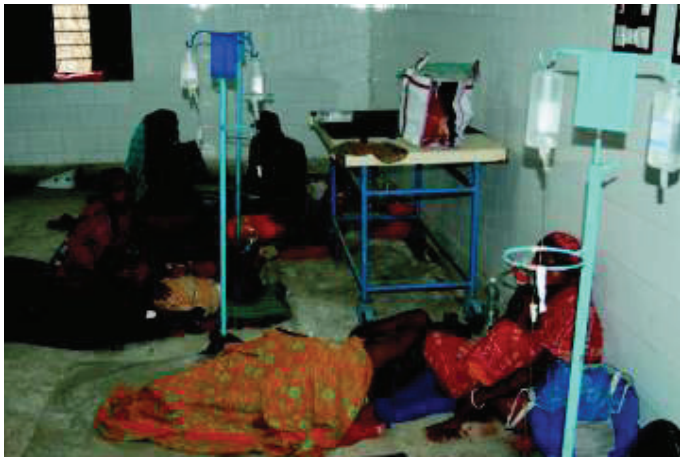


Fig. 3: Diarrhoea cases admitted in Khulna Rural Hospital (from June 2, 2009 onwards)

In all the camps 60% of the admitted cases had severe dehydration and presented with vomiting and diarrhoea (average 17 loose motions in last 24 hours) and 75% of the admitted cases were adults (>15yrs of age). All of them were being treated with IV fluids, mainly Ringer Lactate solution and normal saline, IV antibiotics (Norfloxacin, Amikacin, Ceftriaxone), IV Metrogyl, antiemetics and ORS.

There were 2 reported deaths one in Dulduli medical camp (2½ years old) and one in Sandeshkhali Rural Hospital (5 years old)



Laboratory results

Total number of diarrheal stool specimens analyzed by the NICED team is 14 and among these 9 showed growth on TCBS agar with a typical morphology of *Vibrio cholerae.* Isolated colonies were tested using TSI agar which showed a typical biochemical reaction that correlates to the presence of *V. cholerae.* Confirmation of *V.cholerae* O1 was done using polyvalent anti sera and these organisms were identified as *Vibrio cholerae* El Tor O1 Ogawa.

Water samples were tested from different sources for residual chlorine, but none of the samples showed presence of residual chlorine.

Results of antimicrobial sensitivity pattern of stool samples positive for *Vibrio cholerae*

Organism	Area	Ampicillin	Ciprofloxacin	Furazolidone	Gentamycin	Nalidixic acid	Norfloxacin	Tetracycline	Erythormycin	Azithromycin	Doxycycline	Ceftriaxone	Ofloxacin
Vibrio cholerae O1	Dulduli	R	I	R	S	R	S	R	S	S	I	S	S
Vibrio cholerae O1	Sandeshkhali II	I	I	R	S	R	S	R	I	S	S	S	S
Vibrio cholerae O1	Sandeshkhali II	R	I	R	S	R	S	R	I	S	I	I	S

Recommendation for action to be taken immediately

- Supply of adequate quantity of safe drinking water, pouched water, halogen tablets were being supplied but quantity was insufficient. A Reverse Osmosis (RO) plant had been established on a trial mode - taking water from a river near Dulduli and treating it to make it potable. This was a cost-effective technique which could be expanded to other areas.
- Arrangement for proper sanitation and housing facilities for the displaced population.
- Mobilization of additional medical and para-medical manpower in all the medical units attending to the affected population. The units were extremely understaffed and without adequate replacement there might be more impending problems.
- Rationale of treatment of diarrhoea cases.
    - IV fluids to start with Ringers' Lactate and not with Normal Saline as is being done in several places. Unless patient has gone into renal failure, there is no place for administering IV Normal Saline to dehydrated diarrhoea patients.
    - Antibiotics to be used as per sensitivity pattern of the drugs. Other than cholera and shigellosis, there is no need for antibiotics in diarrhea. Since cholera and shigellosis have totally different clinical presentations, there should be no confusion regarding treatment.

Post-AILA outbreak investigation that influenced antibiotic prescription practice for management of diarrhoea in Purbo-Medinipur, West Bengal

A severe cyclonic storm, AILA crossed West Bengal coast near Sagar Island in Bay of Bengal between 1330 and 1430 hrs Indian Standard Time (IST) on May 25, 2009. Rapid Situation and Response Assessment (RSRA) technique was employed by the team sent from NICED (Dr Samiran Panda, Dr MK Bhattacharya and Dr Hemanata Koley) to the district of Purbo Medinipur to investigate the cases of diarrhea reported in increasing numbers post-AILA from the district. Family members of 32% and local community residents of 66% watery diarrhoea cases had also reportedly suffered from watery diarrhoea. Only 26% of the patients used 'Oral Rehydration Solution' following diarrhoeal attack. *V. cholerae* was recovered from 54% of the stool samples (21/39; 17 *V. cholerae* serogroup O1 Ogawa and 4 non-O1-non-O139), and *Shigella flexneri* 3a from 2 (5%) samples. All the isolates of *V. cholerae* were sensitive to norfloxacin and azithromycin. Resistance to multiple antibiotics was noted in the isolates of *V. cholerae* cultured from stool samples collected from treatment centres as well as from community. Both the isolates of *Shigella flexneri* were resistant to furazolidone, chloramphenicol, co-trimoxazole, ciprofloxacin, norfloxacin, ofloxacin, nalidixic acid, streptomycin and tetracycline. Norfloxacin use increased significantly after we disseminated laboratory findings to the respective health-administrators of the district. Chief Medical Officer of Health of Purbo Medinipur incorporated use of Norfloxacin and Azithromycin (single dose) in the circular issued by him regarding prevention and management of diarrhoea to all the Block Medical Officers of Health. An example of translational research was thus set where laboratory findings were used to influence policy decision and practice.

Outbreak investigations (Laboratory analysis for microorganisms in water samples)

- During the epidemic outbreaks (2009-2010) of diarrhea which spread across different southern districts of West Bengal, results of microbial analysis following examination of samples of potable water collected at different sources, in different parts of West Bengal was reported to the Govt. agencies, as routine activity.
- Water samples had been received from different PHCs of 24 Parganas (N), 24 Parganas (S), East Midnapore, Howrah and Hooghly as well as from endemic and epidemic affected Municipal wards under the Kolkata Municipal Corporation and its adjoining areas. Results have been conveyed to the respective agencies with a copy of the same to State Health Secretariat, Govt. of West Bengal. During the period under report, 155 samples were received from various sources, of which 94 were found to be positive for faecal coliforms and 33 were positive for *V.cholerae* O1 (Table).

Sl. No.	District	No. of samples received	Source				Culture Positive	PCR positive
			Tap	Tube well	Pond	Unknown		
1.	South 24 Parganas	21	17	3	_	1	7	6
2.	North 24 Parganas	73	43	5	8	17	24	15
3.	Hooghly	29	_	9	16	4	9	7
4.	East Midnapore	29	_	12	17	_	14	4
5.	Howrah	3	_	1	1	1	1	1
	Total	155	60	30	42	23	55	33

TRAINING

TRAINING

The Division of Training and Extension, *a WHO collaborating center for research and training on diarrhoeal diseases*, was actively engaged, throughout the reported year in following activities:

Scientist	:	Anup Palit, Scientist E
Staff	:	R. J. Mukherjee, Technical Officer
		A. Jana, Sr. Laboratory Assistant
		A.K. Roy, Laboratory Assistant
		S. Adhikari, Chowkider

I.A. Organised the following international meeting/workshops/seminars/training:

1. "First Meeting of the Regional Technical Advisory Group (RTAG) Prevention and Control of Acute Diarrhoea and Respiratory Infections" April 23-24, 2009 [WHO-APW Meeting].
2. Informal consultation on research to assess communicable disease impact of climate change, Kolkata, India August 24-26, 2009 [WHO-APW Meeting].
3. Regional consultation on cross-border Collaboration in Disease Control, Kolkata, April 28-30, 2009. [WHO-APW Meeting].

I.B. Organised the following meetings of the Institute:

- SAC meeting during August 28-29, 2009 at NICED, Kolkata.
- Biosafety committee meeting
- Organizing meeting for GCLP training, 2009.
- IVI training programmes
- Meeting of NICED scientific forum
- Meeting of NACO from time to time through the year viz. induction training programmes for Medical Technologists
- Observation of National Science Day on March 2, 2010.
- Seminars and oration lectures organised by ISCA, Kolkata Chapter & NICED, Kolkata.
- Oration lecture of Indian Science Congress Association, Kolkata chapter.
- Observation of National Science Day by ISCA, Kolkata Chapter on March 18, 2010.
- NACO Regional Institute meeting for HIV surveillance.
- WHO/TDR La. Survey, March 1-5, 2010.
- Arrangement of meeting for Dr. Rita Colwell.
- Assistance for Annual session meeting of Calcutta University and National Academy of Sciences, Allahabad.

II. Prepared the following documents for the institute:

- Preparation, compilation and submission of 4 (four) years' performance appraisal report for WHO for redesignation of the Institute as a WHO collaborating center for research and training on diarrhoeal diseases.
- The redesignation has been granted by WHO for a 4 (four) year period from April 2010-April 2014.
- Compilation and submission of Annual report of WHO Collaborating Center for research and training on diarrhoeal diseases (2008-2009).
- Project report book (2008-2009).
- Compilation of minutes of SAC, 2009.
- Reports for Institutional Biosafety Committee.
- Minutes of Institutional Biosafety Committee Meeting.
- Highlight of the Institute for DBT.

- Highlight of the Institute for Directorate of Biotechnology, Govt.West Bengal.
- Training modules of different workshops.
- Documents for the Institutional Scientific Audit.
- Report of the Training Programmes.
- Institutional Documents for West Bengal Academy of Science & Technology.

III. Prepared the following documents for ICMR Head Quarter:

- Highlights of the Diarrhoeal Diseases Research carried over by the Institute
- Highlights of the Institutional activities for ICMR Annual Report
- Highlights of the Institutional activities for ICMR SAG Meeting
- Power point presentation for SAG meeting
- Reports of celebration of National Science Day for ICMR
- Reports of celebration of Technology Day for ICMR
- Reports of celebration of ICMR Day for ICMR
- Documents for foreign participation in R&D activities of the Institute
- Documents for Annual Report for Department of Health Research (DHR)
- Documents for National Institute of Science Communication & Information Research
- WHO training details with prospective budget estimate conducted by NICED
- Documents on activities undertaken by NICED in the context of global climate changes on diarrhoeal diseases and cholera.
- ICMR-NBDP (National Biological Development Programme).
- Preparation of PowerPoint slides for training at ICMR Hqrs. for the B.Sc. (Nursing) students.

IV. Submission of Administrative and A/C related documents for WHO, providing Institutional profile

V. Training programmes:

- Training for M.Sc. Biotechnology of Vishwabharati University.
- Training programme for students of National Institute of Homeopathy, August 6, 2009.
- Training programme for Microbiology students of Calcutta University.
- Training programme for Microbiology students of Kalyani University.
- Training programme for M.Sc. (Zoology) students of Kalyani University, 2009.
- Training programme on Clinical Tropical Medicine, Optional Module for Master's programme in International Health.
- Coordinating training programme for WHO nominated International trainee in the Institute.
- NACO workshop.
- Training Programmes of Immunization strengthening Project for mid level managers of the districts of

Eastern states, Andaman & Nicobar islands, Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Orissa, Sikkim, Tripura, West Bengal as per programme schedules.

- Training for final year students of National Institue of Homeopathy, Kolkata

VI. Organized the workshops/meeting for professional bodies at this Institute:

- For Indian Science Congress Association, Kolkata Chapter for observance of Doctor's day, July 1, 2009.
- For Indian Science Congress Association, Kolkata Chapter for observance of Science Day, March 18, 2010.
- For "Sparsh", an NGO, HIV study sensitization programme.
- Meeting public health officials with Dr. Eric Mintz, Diarrhoeal disease Epidemiology, CDC, Atlanta, September 17, 2009.
- West Bengal State AIDS Prevention and Control Society meeting on DBS and whole blood sample collection at NICED, Kolkata, July 6-8, 2009 coordinated by WBSAP&CS.
- For Indian Science Congress Association, Kolkata Chapter for observance of Environment Day, March 5, 2010.

Summary of 4th Technical review meeting of HIV-TI impact assesment study commissioned by National Aids Control Organisation (NACO), India

The 4th Technical Review meeting on the project entitled 'Impact Assessment of Targeted Intervention of HIV in India' was held on December 4-5, 2009 at NICED, Kolkata. It was attended by the representatives from the Lead Research Institute (LRI) PGIMER, Chandigarh, and Partner Research Institutes (PRI) NARI, Pune. The National Institute of Cholera and Enteric Diseases (NICED), Kolkata is involved in this Project as another PRI.

On December 4, 2009 the first session of the meeting was flagged off by Dr. Samiran Panda (one of the Co-PI of the project) with a welcome note followed by broad overview and the purpose of the meeting. Dr. G B Nair, Director of the institute made comments on the organogram and mandate of NICED, HIV related projects, other ongoing and upcoming activities of the institute.

The technical session on the first day was initiated by Dr. Rajesh Kumar (PI) from PGIMER, Chandigarh. His presentation was based on the progress of the assessment study as presented to NACO on November 16, 2009. The purpose of the session was to summarize the activities undertaken so far and to develop a common understanding among all the attendees of the meeting. This was followed by several interactive discussions on development of an analysis plan, modification and finalization of the reporting format and minimization of data gaps, which were spearheaded by scientists from NICED and NARI.

On December 5, 2009 final decisions were made on several issues which included, preparation of mathematical model of HIV epidemic in India by Dr. A S Rao of Indian Statistical Institute, Kolkata, deadlines for the report submission from each partner research institutes and preparation of final draft by LRI, Chandigarh.

Report on the training program on 'Various aspects of HIV / AIDS counseling' conducted jointly by the National Institute of Cholera and Enteric Diseases (NICED), Kolkata and Society for Positive Atmosphere & Related Support to HIV/AIDS (SPARSHA), Kolkata.

In a letter dated December 9, 2009, 'Desun Hospital and Heart Institute' of Kolkata requested Dr. G B Nair, the Director of NICED to organize a one day training program in collaboration with SPARSHA (an organization run by people living with HIV and their friends) for the key staff of the hospital on HIV / AIDS counseling. As a response to this request, NICED and SPARSHA organized the training program on December 18, 2009 at the meeting room of NICED 2 building (2nd floor). Dr. Samiran Panda, Scientist E, NICED was assigned the responsibility for coordinating the training on behalf of NICED and SPARSHA.

The objectives of the above mentioned one day training program were

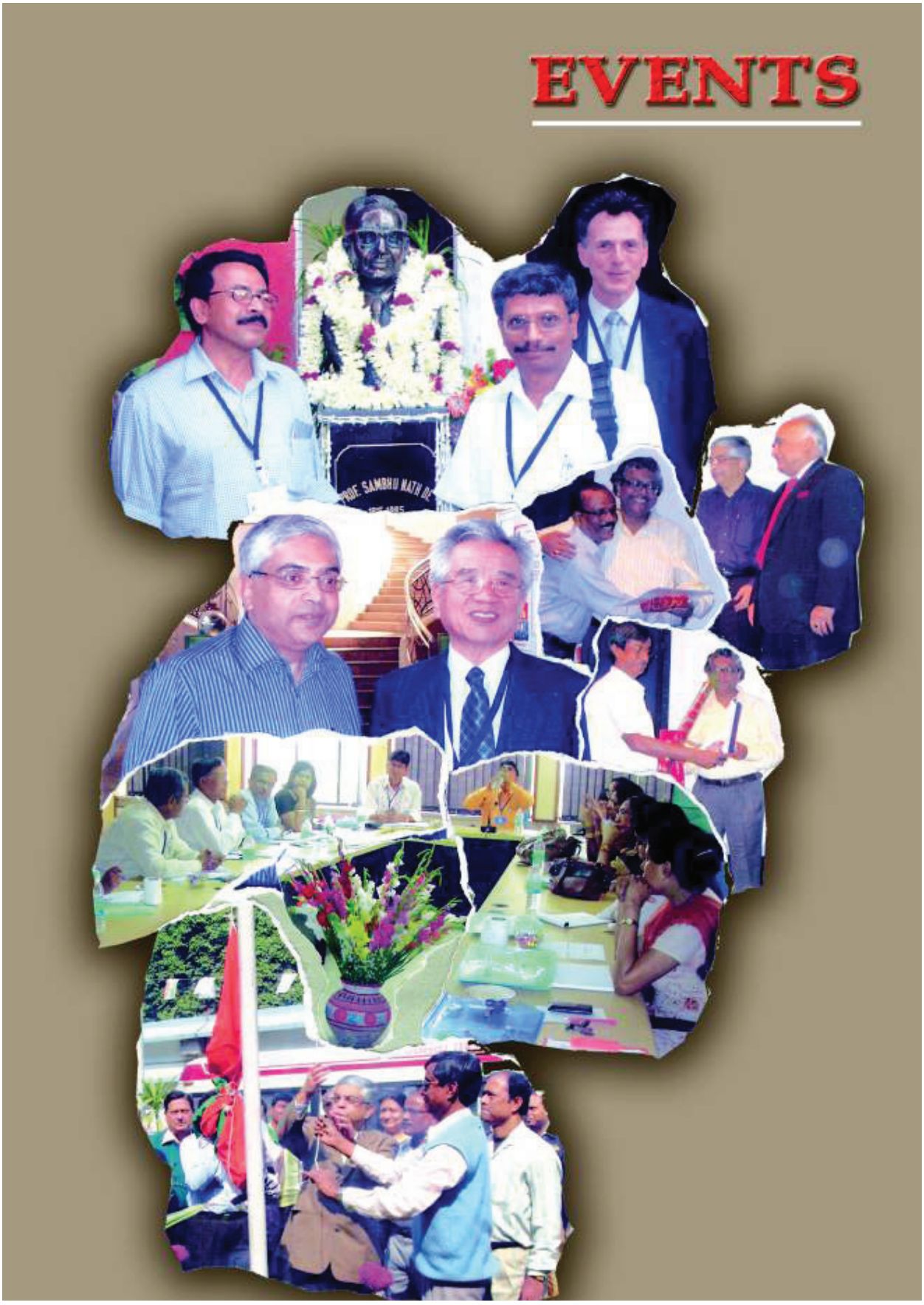
- To increase the knowledge of the training attendees on HIV/AIDS counseling
- To enhance the understanding of the training attendees about interface between counseling and other health care services including the importance of confidentiality and informed consent

The training extended for 5 hrs comprising seven structured sessions, which mostly followed participatory training methods. It started with the welcome note from the Director of NICED. In the second session, interactive training methodology was discussed by Dr. Panda and he initiated the session by asking all participants "why are we all here in this room today?" In the third session, general principles of counseling and specific issues related to HIV/AIDS counseling were discussed. The fourth session dealt with socio economic impact of HIV and the role of counselor in mitigating them. The next session was sharing of experience by a speaker living with HIV, which was followed by counselor's experience of working hand in hand with the people living with HIV and their friends. The last session focused on quality control issues related to HIV test and procedures followed at NICED laboratory before decision is taken about Post Exposure Prophylaxis (PEP).

An innovative idea of this training was that at the end of the program, the trainees were encouraged to provide their feedback. This helped the trainers to identify the strength and weaknesses of this training that was organized. The session ended with distribution of certificates to the participants.

Mr. Tapas Pal, Library & Information Assistant received training at the Libsys Training Programme held during April 20-30, 2009 in Kolkata.

EVENTS



The 13th International Conference on Emerging Infectious Diseases In The Pacific Rim was held in Kolkata, India from April 6-9, 2009 under the US JAPAN Cooperative Medical Science Program, with Enteric Diseases as the focal theme. The meeting was sponsored by the Indian Council of Medical Research, Department of Biotechnology, Indian Ministry of Science and Technology, Ministry of Health, Labour and Welfare of Japan, Ministry of Education, Culture, Sports, Science and Technology of Japan, Ministry of Foreign Affairs of Japan, US Department of Health and Human Services, National Institute of Allergy and Infectious Diseases and US Department of State, Biosecurity Engagement Program.

The First Meeting of the Regional Technical Advisory Group for Integrated Control of Acute Diarrhoea and Respiratory Infections was held in NICED on April 23-24, 2009. The meeting was sponsored by WHO and ICMR.

The Institutional Ethics Committee Meeting was held on July 4, 2009 in NICED, Kolkata to discuss the ethical issues related to the research projects in presence of the members.

A WHO - NICED Scientists Meeting was held on August 24-26, 2009 for informal consultation on research areas to assess communicable disease, with special emphasis to understand the impact of climate change on the disease.

The 37th Scientific Advisory Committee (SAC) Meeting was held in NICED on August 28- 29, 2009 to evaluate the research projects and get the advice of committee members on various other aspects.

Hindi Divas was celebrated on Sept. 14, 2009 in NICED with great enthusiasm and active participation by the staff.

An interactive Session among, CDC, NIE, FETP, State Public Health Officials and NICED scientists was held in National Institute of Cholera and Enteric Diseases (ICMR), Kolkata on September 17, 2009. The participants from CDC: Atlanta, USA were Dr. Eric Mintz, Epidemiologist; Dr. Nancy Strockbine, Microbiologist and Dr. Michele Parsons, Microbiologist. The participants from NIE, Chennai, India were Dr. V. Kumaraswami, Epidemiologist; Dr. Manoj Murhekar, Epidemiologist and Dr. Tarun Bhatnagar, Epidemiologist along with Field Epidemiology Training Programme [FETP] graduates / scholars.

The CHOLDInet RDT Working Group Meeting was held in NICED on October 28, 2009. The Members were Dr. Awa Aidara-Kane, Dr. Cheryl Bopp, Dr. Chaire-Lise Chaigant, Dr. Firdausi Qadri, Dr. Martine Guillerm and Dr. G. Balakrish Nair.

The Third India Probiotics Symposium organised by NICED on *Probiotic Foods in Health and Disease* was held in New Delhi from November 21-22, 2009. The Symposium was sponsored by Yakult Danone India.

The 4th Technical Review Meeting on HIV-TI Impact Assessment Study was held in NICED on December 4-5, 2009.

A Training programme for Health Care Staff of DESUN Hospital & Heart Institute, Kolkata was conducted on *Various Aspects of HIV / AIDS Counseling.* It was jointly conducted by National Institute of Cholera and Enteric Diseases (NICED) and Society for Positive Atmosphere and Related Support to HIV / AIDS (SPARSHA) in NICED on December 18, 2009.

The delegates of Annual Meeting of the Association of Pathologists and Microbiologists, participated in training on parasitology, in the laboratory of Dr Sandipan Ganguly, during APCON that was held in NICED on December 22, 2009

The Republic Day was celebrated in NICED with patriotism and pledging to serve our nation better for a better future.

The new NICED Guest House was inaugurated by Prof. N. K. Ganguly, Former Director General (ICMR), Distinguished Biotechnology Fellow and Advisor, on February 18, 2010. This modernised facility is located in the Dr S.C Pal building of the institute and provides many comforts to visitors.

The Foundation Day of National Institute of Cholera and Enteric Diseases (ICMR) was celebrated with great festivity on February 18, 2010. The celebrations were flagged off with the welcome address to all the retired and current staff who were in their beloved institute. The special guest on the occasion was Prof. NK Ganguly. The Alumni Association was well represented by many retired colleagues who visited the institute on the Foundation Day. The celebrations continued throughout the day and ended with a colourful cultural programme in the evening.

The Dr. S. C. Pal Memorial Oration 2010 was delivered by Prof. Nirmal Kumar Ganguly, Former Director General (ICMR), Distinguished Biotechnology Fellow & Advisor, Translational Health Sciences & Technology Institute, National Institute of Immunology. The oration *Are Dirrhoeal Diseases Still a Problem?* was delivered on February 18, 2010 in NICED amongst past and present staff who had gathered to celebrate the Foundation Day of the Institute. Prof. NK Ganguly was felicitated in profound gratitude after his oration for his immense attachment to the activities of the institute, over the decades.

The staff members who completed 25 Years of service in NICED were felicitated in appreciation of their dedication and devotion on the 'Silver Jubilee' with a personalised memento to honour their good services.

Felicitation of Dr. Kalyan K. Banerjee

Felicitation of Sri Anath Jana

Felicitation of Sri Arun Sarkar

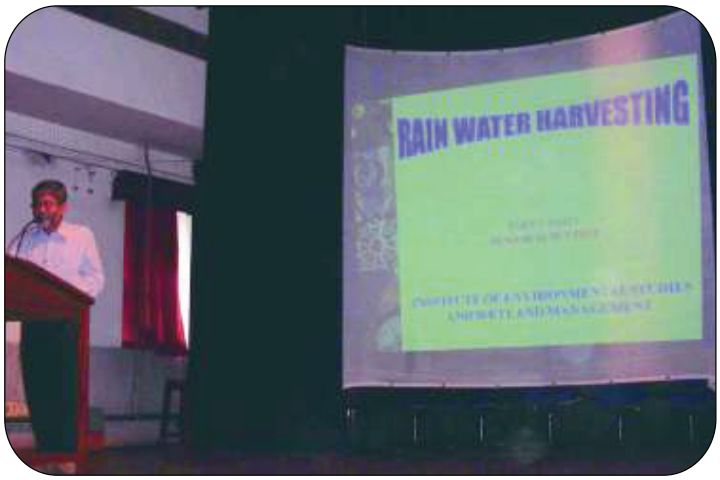
Felicitation of Sri Kanu Dey

Felicitation of Sri M Ali Khan

Felicitation of Sri Swapan K. Shaw

The recipient of the NICED Award 2010 was Dr Dilip Mahalanabis for his selfless association with the scientists of the institute for many decades. The award was presented to him on Feb 18, 2010 to show our gratitude for his outstanding contribution for the development and use of Oral Rehydrating Solution (ORS), for treatment of diarrhoea, that helps save many lives by preventing fatal dehydration.

The Animal Ethics Committee Meeting was held on Feb 19, 2010 in NICED, to evaluate the research projects where animals are used in different experimental procedures and to seek the advice of experts for conducting research with good animal ethics.



On the occasion of Science Day, Dr. Tapan Saha, Senior Scientist, Institute of Environmental Studies and Wetland Management, Kolkata delivered the lecture ***Rainwater Harvesting - a solution for combating the water crisis***, in NICED, Kolkata on March 2, 2010.

International Symposium on "Fifty Years of Discovery of Cholera Toxin: A Tribute to SN De"

Fifty years have passed by since Dr. Sambhu Nath De (1915-1985) discovered the cholera toxin produced by *Vibrio cholerae,* the causative agent of the severe diarrhoeal disease Cholera, in Kolkata in May 1959. The rehydration therapy for replenishing the massive fluid loss in cholera patients which has saved innumerable lives to date should be considered as a direct outcome of Dr. De's discovery of the cholera toxin. His findings also set the stage for the modern views of diseases caused by toxin producing bacteria and helped in the development of a series of cholera and related vaccines. Dr. De's impact on the international scene was undoubtedly great. Dr. De was largely unrecognized in India despite the seminal nature of his work.

Three premier Institutes in Kolkata - National Institute of Cholera and Enteric Diseases (ICMR), Indian Institute of Chemical Biology (CSIR) and Bose Institute (DST) - jointly organized an International Symposium from October 25- 27, 2009 to celebrate this historic event. The title of the symposium was "Fifty Years of Discovery of Cholera Toxin: A Tribute to SN De". Several internationally renowned scientist from all over the world delivered lectures related to *Vibrio cholerae* and other enteric infections. The main areas covered during the symposium were epidemiology of cholera, management of cholera, cholera/diarrhoeal vaccines, pathogenicity of *V cholerae,* molecular epidemiology of *V cholerae,* ecology of *V cholerae, Escherichia coli* and related diarrhoeas.

This symposium was inaugurated at Bose Institute, Kolkata where Dr. De did part of his research. A bust of Dr. De was unveiled by Prof. M. Vijayan, President of Indian National Science Academy, New Delhi and Distinguished Biotechnology Research Professor, Molecular Biophysics Unit, Indian Institute of Science, Bangalore. About 15 talks were delivered by eminent scientists and 40 posters were displayed during the symposium. By the end of this year, the Indian Journal of Medical Research is bringing out a special issue to honour Dr. De's contribution to the science.

Abhisek Ghosal received his PhD degree on December 24, 2009 at the annual convocation of Jadavpur University

K Rajendran, Saswati Sinha and Mukti Kant Nayak received their PhD degree on December 24, 2009 at the annual convocation of Jadavpur University

Souvik Chatterjee received his PhD degree on December 24, 2009 at the annual convocation of Jadavpur University

ALUMNI ASSOCIATION OF NICED

Dr. Archana Dutta addressing the gathering

The National Institute of Cholera & Enteric Diseases (NICED) began its journey in the year 1962. An Alumni Association of NICED was formed and celebrated on the Foundation Day of the Institute, for the first time on February 18, 2008 at the initiative of the present Director, Dr. G. B. Nair. This year too, the foundation day was celebrated on February 18. The Director, Dr. G. B. Nair, reiterated the growth and development of NICED. Dr. Nair felicitated all the alumni on this day for their contribution to the growth and achievements of NICED. The research scholars, scientists and staff members actively participated in the cultural function and enjoyed this gala ceremony that continued throughout the entire day.

Dr. Uma Ganguly addressing the gathering

EXTRAMURAL PROJECTS

EXTRAMURAL PROJECTS

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, AK Mukhopadhyay, H. Koley, S.S. Das, R.K. Nandy, S. Basu
Funding Agency	:	Okayama University, Japan.
Title	:	A randomized controlled trial of the bivalent killed whole cell oral cholera vaccine in Eastern Kolkata, West Bengal, India.
Investigators	:	**G. B. Nair** D. Sur, B. Manna, S. K. Niyogi, B. L. Sarkar, S. Kanungo
Funding Agency	:	Bill and Melinda Gates Foundation, USA
Title	:	Possible role of host genetics in relation to infection, progression and pathogenesis of HIV/AIDS
Investigator	:	**Sekhar Chakrabarti**
Funding Agency	:	DBT-ICMR, Govt. of India
Title	:	Surveillance for dengue fever in eastern Kolkata, West Bengal, India
Investigators	:	**Sekhar Chakrabarti, Dipika Sur** S. Kanungo, B. Manna, Shanta Dutta, Shyamalendu Chatterjee, Provash Sadhukhan
Funding Agency	:	Paediatric Dengue Vaccine Initiative, Seoul, Korea & Bill and Melinda Gates Foundation, USA
Title	:	Study on HIV comorbitdities, ART resistant and behavior factors for HIV transmission in Manipur.
Investigator	:	**Sekhar Chakrabarti**
Funding Agency	:	DBT, Govt. of India
Title	:	Implementation of a novel genotyping assay to understand complex HIV 1 epidemic in North-East and Eastern part of India
Investigator	:	**Sekhar Chakrabarti**
Funding Agency	:	DBT, Govt. of India
Title	:	Global Enteric Multicentric Study (GEMS)-Microbiology
Investigator	:	**T. Ramamurthy**
Funding Agency	:	Bill and Melinda Gates Foundation, USA
Title	:	Study on Impact assessment
Investigator	:	**Samiran Panda**
Funding Agency	:	NACO, Govt. of India
Title	:	Diarrheal Disease in Infants and Young Children in Developing Countries
Investigator	:	**Dipika Sur** T. Ramamurthy, B. Manna & S. Kanungo
Funding Agency	:	Bill and Melinda Gates Foundation, USA
Title	:	Regulation of Mucosal Immune Response by Synthetic Peptides of Porin: A Candidate Adjuvant.
Investigator	:	**T. Biswas**
Funding Agency	:	DBT, Govt. of India
Title	:	Presentation of Shigella Porin by dendritic cell to $CD3^+$ $CD4^+$ cell for the T helper activation: Differentiation and generation of the adjuvant specific memory cells
Investigator	:	**T. Biswas**
Funding Agency	:	DST, Govt. of India

Title	:	Evaluation of anti dirrhoeal activity of three ethonomedical plants
Investigator	:	**Shanta Dutta**
Funding Agency	:	ICMR, Govt. of India
Title	:	Scientific validation of anti typhoid claim
Investigator	:	**Shanta Dutta**
Funding Agency	:	National Innovative Foundation, DST, Govt. of India
Title	:	Impact of climate change on diarrhoeal diseases in India-Phase- I Study
Investigator	:	**Anup Palit**
Funding Agency	:	WHO-NICED-TERI
Title	:	Pattern of assistance for Apex, NRLs and SRLs and the external quality assessments skills (EQAS) of NACO
Investigator	:	**M. K. Saha**
Funding Agency	:	NACO, Govt. of India
Title	:	Early Infant Diagnosis (EID)
Investigator	:	**M. K. Saha**
Funding Agency	:	NACO, Govt. of India
Title	:	HIV Sentinel surveillance
Investigator	:	**M. K. Saha**
Funding Agency	:	NACO, Govt. of India
Title	:	Establishment of hospital based rotavirus surveillance for disease and strains and Noroviral infections in pediatric acute gastroenteritis and asymptomatic children
Investigator	:	**T. Krishnan**
Funding Agency	:	ICMR-CDC, USA
Title	:	Molecular mechanism of enterotoxigenic *Escherichia coli* adherence in the intestine: Host-pathogen relationship
Investigators	:	**Nabendu Sekhar Chatterjee** T. Ramamurthy
Funding Agency	:	Department of Atomic Energy, Govt. of India
Title	:	Comparative analysis of luxO, the quorum sensing master regulator, among O1, O139 and non-O1, non-O139 *V. cholerae* strains
Investigator	:	**R. K. Nandy**
Funding Agency	:	DBT, Govt. of India
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 travellers
Investigator	:	**R. K. Nandy**
Funding Agency	:	University of Texas, USA
Title	:	IVI- Infant Study
Investigator	:	**Alok Kumar Deb**
Funding Agency	:	International Vaccine Institute, Seoul, Korea
Title	:	Novel strategies to combat cholera
Investigators	:	**R. K. Nandy** Dr. Werner Tegge
Funding Agency	:	ICMR, Govt. of India

Title : IVI- NICED Immunomonitoring Laboaratory
Investigator : **R.K. Nandy**
Funding Agency : International Vaccine Institute, Seoul, Korea

Title : Elucidation and analysis of biological function(s) of *Helicobacter pylori* Restriction-Modification systems
Investigator : **A. K. Mukhopadhyay**
Funding Agency : DBT, Govt. of India

Title : Biomedical Informatics Center of ICMR.
Investigators : **Santasabuj Das**
Sandipan Ganguly
Funding Agency : ICMR, Govt. of India

Title : Identification and distribution of HIV-1 encoded microRNAs in North-East Indian population
Investigator : **Santasabuj Das**
Funding Agency : ICMR, Govt. of India

Title : A study on differentiation-induced regulation of the immune response related genes in the intestinal epithelial cells.
Investigator : **Santasabuj Das**
Funding Agency : ICMR, Govt. of India

Title : Enhanced surveillance of severe respiratory infection in Sadar Hospital of Malda, Murshidabad and Birbhum district
Investigator : **M. Chawla-Sarkar**
Funding Agency : DHS, Govt. of West Bengal.

Title : Enhanced surveillance for pH1N1- Multi site study (Project IU511P 00033301)
Investigator : **M. Chawla-Sarkar**
Funding Agency : ICMR-CDC, USA

Title : Gastric aspirate: an indicator of neonatal sepsis
Investigators : **S. Basu**
A.K. Singh, T. Ramamurthy
Funding Agency : DST, Govt. of West Bengal.

Title : To study the presence of common enteric parasites found during regular hand washing
Investigator : **Sandipan Ganguly**
Funding Agency : The Research Foundation of City University of New York, USA

Title : *Entamoeba* STR.
Investigator : **Sandipan Ganguly**
Funding Agency : JHSF, Japan

PUBLICATIONS

LIST OF PUBLICATIONS 2009-10

1. Agrawal AS, M Sarkar, S Chakrabarti, K Rajendran, H Kaur, AC Mishra, MK Chatterjee, TN Naik, MS Chadha and **M Chawla-Sarkar**. 2009. Comparative evaluation of real-time PCR and conventional RT-PCR during a 2 year surveillance for influenza and respiratory syncytial virus among children with acute respiratory infections in Kolkata, India, reveals a distinct seasonality of infection. J Med Microbiol. 58:1616-1622.

2. Agrawal AS, M Sarkar, S Ghosh, M Chawla-Sarkar, N Chakraborty, M Basak and **TN Naik**. 2009. Prevalence of respiratory syncytial virus group B genotype BA-IV strains among children with acute respiratory tract infection in Kolkata, Eastern India. J. Clin. Virol. 45(4):358-61.

3. Alam M, WB Chowdhury, NA Bhuiyan, A Islam, NA Hasan, GB Nair, H Watanabe, AK Siddique, A Huq, RB Sack, MZ Akhter, CJ Grim, KM Kam, CK Luey, HP Endtz, A Cravioto and **RR Colwell**. 2009. Serogroup, virulence, and genetic traits of *Vibrio parahaemolyticus* in the estuarine ecosystem of Bangladesh. Appl Environ Microbiol. 75(19):6268-74.

4. **Basak S**, P Mukhopadhyay, SK Gupta and TC Ghosh. 2010. Genomic adaptation of prokaryotic organisms at high temperature. Bioinformation 4(8): 352-356.

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11. Bhowmick TS, H Koley, M Das, DR Saha and **BL Sarkar**. 2009. Pathogenic potential of vibriophages against an experimental infection with *Vibrio cholerae* O1 in the RITARD model. Int J Antimicrob Agents. 33(6): 569-73.

12. Bhowmick TS, M Das and **B L Sarkar**. 2010. Evaluation of *VcA* VNTR as a strain-typing and phylogeny study method of *Vibrio cholerae* strains. Epidemiol Infect 5:1-13.

13. Bhowmick TS, M Das, W Ruppitsch, A Stoeger, AT Pietzka, F Allerberger, DP Rodrigues and **BL Sarkar**. 2009. Detection of virulence-associated and regulatory protein genes in association with phage typing of human *Vibrio cholerae* from several geographical regions of the world. J Med Microbiol. 58(Pt 9):1160-7.

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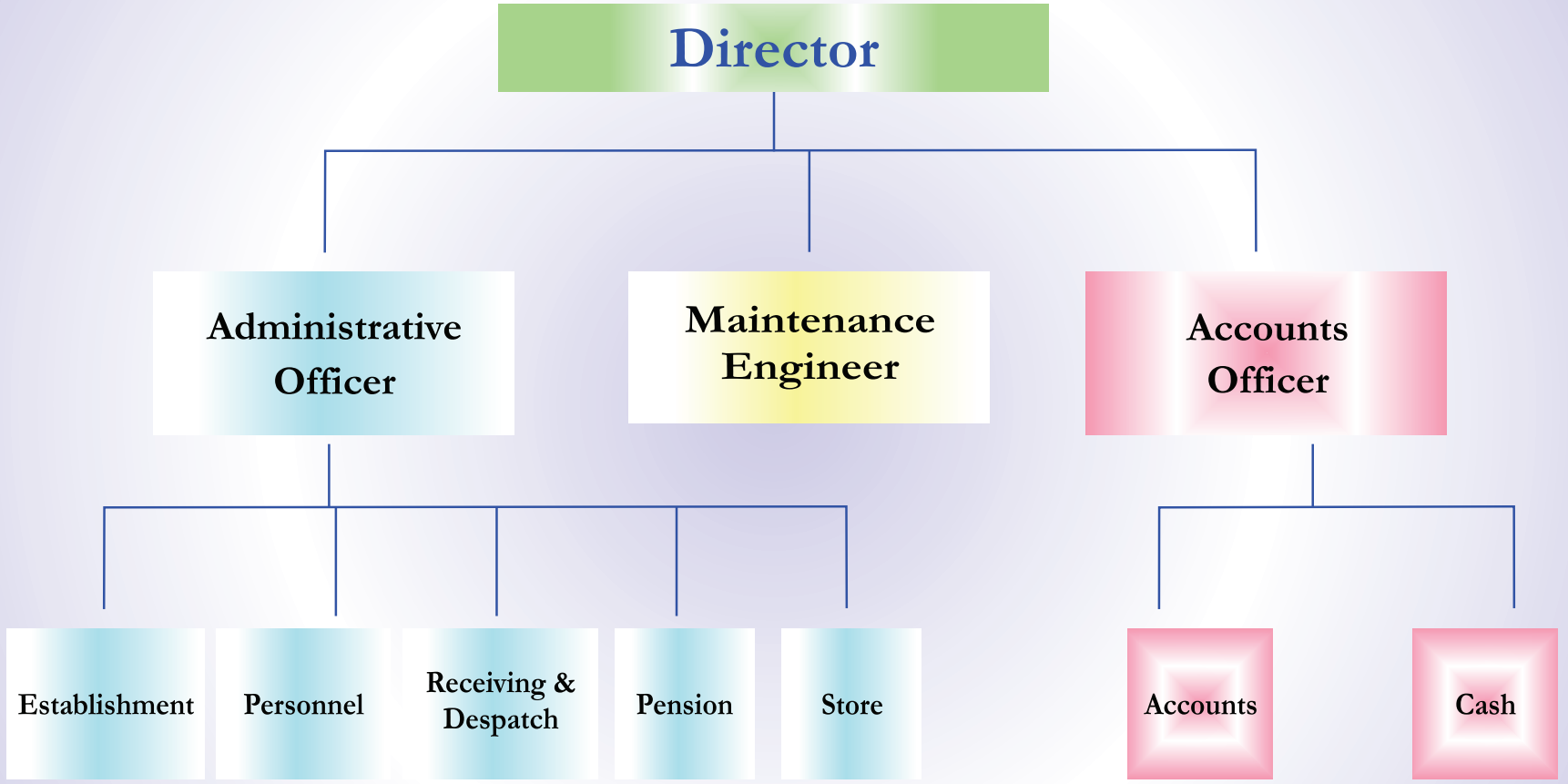

ADMINISTRATION

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

S. Karmakar
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The Administration is involved in the following tasks:

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- Maintaining management information systems (manual or computerised)
- Reviewing and answering correspondence
- Providing secretarial or executive services for committees.

T. K. Chanda
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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 over the years. The Institute is receiving financial assistance from different Government, Non-Government and International Agencies, like, IVI, WHO, UNICEF, DST, DBT, CSIR, CDC etc. in the form of extramural projects such as the Okayama project. There are more than 51 such extramural projects. Two new buildings have also been constructed in the I.D. & B.G. Hospital campus under the Institute to accommodate its expanding activities. 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.

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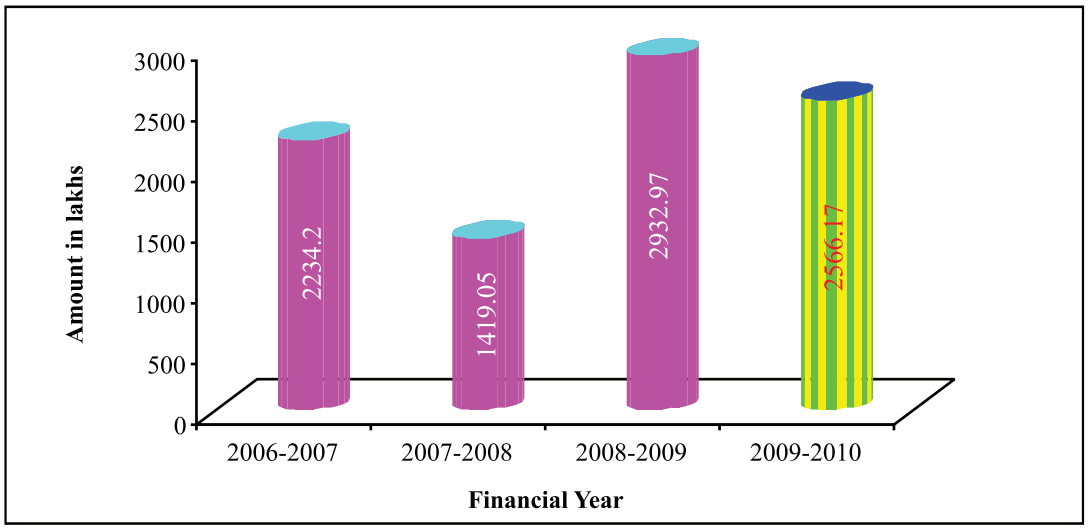

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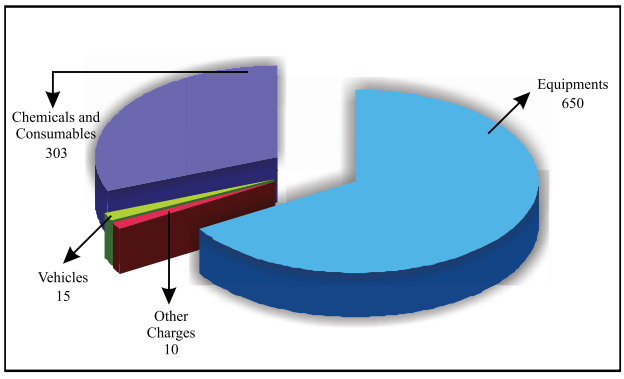

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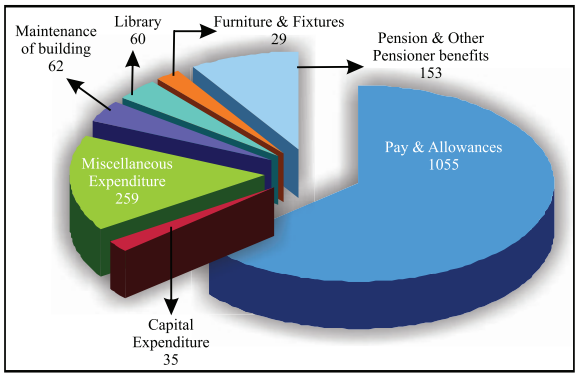

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Dr. Dilip Mahalanabis, a pediatrician and clinician scientist par excellance is the Director of Society for Applied Studies, Kolkata. His path-breaking works on ORT earned him worldwide acclaim. He is the recipient of many prestigious awards for his 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 Crippled 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.

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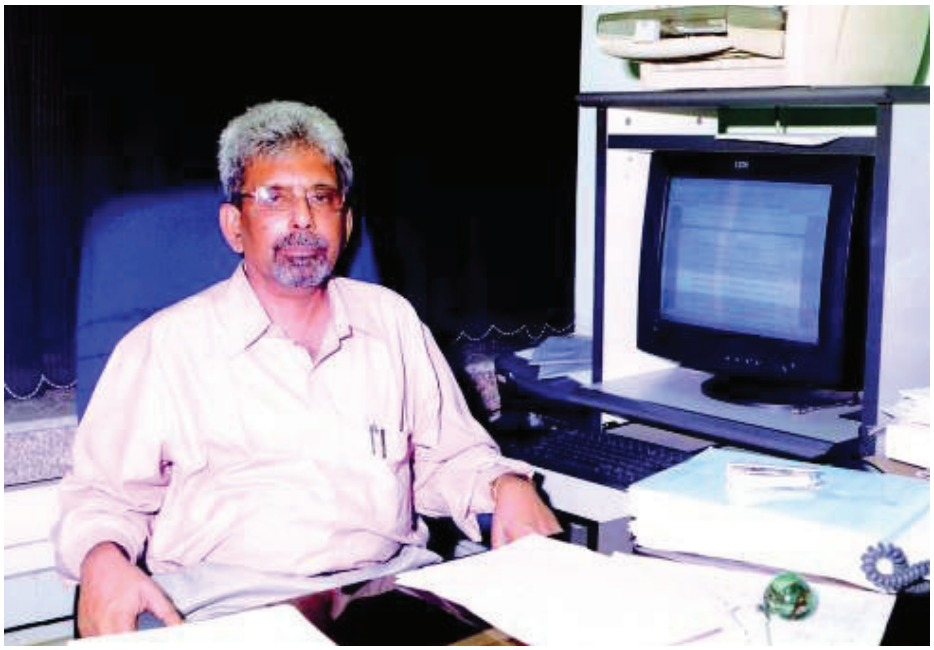

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