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PREFACE

Like that of previous years, National Institute of Cholera and Enteric Diseases continued to pursue its research goal enthusiastically on different aspects of diarrhoeal diseases, organising workshops, training programmes for manpower development, assisted the various State Governments in conducting investigations on diarrhoeal diseases including cholera outbreaks, suggested control measures and provided referral services to several laboratories in different parts of the country. To expand the research activities, the foundation stone was laid down for a new building at the I.D. Hospital campus by Prof. N.K. Ganguly, Director General, Indian Council of Medical Research (ICMR), New Delhi in presence of eminent personnel of the city, created a land mark in the history of the Institute.

Molecular epidemiology of *Vibrio cholerae* revealed clonal diversity among *V.cholerae* O139 strains and emergence of new epidemic clones as evidenced by change in the structure, organisation and localisation of the CTX prophages over a period of seven years. Emergence of *V.cholerae* non O1, non O139 serogroups for causation of cholera like diarrhoea in this part of the country creates a lot of interest to the professionals. Severe inflammatory cell infiltration was observed in the rabbit small intestine by induction of *V.cholerae* O139 strain as compared to non O1, non O139 strain. New virulent clones of *Vibrio parahaemolyticus* comprising of O3:K6; O4:K68 and O1:KUT serovers have also been detected which have potential for causing pandemics. For detection of shigellosis in children, PCR method was found to be highly sensitive as compared to standard culture methods or DNA hybridization method using oligonucleotide probe. Detection of shiga toxin producing *Escherichia coli*, particularly O157:H7 serotype from dairy cattle and beef samples marketed in Calcutta is alarming as it can cause food-borne infection. Indepth molecular characterisation of human Group B Rotavirus (CAL strain) was carried out following the detection of this rotavirus in this region. Various gene segments of CAL strain was amplified by RT-PCR and sequenced and compared with ADRV (Chinese strain) which revealed that CAL strain is the progenitor of ADRV.

The collaborative project entitled "Prevention of Emerging Diarrhoeal Diseases" with Japanese International Cooperative Agency (JICA) is progressing satisfactorily. Research activities of this Institute earned an important international affiliation with the JICA collaborating programme. This collaboration has lent invaluable support on research of molecular biology for bacterial, viral and parasitic enteric pathogens. Scientists and technicians of this Institute are trained in advanced Japanese laboratories. support from CSIR, DBT, WHO, UNICEF and other national and international funding agencies is also acknowledged.

The guidance, support and cooperation received from the office of the Director General, ICMR and members of the Scientific Advisory Committee are gratefully acknowledged. The dedicated and sincere efforts of our scientists, technical and administrative staff and research fellows in enhancing the activities of this Institute deserve my sincere and heartfelt appreciation.

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Dr. S.K. Bhattacharya
Director

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1. Hospital Surveillance Study

1.1. Hospital based surveillance system for diarrhoeal diseases

Principal investigator : S.K. Bhattacharya
Co-ordinator : S.K.Niyogi

Investigators:

D. Dutta, M.K. Bhattacharya, G.B. Nair, P. Das, S. Dutta, P.G. Sengupta, T.N. Naik, D.R. Saha, K. Rajendran and B. Manna

The present study was aimed for systemic sampling of every 5th patient on 2 randomly selected days per week, irrespective of age, sex, economic status, types of acute diarrhoea and degree of dehydration with or without other complaints attending the casualty of Infectious Diseases Hospital, Calcutta.

During the period between April 1, 1999 and March 31, 2000, a total of 1462 diarrhoea patients were enrolled in this surveillance system. Of the 1462 patients, 25.2% were upto 5 years of age. Majority of cases belonged to urban population (87.5%) and were Hindus. The ratio of male and female patients was 5:4.

Types of diarrhoea observed among the patients surveyed is shown in Fig 1. Out of these 1462 hospitalised diarrhoea patients, 95% had watery diarrhoea, 3.0% had bloody diarrhoea and 2.0% had mucoid diarrhoea (Fig.1). Vomiting was predominant feature in 62.1% cases. Prior to hospital admission 46.7% patients received medicines, 44.4% received ORS (among which 64.8% consumed WHO ORS, 27.6% received home available fluid (HAF) and 8.6% received intravenous fluids).

On admission, dehydration was present in 91% cases of which 52% cases had severe dehydration. Patients received tetracycline, furazolidone and norfloxacin in 61.2%, 2.6%, and 24.9% cases respectively. Duration of hospital stay was less than 2 days in majority (96.1%) of cases. Death occurred in 0.3% cases.

So far 1320 faecal specimens were examined. *V.cholerae* was isolated from 17.8% faecal samples, of which 9.5% were *V.cholerae* O1, 5.1% were *V.cholerae* O139 and 3.2% were *V.cholerae* non-O1 non-0139. Shigella spp. and Salmonella each were isolated from 0.5% faecal samples. Rotavirus was detected from 6.4% samples.

Vibrio cholerae O1 strains were uniformly (100%) resistant to furazolidone, nalidixic acid and chloramphenicol but *Vibrio cholerae* O139 strains were uniformly (100%) resistant to ampicillin and furazolidone. The drug resistance pattern of *Vibrio cholerae* non O1 non O139 strains was as follows : ampicillin (100%), cotrimoxazole (53%), nalidixic acid (78%) and tetracycline (56%). *Vibrio parahaemolyticus* strains were uniformly (100%) resistant to ampicillin, furazolidone, neomycin and streptomycin. All the *shigella* strains were uniformly resistant to ampicillin, co-trimoxazole and tetracycline, intermediate sensitive to furazolidone, gentamycin and sensitive to nalidixic acid, norfloxacin and ciprofloxacin.

Highlights of epidemiological, clinical and microbiological features of 1462 patients admitted to the hospital during April, 1999 and March, 2000

Male and female ratio	-	5:4
Age group ≤5 yrs	-	25%
Caste : Hindu	-	(74%)
Locality from where the patients came	-	Urban

Highlights of clinical features :

Character of stool

Watery	-	95%
Bloody	-	2.5%
Mucoid	-	1.3%
Vomiting	-	62.1%
Received drugs before hospitalisation	-	46.7%
case fatality	-	0.3%
Hospital stay (≤2 days)	-	96.1%

Highlights of Microbiological Reports :

V.cholerae O1	9.5%
V.cholerae O139	5.1%
V.cholerae non-O1 non-O139	3.2%
V.parahaemolyticus	2.5%
Shigella spp.	0.5%
Non typhoidal *Salmonella* spp.	0.5%
Rotavirus	6.4%

2. Community Studies

2.1. Impact of zinc supplementation in reducing diarrhoeal morbidity amongst under five children : a community based intervention study

Investigators:

D.N. Gupta; P.G. Sengupta; S.K. Mondal; S. Ghosh; K. Sarkar; B. Manna; D. Sur; A. Pandey; K. Rajendran

Zinc, one of the trace elements though required in small amounts, is responsible for many important functions of the body in relation to metabolism, enzyme regulation, growth and development and immunity. Zinc deficiency is usually associated with protein energy malnutrition, an important public health problem in children. Malnutrition and infection are interrelated and in India about 40-50 percent of the childhood deaths are due to malnutrition. It has been suggested that deficiency of some micronutrient causes diarrhoea and there may be a

cause and effect relationship of diarrhoea with zinc deficiency. Beneficial effect following zinc supplementation has been observed in different studies. There is also evidence to suggest that future attacks of diarrhoea were less following zinc supplementation. Even if it is used in persistent diarrhoea, duration of diarrhoea become less. Till today diarrhea is an important cause of morbidity and mortality in developing countries. This study is therefore, proposed to determine the impact of supplementation of zinc on diarrhoeal morbidity and nutritional status in a cohort of rural children. The study also proposes to compare the effect of a daily and weekly supplementation of zinc on health status of the children.

Four villages having around 7000 population in the existing rural field area have been selected. Children aged between 6-41 months were enlisted. Baseline information of families having the enlisted children were collected (Table-1). Baseline nutritional status of the study children have been determined by anthropometric measurement of length and weight to the precision of 0.1 cm. and 50 gm respectively. Mid upper arm circumference (MUAC) was also measured. This will be repeated every six months for one year. The eligible study children were randomly allocated by serial number into three groups. Supplementation of zinc to the study children started from November 1999. Both zinc and placebo are being provided in 5 ml syrup base. One group is receiving 10 mg elemental zinc as zinc sulfate in 5 ml syrup base daily from Monday to Friday. The second group is receiving only placebo as 5 ml syrup daily from Monday to Friday. The third group is provided 50 mg elemental zinc as zinc sulfate in 5 ml syrup base only on one day and placebo for remaining four days in a week. A total of 83 diarrhoeal episodes have been detected so far through surveillance with one week recall by the resident surveillance workers. Data will be analyzed after decoding the groups.

Table 1 **Salient features of baseline information of families having a study child(6-41 months)**

No of children 6-41 months. initially enlisted	319	
No of families	289	
Total population	1206	
Average person/family	4.17	
Housing condition		
a. pucca	14.2%	
b. kuccha	48.8%	
c. mixed	37.0%	
Defaecation habits		
a. in sanitary latrine	86.2%	
b. in open places	13.8%	
Income of the family/month		
a. upto Rs.2000	78.5%	
b. Rs.2001-5000	19.4%	
c. above Rs.5000	2.1%	
Occupation of Head		
a. daily labourer	57.4%	
b. others	42.6%	
Literacy	Father	Mother
Illiterate	28.8%	48.1%
School level	70.6%	49.8%
H.S.and above	6.6%	2.1%

2.2 Impact of zinc supplementation on incidence of diarrhoea and growth pattern among low birth weight infants of an urban slum

Investigators :

Dipika Sur, P.G. Sengupta, D.N. Gupta, S.K. Mondal, S. Ghosh, B. Manna, A. Pandey, D. Ganguly

In India `Low Birth Weight' is a major public health problem. About 30% of all live births in this country are low birth weight and they become the victims of protein energy malnutrition and infection. Diarrhoea is an important cause of childhood mortality and morbidity in children who are already nutritionally deficient.

Zinc is an essential trace element required for optimal growth and development. Low birth weight infants have higher postnatal requirements for this nutrient and unless replenished, the newborns remain at increased risk of developing zinc deficiency. Further, beneficial effects of zinc supplementation have been noted in the form of reduction of diarrhoeal episodes in children who were zinc deficient.

This study was therefore proposed to estimate the impact of zinc supplementation on growth and diarrhoeal incidence in a cohort of Low Birth Weight infants followed up for one year.

It is a double blind randomized community based intervention study, carried out on a birth cohort of 100 Low Birth Weight babies in the Tiljala slum area of Calcutta. As soon as a low birth weight baby is being identified in the community, informed consent is being taken from the mother and the child is included in the study. Presently 60 babies have been enlisted immediately after birth and randomly allocated into two groups each group receiving either zinc or placebo. Mothers of the study children have been advised to administer the necessary daily dosage to her child for a total span of one year under weekly supervision of female resident workers. These resident workers have been trained to carry out surveillance for diarrhoea and also manage such cases with ORS at the home level. An epidemiological team visits the area regularly for supervision, management of diarrhoea cases and anthropometric measurements of the study children. Growth of these children are measured by length and weight for age which is recorded once every month.

The project was started in October 1999. So far 28 diarrhoeal episodes have been detected. Final analysis will be done after decoding. Some of the demographic features of families of the study children are given in the Table-2.

Table-2 **Demographic features of families of the study children**

Total no. of children enlisted		60
Mean birth weight (in gms)		2250
Literacy		
Father	Illiterate	40.0%
	School level	58.3%
	HS & above	1.7%
Mother	Illiterate	36.7%
	School level	63.3%
	HS & above	Nil
Occupation of head of family		
	Labourer	65.0%
	Others	35.0%
Income of family per month		
	Upto Rs.1,000.00	23.3%
	Rs.1,001.00 to Rs.2,000.00	63.3%
	Rs.2,000.00 to Rs.5,000.00	13.3%
	> Rs.5,000.00	Nil
Drinking water supply		
	Tap	36.7%
	Tubewell	63.3%
	Others	0%
Latrine		
	Sanitary	98.3%
	Others	1.7%
Structure of house		
	Kuchha	3.3%
	Pucca	26.7%
	Kuchhapucca	70.0%

2.3. A rural community based longitudinal study on diarrhoeagenic *Escherichia coli* amongst children below 5 years

Investigators :

S. Ghosh, T. Ramamurthy, A. Pal, S.K. Mondal, D.N. Gupta, D.R. Saha, S. Dutta, D. Sur, T.N. Naik, B. Manna, A. Pandey, K. Rajendra and P.G. Sengupta

E.coli is an important aetiological agent of childhood diarrhoea in the developing countries including India. Recently several groups of *E.coli* have been recognized as the causative agent of diarrhoea which include enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EaggEC), enteroinvasive *E.coli* (EIEC) and enterohaemorrhagic *E.coli* (EHEC). The organisms are associated with childhood as well as adult diarrhoea. They cause any of the types of diarrhoea i.e. acute watery diarrhoea or dysentery or persistent diarrhoea. Role of different categories of *E.coli* as the etiological agent of diarrhoea has been evaluated by a number of hospital and community based studies. There is still a need to study the epidemiology of this organism to determine the magnitude of problem caused by each of these groups in different geographical settings and to identify the prevalent serotypes and the antimicrobial susceptibility pattern of these strains.

Therefore, this systematic study has been initiated to determine the clinico-epidemiological aspects of diarrhoea caused by commonly prevalent *E.coli* groups in a rural community of West Bengal and to find out the antibiotic resistant pattern of these isolated strains.

This longitudinal observational study is being carried out on two cohorts, a birth cohort and a cross sectional cohort. The study area is located around 10 Km from the Institute. A total of 405 children between 0 to 47 months of age have been identified as a cross-sectional cohort in four villages from 1546 families having a population of 6827 approx. for longitudinal follow up. In addition 91 new borns were added to this cross-sectional cohort. The children are being visited once a week for detection of diarrhoeal episodes with a seven days recall. Surveillance is carried out by trained female resident surveillance workers who are provided with an updated computerized list of children every month for house to house visit. Once a diarrhoea case is detected details of the case is recorded by experienced field workers on a predetermined schedule where data regarding onset, type of stool, clinical characteristics, duration, management of the case and outcome are recorded. Every case is examined by a physician and appropriate treatment is provided.

A birth cohort of 53 children out of 91 new born recruited so far, could be observed through weekly surveillance to study the changes in feeding practices and occurrence of diarrhoea. A total of 54 diarrhoeal episodes were noted in different feeding modes. Five out of 54 episodes occurred amongst exclusive breast fed babies who were subjected to 148 child week of observation (incidence of 1.7 episode/child/year). The remaining 49 episodes occurred among children in other feeding modes with 360 child weeks of observation (incidence of 6.9 episode/child/year). Weight, length and mid arm circumference (MAC) were being monitored every 6 months for both cross-sectional and birth cohorts. Fresh faecal specimens collected from 48 diarrhoea cases were processed in the laboratory for isolation and identification of recognized enteropathogens using standard techniques. Diarrhoeagenic *E.coli* could be isolated from 15 (31.2%) faecal samples which were processed completely so far. DNA-colony

hybridization assay further classified 7 of these 15 samples positive for EPEC, 4 as ETEC and the remaining 4 as EaggEC.

2.4. Epidemiological study of typhoid fever in a rural community of West Bengal

Investigator:

K. Sarkar, P.G. Sengupta, D.N. Gupta, S.K. Mandal, M.R. Saha, S. Ghosh, D. Sur, B. Manna, K. Rajendran

This is a community based prospective study :

a. to identify the age specific incidence of typhoid fever and association with demographic variables like latrine, storage of water in the rural community. The study also aimed to find out the drug sensitivity pattern of the causative agent of typhoid fever.

The study is being carried out through active surveillance for typhoid fever by our specially trained resident volunteers in our existing field practice area. Typhoid fever is considered only when patient's blood culture shows positive for *Salmonella typhi*. Stool samples are being collected from family members of suspected typhoid fever cases.

A total of 48 fever cases of more than 3 days duration have been subjected to blood culture of which 3 were positive for *S.typhi*. Regarding antibiogram, one strain was sensitive to all drugs while two strains were resistant to chloramphenicol, co-trimoxazole and amoxycillin, but sensitive to ciprofloxacin. No stool sample was positive for *S.typhi* out of 84 tested till date. The study is in progress.

2.5. Impact of lactation counselling on exclusive breast feeding among rural mothers : An operational research

Investigators : S.K. Mondal, P.G. Sengupta, D.N. Gupta, S. Ghosh, K. Sarkar, D. Sur, B. Manna, A. Pandey, K. Rajendran

Consultants : Manab Roy (Social Scientist), Sanjoy Bhattacharya (Mass Communication)

It is well known that exclusive breast feeding for first 6 months of life protects infants from diarrhoea. Breast fed babies are less likely to suffer from severe episodes of diarrhoea and diarrhoea-related deaths compared to non-breast fed babies. There is very little information regarding the exact duration of exclusive breast feeding in this region. Studies conducted in rural West Bengal have showed that around 70% of infants are weaned before 6 months and are exposed to a higher risk of diarrhoea. Breast feeding has been promoted in hospitals but were completely lacking in follow up of mothers at the home level.

The present study has been initiated with the objective to determine the impact of lactation counselling on promotion of exclusive breast feeding for first 6 months of life in infants and introduction of safe weaning practices thereafter.

The study is an operational research conducted in collaboration with Govt. of West Bengal for promotion of breast feeding in the community. The study will be conducted in two phases. In the first phase, breast feeding practices of infants particularly for first 6 months will be followed. In the second phase, weaning practices by mothers will be observed. Data will be

analyzed to identify the areas for behavioural changes of mothers in relation to feeding practices of their young children. After identifying the areas of behavioural changes, necessary action will be taken to overcome the difficulties for breast feeding as well as for proper weaning.

A survey was conducted amongst mothers of Udaynarayanpur Block to know the feeding practices of their infants including socio-demographic characteristics of the families. For conducting the survey, a specially designed questionnaire was prepared in local language in consultation with sociologist and mass communication specialist. The questionnaire was pre-tested in the field. Locally residing volunteers from Peoples Relief Committee (a Non Governmental Organization) were selected and trained and the survey was conducted under supervision of the investigators. The data were entered subsequently in a PC after translating to English.

A total of 597 mothers, having a child aged within one year and residing in 59 villages of the block, were surveyed. Number of mothers having infants below 6 months were 327 and between 6 to 12 months were 270.

Average family size was 6.13 person/family and average income was Rupees 1829/-month. Electricity was available in 161 (27.0) families, while 143 (24.0) families had Television; Radio was found in 333 (55.8) families. Five hundred and four (84.4) families used tube-wells for drinking purpose while 572 (95.8) families utilised pond water for domestic uses. Sanitary latrine although available in 188 (31.0) families; only 2 (0.3) families used it for disposal of children's faeces, majority of them disposed it in surface water sources.

Mothers' practice of washing their hands, particularly after ablution of their babies was unhygienic, only 117 (19.6) used soap and water, rest used either mud/ash and water or only water.

Literacy status of mothers were recorded, 207 (34.7) of them were illiterate, 79 (13.2) read up to primary level and the rest 311 (52,1) were educated up to secondary level or more.

In majority of the families father of the child was the only earning member of the family and agriculture was the main occupation in 251 (43.0) families. Mothers 556 (93.1) were mostly housewives.

Out of 597 mothers surveyed, 327 had children below 6 months and the rest 270 had children within 6-12 months of age. Prevalence of breast feeding practices of infants below 6 months, at the time of survey are shown in Fig.2. Exclusive breast feeding - 143 (43.7), Predominant breast feeding - 32 (9.8). Complementary feeding - (44.0), and no breast feeding 8 (2.4). However, all the babies of 6-12 months of age were on complementary feeding except only 5 were predominantly breast fed.

3. Studies on *Vibrio cholerae* and Vibrio Phages

3.1. Molecular epidemiology of cholera in India

Investigators : A. Basu, P. Garg and G.B. Nair

Cholera is a severe and sometimes lethal diarrhoeal disease caused by the gram-negative bacterium classified as *Vibrio cholerae*. Cholera is endemic in India but sometimes it appears in epidemic form. At the National Institute of Cholera and Enteric Diseases, Calcutta, a continuous

surveillance on the cholera situation in and around Calcutta in particular, and in India in general, is being conducted. *V.cholerae* O139 serogroup dominated as the causative agent of cholera in Calcutta in 1992-93 and in 1996-97, while the O1 strains dominated during the remaining period as the major serogroup causing cholera. Apart from 1992, the year of its genesis, the isolation rate of the O139 strains did not fall below 3.4% from hospitalised acute diarrhoea patients of Infectious Diseases Hospital, Calcutta. Apart from the initial years of its appearance in 1993 and 1994, the highest number of O139 cholera cases were recorded during the later half of 1998. During September and October 1998, a perceptible increase in the incidence of cholera due to O139 serogroup was observed which led us to characterize these O139 strains in detail.

Dramatic shifts in susceptibility patterns of co-trimoxazole, neomycin and streptomycin were observed during the study period. During 1992-1994, O139 strains were resistant to co-trimoxazole while most of the strains isolated between 1997 and 1998 became susceptible. Increasing trend of resistance to neomycin and streptomycin was observed till 1996 after that period the trend declined Table-3. *V.cholerae* O139 strains isolated throughout the study period were resistant to furazolidone and by and large, to ampicillin. The dominant drug resistance pattern among O139 strains isolated in Calcutta from 1993 to 1998 were AFz (98.3%), AFzNS (96.0%), ACoFzS (49.1%), ACCoFzS (48.1%), AFzNS (32.0%), ACCoFzS (30.0%).

PCR assay of the O139 strains isolated during the seven years period (1993-2000) showed that all the strains have the organelle required for intestinal colonization (amplicon 471 bp with *tcpA* primer pair) and also CTX prophage (amplicon 301 bp with *ctxA* primer pair) with an exception in one strain.

The PFGE profile of the representative O139 strains isolated from Calcutta during 1996-97 and the reference strain MO45 (ATCC 51394) isolated in 1992 from Madras showed similar but not identical pattern. Restriction fragment length polymorphism of CTX prophage indicated that there is a continuous change in the structure and organization of CTX prophage during the study period along with emergence of a new type of CTX prophage. The 1992-93 strains (Fig. 3a) showed two CTX prophages connected by an RS1 element while the 1996 strains (Fig. 3b) showed three CTX prophages arranged in tandem. Most of the 1998 (Fig. 3c) strains from Calcutta exhibited only one CTX prophage while those isolated from other parts of India were identical to the 1996-97 strains or showed two CTX prophages arranged in tandem. In 1996, O139 strains exhibited two type of CTX prophages with the first of the three prophages being an ElTor-type CTX prophage and the second and third CTX prophages being a new type of CTX prophage, with difference primarily lying in *rstR* gene which codes for the repressor proteins of CTX. In 1998, it was observed that two new clones of O139 have evolved probably from the 1996-97 strains with two epicenters namely, Calcutta and Alleppey. Calcutta strains showed only the ElTor-type CTX prophage and not the unique O139 CTX prophage of the 1996 strains while the reverse was the case with the Alleppey strains. Therefore, at this point, there are two different clones of O139 circulating at two different locations with different CTX prophages indicating that reassortment in the genome is taking place in the O139 strains.

This molecular epidemiological study revealed clonal diversity among the O139 strains and emergence of new epidemic clones, as evidenced by the change in the structure, organization and location of the CTX prophages over a period of seven years.

3.2. Study the role of plasmids in adherence mechanism of *Vibrio cholerae* non-O1, non-O139

Investigators : A. Pal, P. Garg, T. Ramamurthy, G.B. Nair and M.K. Chakraborty

To study the role of plasmids in adherence mechanism of *Vibrio cholerae* non-O1, non-O139 was the objective of this study. In the previous studies conducted from February 1996, showed the increased isolation rate of *V.cholerae* non-O1 non-O139 from the hospitalized patients of Infectious Diseases Hospital, Calcutta. Investigations were conducted to address the virulence mechanism of these strains. These strains did not possess any of the known toxin genes as reported in *V.cholerae* O1 and O139 strains. Bacterial adherence and colonization are the key steps, which help enterotoxigenic pathogens to establish infection in the gut. Twenty strains of *V.cholerae* non-O1 non-O139, isolated as sole pathogen were studied for adherence property using HeLa cells and glass surfaces. Only 4 strains showed adherence with both HeLa cells and glass surfaces. It was also revealed that 9 strains contained plasmids. Ten strains showed clump formation in Muller Hilton broth. In this study no correlation between HeLa cell adherence, presence of plasmids and clump formation were observed. The study is in progress.

3.3. Isolation and characterization of the chicken erythrocytes receptor for N-acetyl-D-glucosamine specific hemagglutinin/adhesin

Investigators : D. Sasmal, B. Guhathakurta and A. Datta

N-acetyl-D-glycosamine specific hemagglutinin (HA)/lectin from a strain of *V.cholerae* O1 was purified which have have the capacity to attach the bacteria to intestinal epithelial cells and chicken erythrocytes membrane. The receptor(s) involved in these interactions have not been identified.

This study was undertaken to identify and characterise the receptor(s) in chicken erythrocytes membrane to further establish the lectin-like properties of the purified HA/adhesin.

The chicken erythrocytes were collected in presence of normal saline containing anticoagulant. Erythrocytes were washed thrice with normal saline and freeze dried. The dried cells were subjected to chloroform-methanol in different proportions in a Saxhlet apparatus. Extracts were pooled (CM) and solvents were removed in a rotary evaporator. Total CM extract then suspended in 0.1 M KOH in methanol water. The vessel was then flashed with nitrogen and left with magnetic stirring in the dark for 12 hours at room temperature. The alkaline hydrolysis was stopped by slow addition of 2N HCl. Chloroform water was then added to get chloroform-methanol-water phase. The lower phase was taken for evaporation.

Further work is in progress.

3.4. Studies on the structure function relationship of *Vibrio cholerae* hemolysin

Investigators : K.K. Banerjee and K. Chattopadhyay

Vibrio cholerae hemolysin (HlyA) an extracellular protein with a molecular weight of 65,000, causes lysis of erythrocytes at a concentration of 50 μM and is enterotoxic in a rabbit ligated ileal loop. In common with oligomeric pore-forming toxins of bacterial origin, the mechanism of membrane damage by the HlyA involves binding and insertion of the monomeric protein in lipid bilayer and oligomerization to an amphipathic transmembrane channel leading eventually to colloid osmotic lysis of the target cell. Since we characterized the hemolysin as a bifunctional protein with distinct lipid and sugar binding domains, several reports analyzing the contribution of cell surface carbohydrate receptor and the lipid bilayer matrix to pore formation

have been published. However, a self-consistent mechanism of membrane damage by *V.cholerae* HlyA is yet to evolve from these discrete studies. With this background, we have formulated the following questions :

i) It follows from elementary principles of thermodynamics that a protein (or any molecular species) is stable under a given set of conditions either as a monomer or as an oligomer but not as both. The HlyA is apparently stable as a monomer as well as an oligomer. The reason needs to be explored.

ii) The process of oligomeric pore formation by HlyA can be simulated in artificial lipid bilayer, but the process is about 100 times more efficient in biomembranes. This leaves the role of non-lipid constituents of membrane enigmatic.

iii) The HlyA is an extremely potent cytotoxin in *in vitro* assays and the HlyA is one of the most conserved genetic elements. Yet, there is general consensus that the protein does not contribute appreciably to the virulence of the organism.

The purified HlyA monomer was stable indefinitely in 50% ethylene glycol, however, it self-associated spontaneously in aqueous solution at a protein concentration as low as 5 μg/ml to an oligomer indistinguishable in stoichiometry and biochemical properties from the oligomer formed in lipid bilayer. The HlyA oligomer was about 3-fold less active than the monomer and formed non-stoichiometric aggregates with decreased hemolytic activity on keeping. The results suggested that the HlyA monomer was intrinsically unstable in water and the spontaneous transition to a stable oligomer was merely accelerated in the lipid bilayer plane due to increase in effective concentration and correct orientation. This was supported by the partitioning of the HlyA monomer to Triton X-114 phase, which suggested that the HlyA monomer had a surface hydrophobicity comparable to that of integral membrane proteins.

The domain responsible for oligomerization was found to be located in a 50 kDa carboxyl-terminal fragment of the HlyA monomer. The 50 kDa fragment was devoid of sugar-binding activity, considerably less hydrophobic than the HlyA monomer and was about 100-fold hemolytically less active. The 50 kDa fragment formed oligomers in concentrated aqueous solution, as well as mixed oligomers with the HlyA monomer. The results suggested that lipids or carbohydrates are not essential pre-requisites for induction of self-assembly of HlyA monomer. However, affinity of the HlyA for lipids or carbohydrates facilitates greatly the process by increasing the effective concentration of the protein on liposome or cell surface.

The spontaneous self-assembly of the HlyA monomer raises the question : How does the monomer survive in bacterial culture supernatant? Due to high surface hydrophobicity the protein formed low affinity, non-specific complexes with media components and this association prevented effectively the self-assembly of the oligomer. Removal of media components by chromatography or dissociation of the complex by dilution led to conversion of the monomer to the oligomer with a concomitant fall in specific activity. Since the membrane damaging activity of the HlyA depends critically on the survival of the monomer, it is as much a property of the HlyA polypeptide as of the composition of the surrounding medium. This might explain the lack of agreement between *in vitro* toxicity of the protein and its inability to serve as a virulence factor during disease.

3.5 Sequential studies of Vibrio cholerae O139 and some of its defined virulence phenotypes on intestinal pathology in rabbit ileal loop model

Investigators : D.R.Saha, A.N. Ghosh and G.B.Nair

The study was undertaken to determine the nature and location of lesion in the rabbit small intestine caused by *Vibrio cholerae* O139 and two other non O1 non 0139 strains at different time intervals using histologic and ultrastructural methodology. The strains were introduced in the rabbit at scheduled time interval after proper preparation of the animal. The rabbits were sacrificed at 2, 6, 10, 14 and 18 hours interval and tissues of small intestine were taken, fixed and processed separately for observation under light and electron microscope.

Pathological changes in the small intestine caused by all these strains were examined with appropriate controls. Inflammatory cell infiltrations was observed with all the three strains but it was in severe form in infection caused by *V.cholerae* 0139 strain which appeared to be more virulent (Fig.4). Further work with a new Non 01 Non 0139 strain is in progress.

3.6. Evaluation of new phage typing scheme for *Vibrio cholerae* O1 biotype ElTor strains

Investigators : B.L. Sarkar and A.K. Chakrabarti

In this reference laboratory, during the course of the study, a total of 617 strains of *V.cholerae* received from different parts of the country for serotyping, biotyping and phage typing. Of these, 357 (57.5%) representative strains of *V.cholerae* O1 biotype ElTor were included for phage typing study (Table-4). These strains were grouped under type 2 and 4 with conventional scheme of Basu and Mukerjee. A number of strains were found to be untypeable. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 (71.2%) followed by type 26 (13.8%), 15 (4.5%) and 3 (2.8%). It was found that type 27 is the predominant phage type circulating in our country.

3.7.Search for newer phages to develop a phage typing scheme for *Vibrio cholerae* O139

Investigators : B.L. Sarkar, A.K. Chakrabarti, A.N. Ghosh, S.K. Niyogi and G.B. Nair

Emergence of the new serogroup *V.cholerae* O139 formed the impetus to search for newer phages and to develop a phage typing scheme for *V.cholerae* O139. a total of 5 phages were detected and they differed from each other and also differed from the existing O1 phages in their lytic patterns, morphologies, restriction endonuclease digestion profiles and immunological criteria. A total of 500 *V.cholerae* O139 strains were evaluated for their phage types and almost all strains were typeable. Table-5 shows the *V.cholerae* O139 strains received from different parts of India. The strains clustered into 10 different phage types, of which type 1 (38.2%) was the dominant type followed by type 2 (22.4%) and type 3 (18%).

Additionally, a comparative study of phage types of *V.cholerae* O139 recovered during 1993 and 1994 and 1996 to 1998 showed higher percentage of phage type 1 (40.5%) followed by type 3 (18.8%) during the period between 1993 and 1994, whereas phage type 2 (32.1%) was the next major type during the period from 1996 to 1998 (Fig. 5). The proposed scheme comprising five phages would be useful in the study of the epidemiology of cholera caused by *V.cholerae* O139.

Table-5 Strains of *Vibrio cholerae* O139 received from different parts of the country in 1993-94 and 1996-98

City	Total number of strains	Year	
		1993-94	1996-98
Madras	77	77	-
Madurai	52	52	-
Calcutta	129	41	88
Nagpur	104	47	57
Pune	21	21	-
Vellore	19	19	-
Aurangabad	31	31	-
Ahmedabad	16	16	-
Sevagram	17	-	17
Ambajogai	12	-	12
Alleppy	12	-	12
Pondicherry	10	-	10
Total	**500**	**304**	**196**

3.8. Morphological and genomic characterization of vibriophages by electron microscopy

Investigators : A.N. Ghosh

The project was undertaken to characterize different vibriophages used in the phage-typing scheme of *V.cholerae* by electron microscopy.

Phage morphology was studied by negative staining technique in electron microscopy. The morphology of 15 different phages of *V.cholerae* biotype eltor was determined. The morphology of 13 phages viz. Group I-V of Basu and Mukerjee, N1-N5 and L1, S5, S20 was of similar type. All of them have isometric head and short non-contractile tail. The dimensions of head and tail of these phages were, however, different from one another. Vibriophages D10 (Fig. 6) and M4 are morphologically distinct from these 13 phages. Both D10 and M4 have isometric heads but the tails are very long and contractile. Also M4 has a much bigger head than D10.

The DNA of vibriophages was visualized by the basic protein monolayer technique of Kleinschmidt. The DNA of group I, group II, D10 (Fig. 7) and N4 phage was found to be linear and double-stranded while their length was 43, 38.5, 32 and 40.4 kb respectively.

Partial denaturation maps of the DNA of D10, N4 and group II phages were constructed using alkali and in presence of formaldehyde. It was inferred from the partial denaturation maps that DNA of D10, N4 and group II phages are nonpermuted. Also the presence of cohesive ends in D10 DNA and terminal redundancy in N4 DNA was proved. It was also shown with the help of partial denaturation mapping that during packaging of DNA inside D10 phage head the GC-rich end of the DNA is packaged last which implies that during infection the GC-rich end of the DNA enters first into the host bacterium.

Currently, DNA of vibriophage S20 is being studied. The DNA is linear, double-stranded and is about 50 kb long. Partial denaturation map of the DNA has been constructed using alkali and in presence of formaldehyde. It has been observed that the map is circularly

permuted. Also the permutation is not restricted to any particular segment of the DNA but it is random. Thus although phage S20 is morphologically similar to groupII and N4, the genome of S20 is distinctly different from the genome of groupII and N4 phage which have nonpermuted DNA.

3.9. Bacteriophage MB78 : Regulation of gene expression

Investigators : M. Chakravorty, R. Sharma, P. Datta, P. Mallik and A.N. Ghosh

Bacteria and bacteriophages have contributed much to the present day knowledge of regulation of gene expression. Bacteriophage MB78, one of the few virulent phages of *Salmonella typhimurium* isolated in our laboratory represents a wonderful system to study this interesting aspect of biology. The main aim of the work is to understand structure and function of the phage genome with special reference to the regulation of its gene expression.

A detailed physical map of the phage genome has already been constructed. More than seventy five restriction enzyme sites have been mapped on the 42 Kb phage genome which is circularly permuted and terminally redundant (Fig. 8). A number of restriction fragments have been cloned, sequenced and expressed. Using the promoter cloning vector, pKK232-8, a number of promoter regions recognized by σ 70 RNA polymerase of *E.coli* have been cloned. Some of those have been sequenced and analyzed in detail (Fig.8).

Characterization of a strong promoter that binds with 70 RNA polymerase as well as a host coded factor

Another strong promoter of bacteriophage MB78 was identified and partially characterized. Analysis of the nucleotide sequence upstream of the transnational start site revealed the presence of conserved -10 (TAATAT) and -35 (TTCTCCT) region. The transcriptional start site for the gene was determined by primer extension reaction and thymidine seems to be the transcription start point (Fig.9). Most *E.coli* promoters start transcription with a purine residue. However, there are a few promoters (say 5% of the *E.coli* promoters) for which 5' terminus of the transcript is a pyrimidine. This particular promoter of MB78 belongs to such rare case.

Mutation in a minor structural protein gene interferes with phage morphogenesis

Although a large number of genes (ORFS) of bacteriophage MB78 have been identified, their functions could not be determined in most of the cases. Analysis of a few temperature sensitive mutants of the phage (created by hydroxylamine) revealed that at non permissive temperature the cells infected by one such mutant named as ts23 produces tail less particles (Fig.10B). Normal infectous particles have long tails (Fig.10A). The position of the mutant gene on the phage genome was determined with the help of complementation test. A large number genomic fragments of the phage were already cloned. Host cells were transferred with pUC19 carrying a specific genomic fragment. Such transformed cells were infected with phage MB78 and incubated at nonpermissive temperature. In case the cloned genomic fragment possesses the normal (good) gene which is otherwise mutated in the temperature sensitive mutant, the phage development will be normal. After testing a number of genomic fragments the gene could be located in the SalIG fragment of the phage genome. After sequencing the wild type as well as the mutant gene the site of mutation could also been determined.

Over expression of the 5' upstream region of the major coat protein gene interferes with

phage morphogenesis

In an attempt to elucidate the function of different ORFs identified from analysis of base sequence/phage, development was followed in cells (hosts) carrying some specific genomic fragment of the phage. From a set of such experiments it was realized that cells carrying 793 bp region from the 3' end of the 2.3 kb EcoRIF fragment of the phage genome do not support phage development. Deletion of 138 bp from the 3' end of this 793 bp region reverses the effect. From analysis of the adjacent part of the genome it was realized that this part of the genome (138 bp) contains upstream regulatory elements of the major structural protein gene. Removal of this part of the genome enhanced the expression of the contiguous gene which may be the maturase gene. Taking analogy from Qß RNA phage it is being speculated that the maturase gene of the phage is down regulated in presence of the regulatory element contained in the said 138 bp region. Electron micrograph of phage particles produced in host carrying multiple copies of the upstream regulatory region of the putative maturase gene are mostly empty and without tail fibre. Free knob, free tail fibres tail fibres with knob at one as well as both ends are seen in such preparation (Fig. 11).

Attempts are being made to complete the sequencing of the phage genome for which a few more genomic parts have been cloned.

3.10. Comparative analysis of cytotoxin, hemolysin, hemagglutinin and exocellular enzymes among clinical and environmental isolates of *Vibrio cholerae* O139 and non-O1 non-O139

Investigators : B. Guhathakurta, D. Sasmal and A. Datta

The presence of three major virulence genes toxR, topA and ctxA as well as expression of several putative virulence factors were compared in 12 *Vibrio cholerae* O139 and non-O1 non-O139 strains of clinical and environmental origin. All the strains possessed the gene encoding regulatory protein ToxR. None of the non-O1, non-O139 strains as well as one of the O139 environmental strains carried the genes ctxA and topA. Statistically significant differences in hemagglutinin and hemolysin production were observed amongst the strains depending on the source of their isolation. Expression of extracellular enzymes such as protease, elastase, neuraminidase, phospholipase A and phospholipase C, however, did not vary significantly from the groups of strains isolated from different sources.

4. Studies on Shigella species

4.1. Immunoregulatory functions of porin of *Shigella dysenteriae* type 1

Investigator : T. Biswas

Porin caused an increase in nitrite production at concentrations of 1,10,100 ng/ml and 1 µg/ml by 2.25-, 2.8-, 3.2- and 3.1- fold respectively in a dose-dependent manner ($P<0.05$). The levels of nitrite remained elevated at 10 ng/ml and 1 µg/ml of porin and maximal nitrite level peaked at 100 ng/ml of porin (Fig.12). Addition of N^G-monomethyl-L-arginine with 1,10, and 100 ng/ml of porin suppressed the porin mediated nitrite production by 85%, confirming the specificity of porin mediated NO release. Addition of 100 U/ml of IFN- and 10 µg/ml of LPS enhanced the release of nitrite by 1.6 ($P=0.02$) and 1.9-fold ($P=0.013$) respectively compared to the untreated control. The stimulatory effect of LPS could be enhanced by addition of 1,10 and 100 ng/ml of porin. The stimulation of porin along with LPS was of 1.4- and 1.2-fold compared

to that attained by 1 and 10 ng/ml of porin alone ($P<0.05$). Polysaccharide moiety (PS, 200 ng/ml) could substitute for LPS for nitrite production and co-stimulate in presence of porin by enhancing the nitrite level, unlike the lipid A moiety. PS stimulated the nitrite production of elicited macrophages by 1.6-fold compared to untreated control ($P=0.023$). It also enhanced the stimulatory effect of 1 and 10 ng/ml of porin by 1.3- and 1.25-fold, respectively ($P<0.005$).

Porin mediated IL-1 release by elicited macrophages was determined by the thymocyte comitogenic activity of cultures grown with macrophage supernatants. Addition of 24-h old supernatant of macrophage culture in presence of 1 and 10 ng/ml of porin, resulted in 5.5-fold increase in thymocyte growth ($P<0.005$). The specificity of porin mediated IL-1 release by elicited macrophages was determined by the thymocyte comitogenic activity of cultures grown with macrophage supernatants pre-absorbed with 1:20 dilution of anti-IL-1 antibody. More than 60% depletion of thymocyte proliferation was found when grown with 1 or 10 ng/ml of porin treated macrophage supernatants which were subsequently absorbed with rabbit anti-mouse IL-1 antibody. IL-1 activity of the culture supernatants pre-absorbed with normal rabbit IgG was not depleted and was similar to that of porin treated 24 h-old macrophage culture supernatant. Addition of 24 h-old supernatant of macrophage culture in presence of 10 μg/ml of LPS with 1 or 10 ng/ml of porin, showed rise in stimulation by the two modulators by increasing the thymocyte proliferation 1.8- and 1.7-fold, respectively, in comparison to cell growth obtained by macrophage culture media containing LPS alone ($P<0.01$), and 1.5-fold more than the thymocyte growth obtained by 1 or 10 ng/ml of porin alone ($P<0.001$). A 5-fold increase of thymocyte growth was observed ($P=0.002$) in presence of 24 h-old supernatant of macrophage culture with 200 ng/ml of PS. PS also enhanced the thymocyte growth by augmenting 1.4-fold the effect observed by 1 and 10 ng/ml of porin only ($P=0.01$). The tight association of the two bacterial outer membrane components, porin and LPS, could be a necessary co-signal for boosting the release of the two proinflammatory mediators, namely NO and IL-1, which may be associated with the inflammatory response of the colon during *Shigella* invasion.

4.2. Antigenic recognition of *Shigella dysenteriae* outer membrane proteins using human convalescent sera and to evaluate their role in cell mediated immune response in shigellosis

Investigator : A.K. Sinha

In continuation during this period, we have also studied the release of Interferon- (IFN-) in 11 acute phage sera samples collected from hospitalised patients infected with *S.dysenteriae* 1 (day 1-2 of hospitalization) and convalescent sera collected in two different intervals on day 14 and on day 30 after on set of the diseases. IFN- also detected in sera samples from 4 normal healthy individuals used as control.

IFN- was measured using commercial quantative sandwich EIAs kits (Genozyme, USA). The minimum detectable concentration was 100 pg/ml of IFN- . IFN- levels were lowest (mean 0.4 ng/ml) in acute sera samples and the levels increased gradually in the convalescent sera collected on day 14 (mean 1.7 ng/ml). Maximum concentration persisted upto day 30 (mean 2.1 ng/ml). This study revealed that the lack of host defence activity may be linked to delayed recovery of IFN- and release of IFN- acts as indicative in development of immunity against shigellosis.

The study is in progress.

4.3. Genetic studies on virulence mechanisms of *Shigella dysenteriae* 1 in relation to

vaccine development

Investigators : R. Kumar, D. Biswas and S. Roy

Over the past 50 years a great deal of effort has been dedicated to develop a safe and effective vaccine against *Shigella dysenteriae* 1 which is most virulent and epidemic potential organism, however, till date, there is no vaccine with proven efficacy. Characterization of the major virulence factor(s) of *S.dysenteriae* 1 will help in designing a vaccine strain. The LPS somatic antigen plays a critical role in the pathogenesis of *Shigella*.

A hybrid strains of *S.dysenteriae* 1 which carries LPS biosynthesis genes of *Salmonella typhimurium* has already been constructed. A 60 KDa IpaH protein was not secreted into the culture supernatant by the hybrid strains. Synthesis of lipopolysaccharide of the hybrid strains was increased within the region of 43 to 67 KDa in comparison with wild type *S.dysenteriae* 1 strain. The hybrid strain showed cross reactivity with *S.dysenteriae* 1 antisera but weak reaction with *Salmonella typhimurium* antisera.

Ligated ileal loop (10 cm) were prepared in anesthesized rabbits (Ca 1.5 kg). 10^8 CFU/ml cells of hybrid strain was inoculated in the ligated ileal loop. Separate rabbit was used for wild type *S.dysenteriae* 1 as positive control. Fluid accumulation within the loops was recorded after 12 hrs and 18 hrs intervals and the volume/length ratio was calculated. FA ratio of the hybrid strain was 0.4 whereas in case of wild type strain it was 1.6. Infected loops were then opened, the aspect of the mucosal surface was recorded and portions were fixed in 10% buffered formalin. Specimens were processed by standard histopathological procedures and was stained for examination under microscope. Dissolution of the intestinal villi tips was observed by the wild type *S.dysenteriae* 1 (Fig. 13) whereas most of the villas was unaffected in the ileal loop inoculated with hybrid strain (Fig. 14). Antisera has been raised in rabbit against LPS (hybrid strain) check cross-reactivity. Both hybrid and virulent *S.dysenteriae* 1 strains reacted with antisera 1:2560 dilution and *Salmonella typhimurium* 1:640 ratio.

4.4. Studies on possible virulence factors in Shigella species

Investigators : B. Guhathakurta, D. Sasmal, R. Kumar and A. Datta

Preliminary screening of the recent isolates of *Shigella dysenteriae* type 1 and *Shigella flexneri* exhibited a correlation between expression of contact hemolysin with production of keratoconjunctivitis in guineapigs. A *Shigella flexneri* strain, cured of the large 220-kb virulence plasmid, expresses adhering and invading ability in confluent monolayers of HeLa cells similar to its parent strain. Invasion by both the parent and the cured strains resulted in alteration of the monomeric actin(G) in the total actin pool of HeLa cells. Other indicators of invasive characteristics of virulent *Shigella* strains such as production of keratoconjunctivitis in guineapig eye *in vivo*, congo red binding and expression of contact hemolysin however, indicated loss of invasive properties in the plasmid cured strain. Further, pretreatment of bacterial cells with parabromophenacyl bromide (p-BPB), a specific chemical inhibitor of phospholipase A, adversely affected adhesion to and invasion of HeLa cells *in vitro*, irrespective of the presence of the 220-kb plasmid indicating the possible involvement of the enzyme phospholipase A in the invasion process. Adherence of both the strains to guinea pig colonic epithelial cells (CECs) *in vitro* was reduced significantly on pretreatment of bacteria or CECs with p-BPB. Expression of exocellular enzymes viz protease, elastase, phospholipase A and phospholipase C were not related to the large plasmid.

4.5. Evaluation of DNA amplification method with conventional microbiological techniques for detection of *Shigella* and enteroinvasive *Escherichia coli* in stool samples from children with acute diarrhoea

Investigators : S. Dutta, S. Chakraborti, S.K. Niyogi, P. Dutta, B. Manna

The objective of the study was to evaluate P.C.R. method using primers derived from invasion plasmid antigen H (ipaH) sequences to detect *Shigella* and enteroinvasive *E.coli* (EIEC) infection. Study also aimed to determine the actual disease frequency caused by these organisms by PCR. Firstly, various culture concentrations of *Shigella* strains and cultures of other bacterial strains (like *Salmonella*, EPEC, ETEC, EHEC, EAggEC) were tested by PCR using primers derived from ipaH sequence to determine the sensitivity and specificity of PCR test procedure. Subsequently direct PCR was performed on enriched stool samples collected from 300 hospitalized diarrhoeal children. All samples were also tested by conventional microbiological methods using enrichment selective media and agglutination with commercially available antisera (Denka Seiken Co. Ltd., Japan). The enriched stool samples were also blotted onto nylau membrane to get macrocolonies and colony hybridization was performed using ial probe, non-radioactively labelled and detected by chemilumiscence [ECL, amersham, life science]. All three methods were compared and statistically analyzed. Identity of the PCR amplified band was done by southern hybridization with DNA probe. The PCR was found to be highly sensitive and specific test for detection of shigellosis in children. Test procedure is also simple, rapid and convenient. It is capable to detect the infection caused by these organism even after antibiotic therapy. Added advantage of this test is that it can be used to detect the treatment failure cases. The comparison of performance of three methods is given in Table-6.

Table-6 Combination of results obtained by the three different methods for the diagnosis of shigellosis from stool specimen

Possible results	CM/probe	CM/PCR	Probe/PCR
+/+	26	24	24
+/-	0	2	2
-/+	0	19	19
-/-	271	252	252

CM, Culture method; probe, hybridization with oligonucleotide probe

4.6. Molecular characterization of multi-drug resistant *Shigella flexneri* in Calcutta

Investigators : S.K. Niyogi, G.B. Nair, P. Dutta, D. Dutta and S. Yamasaki

Shigellosis is a major public health problem in developing countries. Increased incidence of antibiotic resistance in *Shigellae* constitutes a major concern. High frequency of resistance in *Shigella flexneri* to many of the first line antimicrobial agents (multi-drug resistant) have been reported in recent years from Calcutta. Most of the conventional typing methods are based on the phenotypic properties of the micro-organisms and offer little strain discriminatory information. The objective of this study is to analyze clonal relationships among isolates of multi-drug resistant *Shigella flexneri* using different molecular typing methods to determine changes at the genetic level and to understand their implications in the epidemiology of the disease.

Antimicrobial sensitivity of 50 *Shigella flexneri* strains revealed 8 drug resistance pattern and 73% strains were multidrug resistant. Plasmid profile analysis of the selected strains revealed four band patterns in six strains.

Further study is in progress.

5. Clinical Studies

5.1. Efficacy and safety of a sucrose based hypo-osmolar ORS solution in adults and older children with cholera : a clinical trial

Investigators : D. Dutta, M.K. Bhattacharya, S.B. Ray, G.B. Nair, D. Sarkar and A. Biswas

Oral rehydration salts solution (ORS) recommended by WHO/UNICEF is universally accepted for the treatment of dehydrating diarrhoea in all age groups. However, it cannot reduce the stool output and duration of diarrhoea. Furthermore, there has been concern about the sodium in infants and small children and the European Society of Paediatric Gastroenterology Nutrition recommends an ORS with a sodium concentration of 60 mmol/l and an osmolality between 200 and 250 mmol/l. Several recent studies have evaluated the safety and efficacy of reduced sodium and glucose based ORS solutions (hypo-osmolar). Recently a randomized clinical trial was conducted in children with acute non-cholera diarrhoea to evaluate a sucrose based hypo-osmolar ORS which showed that a sucrose based hypo-osmolar ORS is highly absorption efficient as compared to WHO/UNICEF ORS (unpublished observation). This study was targeted to evaluate the efficacy of a hypo-osmolar ORS solution containing appropriate amount of sucrose in place of glucose.

A total of twenty eight adult male patients with dehydrating acute watery diarrhoea resimbling cholera were included in the present study. The cases received either sucrose based hypo-osmolar oral rehydration solution or standard oral rehydration solution; in addition they also received 300 mg of doxycycline once at the time of inclusion. The range of age of the patients was 14-55 years. The mean duration of diarrhoea was 29.53 ± 5.67 hours. No hyponatraemia was observed in any of the patient in the present series. All the cases were hydrated successfully and recovered.

The study is in progress.

5.2. Impact of supplementation of zinc, zinc and vitamin A and combination of micronutrients and vitamins on acute watery diarrhoeal in mild to moderately malnourished children

Investigators : P. Dutta, U. Mitra, A. Datta, S.K. Niyogi, S. Dutta, B. Manna, A. De, K. Roy and M. Basak

Oral rehydration salts solution (ORS) has successfully been used for the treatment of dehydrating acute watery diarrhoea to replace fluid and electrolytes. However, ORS does not reduce the duration and volume of diarrhoea as a result of which scientists are searching for adjunct therapy which may reduce the volume and duration of diarrhoea. Effective adjunct therapy can satisfy not only the users and treatment providers but also ultimately reduces the misuse of ineffective and often harmful antidiarrhoeal drugs.

Impact of supplementation of zinc, zinc and vitamin A and combination of micronutrients and vitamins (zinc, copper, iron, selenium, vitamin B12 and folate) as adjunct therapy to ORS were evaluated on outcome variables (stool output, duration of diarrhoea,

consumption of fluid and weight gain or loss) of acute dehydrating watery diarrhoea among malnourished children.

A double blind, randomized, placebo controlled clinical trial was conducted with 87 male children (for ease of collection of stool and urine separately) aged between 6 to 23 months, having body weight <75% Harvard Standard weight for age, suffering from acute dehydrating watery diarrhoea for a period of 72 hours or less. Children who were severely malnourished and associated with other systemic or chronic underlying illness, need extensive care, received antibiotic before admission and also received vitamin A during previous 6 months were excluded from the study.

After thorough clinical examination and fulfilment of inclusion and exclusion criteria, children were randomized into four treatment groups according to a random number table. Children were weighed unclothed using owing scale with a sensitivity of 20 gms. These children received, blindly a syrup containing either 20 mg elemental zinc/day or 20 mg elemental zinc/day with single dose of vitamin A (1 lac or 2 lacs i.u. for less than 1 year or more than one year respectively) or a combination of 20 mg zinc, 10 mg iron, 2 mg copper, 40 μg selenium all in elemental form and 1.4 μg vitamin B12 and 100 μg folate per day with a single dose of vitamin A as mentioned before or placebo syrup for micronutrients and vitamin A for 14 days even after cessation of diarrhoea and after discharge from the hospital.

Randomization was done to allocate the patients to specific bottle of supplementation or placebo which were prepared by Aurio Pharma Laboratories, Calcutta according to our specification in identical looking bottles and with similar taste. Code of these bottles was kept with a senior person who was not associated with the study.

After allocation in the study, all the children were assessed for detection of degree of dehydration. Children with "some" dehydration received standard ORS for correction of dehydration and maintenance, however, who developed severe dehydration during study period received intravenous Ringers' Lactate solution as per WHO guidelines.

Children received breast milk or normal diet during that period. Children were followed up till recovery or upto 5 days if not recovered within that period. Following records were kept in predesigned proforma to compare between the groups - (i) number of stools in 24 hours; (ii) stool output (gm/kg/day); (iii) urine output (ml/kg/day); (iv) liquid food (ml/kg/day); (vii) weight gain or loss; (viii) requirement of I.V. fluid (ml/kg/day).

These children attended the hospital on day 15 and 30 of hospitalization and the following parameters were recorded to compare between the study groups - (i) weight; (ii) length; (iii) mid arm circumference.

Results could not be analyzed as the study is still continuing in double blind fashion.

5.3. Evaluation of comparative efficacy of Erythromycin and Azithromycin in the treatment of cholera in children

Investigators

M.K. Bhattacharya, D. Dutta, S.B. Ray, G.B. Nair, A. Chatterjee, K. Chatterjee and A. Biswas

Cholera is a clinical syndrome characterized by the passage of voluminous rice watery stool and frequent vomiting which rapidly leads to dehydration, hypovolemic shock, and

acidosis. Death can ensue if prompt and appropriate treatment is not initiated. Cholera still ranks high in the etiology of the diarrhoeal diseases in Calcutta.

Acute watery diarrhoea caused by *Vibrio cholerae* is an important cause of hospitalization at the I.D. Hospital, Calcutta. Several drugs, namely tetracycline, furazolidone and trimethoprim-sulfamethoxazole (TMP-SMX), have been found to be effective in reducing stool volume, duration of diarrhoea and faecal excretion of vibrio in patients with cholera. It has been observed recently that all the isolated strains of *V.cholerae* O1 and O139 in Calcutta are resistant to furazolidone. Although erythromycin may be used as an alternative to furazolidone. However, in some children it causes gastritis, vomiting. Several *in vitro* studies showed that new macrolide, azithromycin, a synthetic derivative of erythromycin, is highly susceptible against common diarrhoeagenic enteropathogens. Moreover, azithromycin is synthetically obtained from erythromycin by replacing 9a carbonyl in the aglycone ring within a methyl substituted nitrogen. This substitution confers unique pharmacological and microbiological properties, as well as avoids the formation of the hemiketal product, responsible for the common side effects seen with erythromycin. The present study was undertaken to compare the efficacy of azithromycin and erythromycin in the treatment of cholera in children having "some" or "severe" dehydration.

Male patients with acute watery diarrhoea with <24 hrs. duration with "some" or "severe" dehydration aged between 2-10 years were enrolled for the study. After fulfillment of inclusion and exclusion criteria twenty male children with a history of severe dehydrating diarrhoea were included in the present study. The age range of the cases was 3 years to 9 years. All patients received either erythromycin or azithromycin along with placebo. The mean duration of diarrhoea of twenty male child was 33.5 hours. All cases were recovered successfully. However, the data of outcome variables could not be compared as this study is in progress in double blind fashion.

The study is in progress.

6. Studies on other Enteric Organism

6.1. Search for virulence traits and determination of the mechanism of pathogenicity of *Klebsiella pneumoniae* isolated from childhood diarrhoea cases

Investigators : S.K. Niyogi, G.B. Nair, S. Dutta and A. Pal

The project was undertaken with the objective to determine if *Klebsiella pneumoniae* produce any known enteric toxin(s). *K.pneumoniae* strains isolated as a sole pathogen from acute diarrhoea cases admitted to the B.C. Roy Memorial Hospital for Children, Calcutta were included in this study.

Only eight strains of *K.pneumoniae* procuded fluid accumulation in rabbit ileal loop assay. All the strains were negative for ST when tested in suckling mice assay.

Twenty nine *K.pneumoniae* strains isolated during recent past as sole agent, were tested for their ability to adhere to HeLa cells.

Eight strains of *K.pneumoniae* showed aggregative adherence that was distinct from the stacked brick enteroaggregative pattern shown in enteroaggregative *E.coli* strains. These strains carried a large molecular weight plasmid similar to strains of other adherent *E.coli* like localised adherence *E.coli* and enteroaggregative *E.coli*.

Infection of cultured HeLa cells with *K.pneumoniae* strains resulted in significant elevation of intracellular free calcium determined quantitatively, with Fura-2 AM, a calcium sensitive fluorescence dye. We speculate that aggregative adhesion of *K.pneumoniae* caused elevation of intracellular free calcium levels in HeLa cells.

Further study is in progress.

6.2. Studies on the role of enteroaggregative *Escherichia coli* (EAggEC) as a cause of diarrhoea with reference to its virulence properties

Investigators : S. Dutta, G.B. Nair, M.R. Saha, S.K. Niyogi, P. Dutta

The objective of the study was to study the virulent attributes of EAggEC strains isolated in a hospital based case control study conducted in the year 1996-97. Twenty five isolates of EAggEC from cases of acute diarrhoea and 3 isolates from controls were subjected to test for virulence factors by available probe primers and comparison were made between these two groups of isolates. All identified EAggEC were tested for LT, ST, VT genes by PCR method using suitable primers. All strains showed negative results. Salt aggregation tests (SAT) performed with the strains using various molar concentration of ammonium sulphate. Almost all strains showed agglutination (+++) with 1.0 (M) and lower concentration of ammonium sulphate, indicating their adhesive properties. Almost 60% strains were positive for EAST. All strains were negative for *hly* and *eae* genes. Total protein excreted by the bacterial cells in the media has been precipitated by ammonium sulphate (50% and 80%). Precipitates were collected by centrifugation were dissolved and dialysed in tris glycine buffer. SDS-PAGE was run and the results were compared and analyzed (Fig. 15). The strains would also be tested for their plasmid profiles and plasmid encoded toxin (PET) gene by available probes and primers. Further studies are in progress.

The bacterial pellet would be sonicated to release the contact protein and any difference in protein profile would be noted between the strains isolated from cases and controls.

6.3. Search for Shiga toxin producing *Escherichia coli* including O157 : H7 strains in animals, animal products and hospitalized acute diarrhoea cases in Calcutta

Investigators : S. Dutta, S. Ghosh, P.G. Sengupta, P. Dutta

The objectives of the study were to determine whether dairy cattle are potential sources of shiga toxin producing *Escherichia coli* (STEC) including O157:H7 serotype. The study also aimed to ascertain the presence of STEC in raw foods (beef, pork, milk) marketed in Calcutta and in animal handlers residing inclose association with animals. Investigations were carried out to determine the role of STEC in the causation of acute diarrhoea among hospitalized children. All samples were screened for STEC by PCR using VT common primers. All positive PCR samples were further plated onto SMAC medium, blotted onto nylon and hybridisation was performed using stx1 and stx2 oligonucleotide probes. The signals were detected by nucleic acid detecting reagents (ECL; Amasrham, Life Science).

Thirteen strains of STEC were isolated from dairy cattle and beef samples marketed in Calcutta. Clinical samples yielded no STEC. Of 13 strains 7 belonged to O157:H7 serotype. All O157:H7 strains were multidrug resistant and possessed important virulent factors, which included shiga toxin production and the presence of *eae* and *hlyA* genes. Bacterial DNA was extracted from all O157:H7 strains, the DNA was digested with EcoRI restriction enzyme and electrophoresed onto 1% agarose gel. The digested fragments were analyzed. Restriction enzyme digested DNA pattern showed almost identical pattern for all isolated O157:H7 strains.

The electrophoretically separated fragments would be blotted onto nylon membrane and southern hybridization would be carried out using Stx1 and Stx2 probes for RFLP typing. The results will be analyzed. Further studies are in progress.

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

Investigators : M. K. Chakrabarti, A. Pal and K.M. Hoque

The principal objective of this study is to purify and characterize the receptor for *E.coli* STa from a high density receptor system and also to evaluate the mechanism of action of STa. Initially, STa was purified to homogeneity and receptor assay was done with iodinated STa. It has been reported earlier that binding of ^{125}STa to the brush border membranes of rat, rabbit hamster and guineapig was specific, time and temperature dependent. A single class of receptors was present in all the tested animals and the number of receptors remained lower in hamster in comparison to rat, rabbit and guineapig. Autoradiographic demonstration of SDS-PAGE of intestinal brush border membranes showed STa binding proteins of apparent MW of 160 kDa in rat 118 kDa in guineapig, 140 and 38 kDa in rabbit and 65 KDa in hamster. It was also reported that STa binds to a single class of receptors in COLO-205 human colonic carcinoma cell. Binding was specific, time and temperature dependent. STa binding protein with MW of 95 kDa was detected in this cell line. STa was found to stimulate G-cyclase in COLO-205. It has been found that besides stimulating cGMP STa also involves two potential intracellular signals. It increases rapidly inositol triphosphate and cytosolic free calcium in COLO-205 cells prelabelled with myo[2-^{3}H] inositol resulted in a rapid rise of [3H] inositol triphosphate. Using fluorescent indicator, Fura 2AM, intracellular free Ca^{2+} has been found to increase 5.12 fold compared to control. Suspension of cells in calcium was chelated with EGTA. This effect was not observed with cells that were pretreated with dantrolene, which suggest that the intracellular calcium rise might be due to mobilization of intracellular stores. This study demonstrated for the first time a change in cytosolic calcium in cultured human colonic cell by STa, which was accompanied by inositol tri phosphate activation.

We then examined the effect of STa in the activation of Protein kinase C (PKC) in COLO-205, as PKC acts as an important effector molecule in the intracellular signal transduction process. The activity of PKC in the membrane fraction of COLO-205 cells were determined by measuring the transfer of γ^{32}P ATP to histone IIIS. To prepare membrane fraction, cells were incubated with different concentration of STa at 37°C for 1 min. Cells were then washed, homogenized and centrifuged at 100,000 g for 1 hr. The pellet resuspended in 20 mM Tris-HCl, homogenized and centrifuged at 100,000 x g for 1 hr. Supernatant served as membrane bound PKC. STa increased PKC activity in COLO-205 in a dose dependent manner. Maximum PKC activity was noticed with 4 nM STa (Fig.16). In the time course study it was found that PKC activity started increasing as early as 40s and became maximum at 1.5 min (Fig.17). Thereafter, the enzyme activity was decreased but remained higher than the basal value even up to 6 min. It is known that PKC is inactive in its basic state but is activated by diacylglycerol (DG) in the presence of Phosphatidyl serine (PdSer) and Calcium. In their presence, a 5.7 fold enzyme activity was noted in STa treated cells (230 ± 8 nmole/mg protein/min) in comparison to that of control cells (40 ± 3 nmole/mg protein/min). However, in absence of these substances a considerable enzyme activity was noted in STa treated cells (78 ± nmole/mg protein/min) but a negligible amount of enzyme activity was found in the control cells. To separate the PKC activity from that of other kinases and to determine the specificity of STa mediated action of PKC, the inhibitory effect of staurosporine on the activity of PKC was

tested in COLO-205. A complete inhibition of PKC activity was found both in control as well as in toxin treated cells with a dose of 0.6 μM of staurosporine. The inhibitory effect of staurosporine is pronounced in STa treated cells (70%) as compared to that in control (45%) with 0.1 μM of staurosporine suggested that elevated enzyme activity in response to enterotoxin was due to the modulation of existing enzyme to a state of higher catalytic activity (Fig.18). Further study is in progress.

6.5. Molecular characterization of *Vibrio parahaemolyticus* O3:K6 strains harboring the gene *tdh*, isolated from clinical samples in Calcutta

Investigators : T. Ramamurthy, N.R. Chowdhury and G.B. Nair

Continued surveillance of diarrhoea caused by *Vibrio parahaemolyticus* implicated the predominant association of the O3:K6 serovar of *V.parahaemolyticus* with the disease in 1998 and also in 1999. Considering the sudden upsurge of a single serovar of *V.parahaemolyticus* from 1995 onwards in Southeast Asia and in different parts of the world, the O3:K6 strains isolated from 8 different countries (India, Bangladesh, Japan, Vietnam, Thailand, Taiwan, Korea and USA) were compared and analyzed by arbitrarily primed PCR (AP-PCR), ribotyping and pulsed field gel electrophoresis (PFGE). All the O3:K6 strains exhibited identical AP-PCR profile which was distinct from the AP-PCR profile of the strains belonging to other serovars. Southern hybridization of the *rrn* genes with a specific rRNA probe yielded 11 bands ranging in size from 23.0 kb to 4.0 kb for all the strains tested. Further characterization of the strains was done by PFGE and the pattern obtained for all the strains showed no polymorphism. Thus, from the molecular characterization by AP-PCR, ribotyping and PFGE of the O3:K6 strains isolated from different geographical regions documented the possibility of common ancestral origin. The O3:K6 strains, isolated in Calcutta from 1995 onwards (referred to as new O3:K6) were also compared with the O3:K6 strains isolated before 1995 (referred to as old O3:K6). AP-PCR, ribotyping and PFGE profiles of the old O3:K6 isolates were significantly different from the new clone. The *toxRS* sequence analysis of the old and new O3:K6 strains showed that they differ invariably in 7 base regions within the 1,364 bp sequence representing 95% of the *toxRS* region. Consequently, a PCR based method known as group-specific PCR (GS-PCR) was developed utilising 2 of the 7 unique bases present in the new clone to distinguish the old and the new O3:K6 isolates (Fig.19). Interestingly, strains belonging to 2 other serovars of *V.parahaemolyticus* such as O4:K68 and O1:KUT were also found to be positive for the 651 bp amplicon produced by GS-PCR thereby indicating that they belong to the same clone as the O3:K6 strains (Fig. 20). This data was substantiated by the other 3 molecular typing methods.

The emergence of the new clone, comprising of strains belonging to 3 different serovar, having enhanced virulence potential to be capable of causing a pandemic spread is not yet understood but requires close monitoring to prevent the rapid spread of this bacterium.

6.6. Differentiation of *Salmonella typhi* by molecular methods

Investigators : M.R. Saha

Co-investigators: T. Ramamurthy, G.B. Nair, P. Dutta, U. Mitra

Typhoid fever even today continues to be a global health problem specially in the tropic and sub-tropics. It has been estimated that more than 12 million cases occur annually in the developing world, of which as many as 7.7 million cases occur annually in India alone. Several epidemic of typhoid fever caused by multi-drug resistant strains of *S.typhi* have been reported

from different parts of the world from time to time. On recent years, there have been a number of molecular methods developed to detect plasmid and chromosomal DNA. The present study was undertaken to determine spatial and temporal variation of clonal type in Calcutta with the retrospective strains of *S.typhi* isolated since 1990 and strains of *S.typhi* of recent origin by molecular analysis.

Initially, 23 *S.typhi* isolates during the period of 1990 to 1998 showing different antimicrobial resistance patterns and phage types were included. The strains were studied by pulsed field gel electrophoresis. Different pulso types of the isolates were observed of which 5 strains showed similar pulso-type 1 and one strain exhibited one band difference. Four strains showed similar pulso-type 2. The remaining 13 strains showed diverse patterns of pulso-types.

Two hundred and sixty one *S.typhi* strains isolated during 1990-91 were tested with Vi antiserum. Of that, 70 (26.8%) strains did not agglutinate with vi antiserum (Vi negative strains). All the 70 vi negative strains were phage untypable. Nine *S.typhi* isolated during that period were tested by slide agglutination using vi antiserum (Denka Seiken, Japan), 4 (44.4%) strains remained vi non-agglutinable.

To detect the presence of vi antigen encoding gene in the chromosome, all the 74 vi negative strains were tested by polymerase chain reaction (PCR) based on the ViaB sequence. Interestingly, 72 (97.3%) out of 74 *S.typhi* strains that did not agglutinate with the vi antiserum, were found to harbour the vi encoding gene. Our finding suggest that ViaB PCR can reinforce the clinical diagnosis of typhoid fever in Vi agglutination negative *S.typhi* strains.

The study is in progress.

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

Investigators : D.R. Saha, S. Dutta, A. Chowdhury and G.B. Nair

This project was undertaken to determine the association and tissue response to *Helicobacter pylori* with different diseased conditions along with the virulence genetic pattern of the organism. Endoscopic biopsy samples were collected from fundus and antrum of the stomach from patients attending Gastroenterology Division of S.S.K.M hospital, Calcutta. Five bits of tissue were taken – one for Rapid urease test, two in Brucella broth with 15% glycerol for culture and two in buffered formalin for histopathological examination. Formalin treated tissues were processed for paraffin embedding and serial thin sections were cut, stained by haematoxylene and eosine to see the histological changes by light microscopy. Modified Giemsa stain was done to detect *H.pylori*. So far one hundred twenty samples have been processed and examined. A good number of patients have been suffering from chronic active gastritis showing histological presence of *H.pylori*. The study is in progress.

7. Studies on Parasitic Diarrhoea

7.1. Studies on electron dense granules of *Entamoeba histolytica* containing collagenase activity

Investigators : P. Mukhopadhyay, A. Debnath, S. Sengupta, A. Akbar and P. Das

During the course of invasive intestinal amoebiasis, *E.histolytica* actively penetrates the mucosa of host intestine. But the exact mechanism and the factors by which parasite destroys the human tissue are still unclear. In recent years some progresses have been made on the

molecular mechanism of pathogenesis. The major objective of the present study was to characterize the electron dense granules of *E.histolytica* in greater detail and clone the genes coding for these proteins.

Earlier, we characterized the electron dense granules (EDG) produced during the incubation of pathogenic (HM1:IMSS or HM2) *E.histolytica* with human collagen type I (purified from human placenta) and Ca^{2+}. Analysis of EDG showed 8 fold more collagenase activity than whole trophozoites. Chemical and elemental analysis of purified EDGs showed presence of inorganic phosphate, pyrophosphate and Na, Mg, S, Cl, K, Ca and Fe as measured by Scanning transmission electron microscopy. In SDS PAGE six polypeptides with apparent molecular weights of 108, 106, 104, 97, 68 and 59 kDa were found exclusively in EDG preparation. Similarly, two protease activities with apparent molecular weights of 40 and 85 kDa were detected only in EDGs.

During the year purified EDG was used in raising antibodies in rabbit. This antibody was used in immunoscreening of the cDNA library constructed from pathogenic *E.histolytica*. Inserted DNA has been released and approximate size of the cloned cDNA has been determined. Subcloning and characterization of subcloned fragments are under progress.

Attempts were made to amplify the genes encoding for collagenase of *E.histolytica*. After computer search, two oligos were designed from most conserved sequences of collagenase genes of other organisms for PCR. Using *E.histolytica* genomic DNA as template and oligos, four bands (TE51, TE52, TE53, TE54) were amplified by PCR. The largest fragment (TE51) containing 585 bp has been sequenced and homology analysis shows its homology with *E.histolytica* gene (Ehvmal) encoding the catalytic peptide of a putative vacuolar proton-transporting ATPase (V-ATPase) (Accn. No. U04849). In dot blot (Fig. 21) and Southern hybridization, the fragment (585 bp) recognizes only *E.histolytica* genomic DNA and not other enteric pathogens. Characterizations of other three fragments are under progress.

7.2. Studies on multiple genes in *Entamoeba histolytica* during human collagen type I and Ca^{2+} interaction : Use of mRNA differential display

Investigators : A. Debnath, P. Mukhopadhyay, S. Sengupta, A. Akbar and P. Das

Amoebiasis, a parasitic infection in man due to the protozoan *Entamoeba histolytica,* is an invasive enteric illness that can spread to multiple tissues, particularly mucosa and submucosa of host intestine. Because, collagen is major component of the extracellular matrix and basal lamina of human intestine, it is thought that collagenase which was detected in pathogenic *E.histolytica* is one of the important factors of tissue lysis during invasive amoebiasis. Till date no study has been conducted about the expression of collagenase genes in *E.histolytica.* In this study attempts were made to clone and sequence some of the genes that are differentially expressed during activation with human collagen type I and Ca^{2+} and compare homology analysis with the sequences in GenBank.

During the period, total RNA and mRNA from trophozoites of pathogenic *E.histolytica* incubated in presence or absence of human collagen type I and Ca^{2+} was isolated and kinetics of mRNA synthesis was studied. The kinetic study has shown that 2-hrs incubation of *E.histolytica* with human collagen type I and Ca^{2+} are optimal for maximum synthesis of mRNA (Fig. 22). To synthesize the cDNA, degenerate 14 mers have been identified. Conditions and oligos for cDNA amplification by PCR have been optimized. Running of amplified products in denaturing gel has been standardized. Preliminary results showed approximately 15 differentially expressed

bands in pathogenic amoeba incubated with human collagen type and Ca^{2+} (Fig. 23). Further characterizations are under progress.

8. Studies on Viral Diseases

8.1. Nucleotide Sequence of VP7 gene of rotavirus detected from Calcutta

Investigators : S. Chakrabarti, D. Khetawat, P. Dutta

The gene coding for outer capsid protein of VP7, of rotaviruses, detected among children suffering from diarrhoea in the eastern part of India, was studied. Fecal RNAs, isolated from stool samples, were hybridized with the ID45/2 cDNA, previously isolated and sequenced in our laboratory. Some of the samples didn't hybridize and one such sample, WD33, was chosen for further study.

The full length gene, coding for VP7, was synthesized by combined reverse transcriptase-polymerase chain reaction. The PCR product was cloned by direct ligation into TA Cloning vector, PCR^{TM} 2.1 (Invitrogen, USA). The clone was characterized by digestion with different restriction enzymes. In Multiplex - PCR, a 748 bp fragment (specific for G1 serotype) was amplified, using VP7 cDNA from WD33 strain as the template in comparison to the full length gene (1062 bp). It indicates that the WD33 strain might be of Wa like strain having G1 serotype specificity. For sequencing, WD33 cDNA was digested with restriction enzymes EcoRV, EcoRI, HincII, BamHI and each of the fragments were cloned into pUC18 vector. All the subclones were sequenced by Sanger's dideoxy method using the Sequenase Version 2.0 (United States Biochemical Co., USA). The overlapping regions were sequenced across and the complete nucleotide sequence of the VP7 gene of WD33 strain was obtained (Fig. 24). The VP7 gene was found to be 1060 bp in length, which is 2 bp shorter than the commonly reported full length VP7 gene. It possessed two in-frame initiation colons (nt.47-49 and 134-136), a common feature to all Group A human rotaviruses. The first open reading frame (ORF) beginning at nt. 47-49 and ending at nt. 1025-1027, was found to code for a polypeptide of 326 amino acids. A deletion of 2 bp compared to other VP7 gene was noticed at the 5' untranslated region. The sequence also revealed the presence of conserved cysteine and proline residues together with the potential glycosylation sites at nt. 69-71 (NST) and 238-240 (NLT). One additional proline residue was found at the amino acid position 67. The nucleotide sequence data have been submitted to EMBL databases and have been assigned the accession number, Y18786.

8.2. Detection and molecular characterization of Rotaviruses

Investigators : T.N. Naik, T. Krishnan, S. Das, A. Sen

Rotaviruses are the major cause of acute gastroenteritis of human infants and young of a variety of mammalian and avian species throughout the world. Interestingly a gradual increase of infections among older age group besides children has been observed for last 2-3 years. The antigenic diversity of the group specific antigen (VP6) and serotype specific antigens (VP4 and VP7) of Group A rotaviruses continues to overwhelm researchers in the field.

Subgroup nature

A total of 749 clinical samples received from Infectious Diseases Hospital and B.C. Roy Children's Hospital, Calcutta were screened by polyacrylamide gel electrophoresis during April 1999 to March 2000. Among these 81 (10.82%) samples were positive for Group A rotaviruses.

The VP6 nature of rotavirus positive samples showed that SGI (42.5%) was the dominant subgroup for 1999-2000 followed by SGII, SGI&II and non SGI&II with 28.75%, 12.5% and 0.25% respectively. The most interesting finding was the appearance of a new strain which did not react with either Group A specific or any of the subgroup specific monoclonal antibodies or polyclonal antibodies, although a clear Group A specific RNA electrophoresis profile was observed in polyacrylamide gel and which constitutes 10% of the positive samples detected during above period.

A number of nonreactive [for VP6] positive rotavirus samples were taken for PCR amplification and sequencing by dideoxynucleotide dye terminator method on a ABI Prism 310 DNA sequencer apparatus. The sequence analysis is in progress.

Serotype nature

The G-serotypes GI, G4 and dual G-types GIG3 and GIG4 were recorded during current study period by serotyping ELISA, however 78% of the rotavirus positive samples could not be serotyped. We have started G-typing and P-typing by RT-PCR in order to resolve the untypable samples.

Presently we are carrying out RT-PCR and sequence analysis of the genome segments representing Group A specificity [VP6], G-serotype specificity [VP7] and P-genotype specificity [VP4] and also gene 11 of human Group A rotaviruses.

8.3. Comparative genome analysis of human Rotaviruses prevalent in India (DBT)

Investigators : T.N. Naik, T. Krishnan, A. Sen, S. Das

Since 1997, a total of five isolated cases of human Group B rotavirus [HuGBR] infection among adult diarrhoea patients admitted to Infectious Diseases Hospital, Calcutta exhibiting "cholera-like diarrhoea".

The re-emergence of HuGBR in India necessitated the development of a sensitive and rapid diagnostic technique for easy detection of this potentially virulent pathogen. We designed several oligonucleotide primers from sequences of several gene segments of ADRV strain that had been determined previously and deposited in the GenBank database. The suitability of the primers were determined by RT-PCR amplification of genes from the different CAL isolates of HuGBR. Altogether, 32 primers were selected for detection of gene segments coding for viral structural proteins VP4, VP6 and VP7 and viral nonstructural proteins NSP1, NSP2, NSP3, NSP4, NSP5 and a putative fusion protein which is suitable for amplification of human Group B rotaviruses. Further standardization has resulted in a convenient and rapid RT-PCR assay for several of these genes that can be carried out under identical reaction conditions in a clinical diagnostic laboratory (Fig. 25; Table - 7).

Group B rotaviruses are unique in possessing a novel gene that encodes a protein with a low level of similarity with known fusion proteins of orthomyxoviruses. This protein has been suggested to play an important role in the pathogenesis of the virus as syncytium formation is a hallmark of Group B rotavirus infections. The gene encoding this putative protein has been identified to be located on gene segment 6. Gene segment 6 has two overlapping reading frames encoding the putative fusion protein [ORF 1] and NSP1 [ORF 2]. Sequencing of both genes was carried out using a pair of nested primers flanking both the reading frames. Sequence analysis showed a high degree of conservation at the amino acid level for the first ORF suggesting that

this protein may be functionally important. Analysis of the gene encoding the NSP2 viral protein showed the protein to be different and 22 amino acids longer than the cognate gene product from ADRV as a result of a single base insertion that results in a frameshift mutation. This resulted in an altered, extended carboxyl-terminal in CAL-NSP2. It is also noted that there is a very high degree of homology between CAL strain and IDIR (Infectious Diarrhoea of Infant Rat) strain of murine Group B rotavirus after the frameshift mutation. This mutation might have resulted in the development of a highly epidemic strain like Chinese ADRV which caused an epidemic affecting more than one million adults in 1982-84 in China. Therefore CAL strain may be a progenitor of ADRV strain. This mutant gene has been cloned and will be used for further analysis of its function. Sequencing of the NSP2 gene from sequential isolates of the virus detected from Calcutta over a period of three years did not reveal any changes over the years. The gene segment encoding NSP4 viral protein has been determined for the first time in this Group of rotaviruses. The cognate NSP4 protein from Group A rotaviruses was recently demonstrated to function as a viral enterotoxin and is believed to play an important role in rotavirus-induced diarrhoea. There is a low level of sequence homology at the amino acid level between the CAL protein and the few cognate genes from Group A rotaviruses whose sequence has been determined.

8.4. National HIV Reference Centre

Investigators : S.K. Bhattacharya and T.N. Naik

The National HIV Reference Centre of the Institute is funded by National AIDS Control Organization of Ministry of Health and Family Welfare, Government of India since 1992. The activities of the reference centre includes (1) serosurveillance for HIV infection, (2) confirmation of serum samples received from different surveillance and zonal blood testing centres located in different states of Eastern India, (3) training of manpower (doctors, medical laboratory technologists) for HIV surveillance and laboratory diagnosis of HIV infection as and when requested by State Health authorities, hospitals or service organization and (4) HIV kit evaluation

Between April 1999 and March 2000 a total of 915 serum samples were screened by highly sensitive ELISA and positive samples were confirmed by either highly specific ELISA or lineimmunoassay (Table)

National HIV Reference Centre

Division of Virology,

National Institute of Cholera & Enteric Diseases

Calcutta 700 010

Samples Screened for Human Immunodeficiency Virus (HIV)

Antibody by ELISA and/or Confirmatory Test

From 1st April, 1999 to 31st March, 2000

Source of Samples	No. of Tested	No. or Positive
A. WEST BENGAL		
1. Antenatal Mother	1	0
2. Blood Donor	18	11
3. Border Security Force	3	2
4. Drug Users	1	0
5. Eastern Command Hospital	95	84
6. Foreign visit (for Indian)	NT	0
7. High Risk Group	NT	0
8. Patients with blood diseases	2	1
9. Commercial Sex Worker	174	21
10. Foreigners	9	0
11. Miscellaneous	347	18
Sub Total	**650**	**137**
B. OTHER STATES		
1. Bihar	28	4
2. Meghalaya	5	3
3. Mizoram	37	32
4. Orissa	195	173
GRAND TOTAL	**915**	**349**

9. JICA/NICED Project for Prevention of Emerging Diarrhoeal Diseases

This Institute entered into a collaborative project with Japan International Cooperation Agency (JICA) entitled "Prevention of Emerging Diarrhoeal Diseases" since February 1998. The aim of this project is to strengthen the research capability of this Institute and to establish rapid and accurate diagnostic, appropriate therapeutic, and effective prevention methods for the control of emerging diarrhoeal diseases with ultimate objective of reduction of diarrhoeal diseases morbidity and mortality. The three major components of the project are:

i) Provision of sophisticated equipment

ii) Visit of Japanese experts at NICED

iii) Training of NICED scientists in Japan or in a third country where another JICA project is being implemented

Dr.S. Yamasaki, Division Chief,International Medical Centre of Japan was overall in charge of the project and being a specialist in molecular bacteriology,had introduced several molecular techniques for rapid identification of enteric pathogen like *Vibrio cholerae*, *V. parahaemolyticus*, different types of *E. coli*, Shigella spp., Salmonella typhi,etc. These techniques have been utilized by the technical staff of the Microbiology Department.

Mrs. M.Harui, Coordinator of the project is efficiently coordinating all administrative and counterpart training programs for the NICED scientists and staffs.

Three Short term Japanese experts visited this Institute. Dr. S. Nakata, Virologist, Assistant Professor, Sapporo Medical University introduced the new methods for detection of Norwalk virus (NV) and Sapporo virus (SV) from diarrhoeal stool samples. He delivered a lecture on calicivirus gastroenterititis. Dr. H.Kurazono, Molecular Epidemiologist, Professor, Department of Medical Technology, School of Health Science, Okayama University trained the scientists and staffs about bead-ELIZA technique for a heat-labile enterotoxin produced by *Helicobactor pylori*. Dr. T. Dohi, gastroenterologist and mucosal immunologist, Director,Department of Gastroenterology, Research Institute, International Medical Centre of Japan, worked for host-defense system in prevention of diarrhoeal diseases. She discussed on socio-economic conditions of the risk-groups in and around Calcutta regarding susceptibility to cholera. She also extended her knowledge and experiences in mucosal immunology for evaluation of immunity against diseases.

In the reporting year, Mr.R.B.Bose, Laboratory Technician, Division of Microbiology and Dr.M.K.Saha, Research Officer, Division of Virology were sent to Japan. Mr.R.B.Bose worked at the National Institute of Infectious Diseases, Japan to learn the recent technique of raising antisera for serotyping of *V.cholerae*. He will complete his training in May,2000. Dr. M.K.Saha is receiving training in molecular virology of hepatitis virus at the National Institute of Infectious Diseases, Japan since March, 1999. Dr.A.Pandey, Research Officer, attended a course for 10 months and obtained Masters Degree on "Master of Primary Health Care Management Program" at ASEAN Institute for Health Department, Mahidol University, Thailand. Mr. S.K. Bhowmik, Technical Assistant is studying Masters course in Molecular Microbiology at the school of Medicine,Tsukuba University in Japan since April 1999 under the

Japanese Government scholarship program.

Dr. Y.Takeda, Director General of the National Institute of Infectious Diseases, Japan and Mr. T. Ishizaki, Official, the Medical Cooperation Department, JICA Headquarter, Japan visited NICED during 25-29 September, 1999 as the Management Consultation Team for the JICA project and expressed their satisfaction for smooth functioning of the project and appreciated the excellent progress of the project activities for achieving its goal.

10. Training and Extension

10.1. Training Course on Antimicrobial Resistance Monitoring

The antimicrobial resistance has been recognised globally as an important emerging problem. Rational use of antibiotics is considered as an important method of preventing development of resistance in bacteria. The development of effective policies for rational use requires generation and rapid analysis of reliable data from microbiology laboratories. To achieve this objective one of the above WHO assisted training course was organised jointly by NICD, Delhi and NICED, Calcutta. It was held at this Institute during March 22-26, 1999. This hands-on training course for middle level microbiologists was conducted as per the schedule developed by WHO, which combines training on antimicrobial susceptibility testing as per the National Committee on Clinical Laboratory Standards (NCCLS) method and the use of computers in rapid analysis of data through WHONET software.

There were altogether 15 participants from different medical colleges and other related institutes including this institute. There were also four nominated observers from this institute. The training course was found to be very much useful to improve this laboratory skill and reporting technique.

10.2. Training course on clinical management of cholera and other diarrhoeal diseases

Four UNICEF sponsored workshops on management of cholera and other diarrhoeal diseases were conducted at Dibrugarh and Guwahati, Assam one at each station and two at Malda, West Bengal during 9-11 November, 27-29 November, 14-16 December, 1999 and 22-29 March, 2000 respectively. Aim of these workshops was to orient the health functionaries with clinical management, prevention and control diarrhoeal diseases with special emphasis on cholera and shigellosis. Participants were doctors, nurses and paramedical staff who were directly involved with health management aspects. Participants were assigned for group work and group leaders presented their views. Participants interacted freely with the course facilitators during various sessions.

10.3. Others

In addition to the training programme mentioned earlier, 4th semester batch of M.B.B.S. students of Calcutta National Medical Colleges and BHMS students from the National Institute of Homeopathy visited the institute to acquaint themselves with different facets of diarrhoeal diseases and its management. BHMS students were also apprised of the present situation of AIDS in the country. Lectures on respective topics were delivered by Dr. S. Chakrabarti, Deputy Director (Sr. Grade), Dr. P.G. Sengupta, Dr. P. Dutta, Dr. D.N. Gupta, Deputy Directors, Dr. S.K. Mondal, Assistant Director and Dr. D. Sur, Senior Research Officer.

M.Sc. (Physiology and Botany) students of Vidyasagar University with special paper in

Microbiology visited the institute to get oriented with the different aspects of biomedical research. Some students of M.Sc. (Physiology) of Vidyasagar University and M.Tech (Biotechnology) of Jadavpur University carried out their dissertation work at this institute under Dr. M.K. Chakrabarti, Deputy Director.

The students of M.D. (Microbiology), Calcutta University were deputed to this institute for acquiring advance knowledge in different aspects of enteric organisms.

Besides the above, weekly seminars on different aspects of diarrhoeal disease research were held regularly.

10.4. Guest lectures

The following scientists delivered lectures at this institute on various topics as mentioned below :

Speaker	Date	Topics
Dr. Bibek Roy, Professor of Food Microbiology, University of Wyoming College of Agriculture, USA	27.8.99	Bacteriocin of gram positive bacteria
Dr. Suji Nakata, Assistant Professor, Dept. of Paediatrics, Sapporo Medical University, School of Medicine, Supporo, Hokkaido, Japan	3.12.99	Molecular detection and differentiation of Norwalk like viruses and supporo like viruses in human gastroenteritis
Dr. Ranjit Roy, Associate Professor, School of Medicine, St. Louis University, USA	20.12.99	Molecular mechanism of Hep C virus mediated disease progression

10.5. Training of W.H.O. Fellow

During the period under report the institute imparted training on different aspects of diarrhoeal diseases to the following W.H.O. fellows :

Name	Country	Period
Dr. Md. Farooq	Bangladesh	29.3.99 to 16.4.99
Mrs. Umme Habiba	- do -	- do -
Ms. Thuza Myint	Myanmar	31.5.99 to 9.7.99
Mr. Md. Habibullah Akunji	Bangladesh	19.7.99 to 10.9.99

10.6. Training/visit of the Institute staff

Dr. M.K. Bhattacharya, Senior Research Officer attended Summer Epidemiology Programme in HIV epidemiology at John's Hopkins University during 6th June to 3rd July, 1999 under the sponsorship of Fogarty AITRP, USA.

Dr. D. Sur, Senior Research Officer attended training programme on `computerised data management and statistical analysis' organised by Health Research & Development Centre, Calcutta during January 7-28, 2000.

Dr. A. Pal, Senior Research Officer under went training in Ussing Chamber at the Department of Gastroenterology, Christian Medical College, Vellore during September 24 to October 6, 1999.

Shri K. Rajendran, Technical Officer (Bio-stat), obtained training from "Winter School" Training Program Titled "Statistical Exploration of Patterns in Spatial and other types of large data" from the Indian Statistical Institute, Calcutta during February 8-25, 2000.

10.7. Honours/Awards to the Institute Staff:

Dr. G.B. Nair, Deputy Director, received the prestigious Shanti Swarup Bhatnagar prize in Medical Sciences of CSIR for the year 1998 for his contribution in cholera research from Hon'ble Prime Minister of India on February 21, 2000.

Dr. M.K. Chakrabarti, Asst. Director, was awarded Ramendra Sunder Sinha Memorial Oration, 1999 of the Physiological Society of India at the 87th Session of Indian Science Congress held at Pune, during January 3-7, 2000. *Escherichia coli* heat stable toxin : Receptor specificity and signal transduction mechanism.

Dr. D. Sur, Senior Research Officer was awarded Fellowship of Indian Public Health Association at All India Conference of Indian Public Health Association held at Pune during April 15-18, 1999.

10.8. Ph.D. award to the Institute scholars

1. Name of the scholar : Alok K. Chakrabarti

   Title of the thesis : Study on bacteriophages of *Vibrio cholerae* serovar O139

   University : Calcutta

   Year : 1999

   Supervisor : Dr. B.L. Sarkar

2. Name of the scholar : Jayanta Bhattacharya

   Title of the thesis : Studies on the binding of *Escherichia coli* heat-stable enterotoxin to the intestinal epityelial cells and brush border membranes of different animals

   University : Calcutta

   Year : 1999

   Supervisor : Dr. M.K. Chakrabarti

10.9. Other activities of the Institute staff

Dr. S.K. Bhattacharya, Director, acted in the following capacities :

i) Conducted Ph.D viva voce examination at PGI, Chandigarh.

ii) Expert Member during selection of R.O's of Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar.

Dr. S. Chakrabarti, Deputy Director (Senior Grade), acted in the following capacities :

i) Member from Ministry of Health & Family Welfare, Govt. of India in a study tour to Thailand in connection to prevention of HIV-1 transmission from mother to child.

ii) Delivered Guest lectures to the students of M.Sc. in Physiology and M.D. in Biochemistry of Vidyasagar and Calcutta University respectively.

Dr. P. Dutta, Deputy Director, acted in the following capacities :

i) Editor, Indian Journal of Public Health (official publication of Indian Public Health Association).

ii) Divisional Editor (Gastroenterology) of The Child and New Born (Official Publication of Indian Academy of Pediatrics, WB Branch).

iii) Central Council Member of Indian Public Health Association.

iv) Executive Member of Indian Public Health Association (West Bengal Branch).

v) Executive Member of Indian Academy of Pediatrics (West Bengal Branch).

vi) Vice-Chairperson of Souvenir Committee of XI National Pulmology Conference of Indian Academy of Pediatrics held at Calcutta.

vii) Invited as a guest speaker in the East Zone Conference of Indian Academy of Pediatrics held at Imphal, Manipur.

Dr. P.G. Sengupta, Deputy Director, acted in the following capacities :

i) Member of Central team jointly organised by central health services & ICMR. Investigated the course of outbreak of diarrhoeal diseases in super cyclone affected area of Orissa.

Dr. G.B. Nair, Deputy Director, acted in the following capacities :

i) Conducted viva-voce examination for M.Sc. Part II (Paper IV) on Advanced Microbiology at the Biochemistry Department, University College of Science, Calcutta.

ii) External member of the selection committee for the award of Junior Research Fellowship held at IICB,

iii) Expert member of the assessment committee meeting for S&T Staff of IICB.

iv) External examiner for conducting the viva-voce of final year M.Tech students of Department of Biotechnology and Biochemical Engineering, IIT, Kharagpur.

v) Investigated a cholera epidemic in Kottayam district at the behest of the Indian Council of Medical Research.

vi) Member of the Expert Committee for interviewing the candidates for Senior Research Fellows and Research Associated in subject Medic-11.

Dr. M.K. Chakrabarti, Deputy Director, acted in the following capacities :

i) Organised a seminar on "Environment and Health" at Serampore College, Serampore, West Bengal.

ii) Conducted viva-voce examination of Ph.D students of Jadavpur and Calcutta University.

iii) Honorary lecturer of M. Tech. (Bio. Tech) and M.Sc. Part II course (Physiology and Botany) of Jadavpur and Vidyasagar University respectively.

iv) Member of Editorial Board of Indian Journal of Physiology & Allied Sciences and U.G Board of studies in Physiology of Calcutta University.

v) Member of board of examiners of post graduate department of Jadavpur, Calcutta, Burdwan and Tripura university.

vi) Honorary Assistant General Secretary of the Physiological Society of India.

vii) Joint Organising Secretary of 3rd Congress of Federation of Indian Physiological Societies (FIPS).

viii) Recorder, Section of Physiology, Indian Science Congress.

Dr. P. Das, Deputy Director, acted in the following capacities :

i) Members in board of examiners of Calcutta University for setting theory and practical examination question papers for M.Sc. Zoology (Immuno-parasitology Spl.) 6th year students.

ii) Honorary lecturer by Calcutta University to take both theory and practical classes on modern immunological aspects for M.Sc. 6th year Zoology (Immuno-parasitology Spl.) students.

iii) Resource person for the UGC sponsored Academic Staff College of Calcutta and Visva Bharati University's Orientation and Refresher Courses.

iv) Honorary lecturer for taking classes of 6th year Biotechnology students of Visva Bharati University.

v) Resource person for UGC sponsored academic staff college of Calcutta University, for teachers orientation programme, Department of Zoology.

vi) Member of ICMR "Intestinal Protozoan Task Force Committee".

vii) Referee of Journal of Post Graduate Medicine.

Dr. S.K. Niyogi, Assistant Director, acted in the following capacities :

i) Member of Central team jointly organised by central health services & ICMR. Investigated the course of outbreak of diarrhoeal diseases in super cyclone affected area of Orissa.

Dr. K.K. Banerjee, Assistant Director, acted in the following capacities :

i) Lecturer in the workshop "Man, Microbes and Environment" conducted by JBNSTS, Calcutta.

ii) Honorary lecturer of the Department of Biochemistry, UCM, Calcutta University.

Dr. A.K. Sinha, Assistant Director, acted in the following capacities :

i) Hony, Associate Editor, Indian Medical Journal.

ii) Hony. Treasurer, Society of Haematology and Blood Transfusion of West Bengal State Branch.

Dr. D. Sur, Senior Research Officer, acted in the following capacities:

i) Member of Editorial Board, Indian Journal of Public Health (official publication of Indian Public Health Association).

ii) Treasurer, Indian Journal of Public Health (official publication of Indian Public Health Association).

iii) Central Council Member of Indian Public Health Association.

iv) Joint Secretary of Indian Public Health Association (West Bengal Branch).

v) Member Standing Committee Bengal Task Force of Body Friendly, Hospital Initiative under UNICEF, Calcutta.

Dr. S. Dutta, Senior Research Officer, acted in the following capacities:

i) Supervised a NVPH student of Calcutta University as co-guide.

11. National Science Day Celebration, 2000

In connection with celebration of the National Science Day, the Institute organised an one day programme on February 29,2000. The theme for the year was "Recreating interest in basic sciences".

Dr. S.K. Bhattacharya, Director of the Insitute, in his inaugural speech explained about the theme of the celebration and also emphasised on the importance of recreating interest in basic science.

Shri D. Kohali from the Faculty of Department of Sanitary Engineering, All India Institute of Hygiene & Public Health, Calcutta was invited to deliver a scientific lecture on environmental pollution and health. Shri Kohali explained how environmental pollution and health and diseases are inter-related to each other. He also suggested its prevention and central measures.

12. Brain storming session on *Helicobacter pylori*

A brain storming meeting on *Helicobacter pylori* was organized at NICED, Calcutta on April 1st, 1999 by Dr. G.B. Nair to coincide with Dr. Douglas E. Berg's visit and was attended by many Indian scientists working with *H.pylori* from various parts of the country. The participants agreed that *H.pylori* research in India should emphasize features such as Hp epidemiology, pathophysiology associated with infection, appropriate treatment regimens and molecular genetic characterization of strains from different parts of India, especially in relation to peoples of different ethnicities. The need for further development of good animal model was also highlighted.

13. Referral Service from Vibrio Phage Reference Laboratory

During the year 1999-2000 the phages and propagating strains have been supplied from Vibrio Phage Reference Laboratory to the following Institutes within India and abroad.

1.	Dr. Kai Man Kam Department of Health The Government of the Hong Kong Special Administrative Region Sai Ying Pun, Hong Kong	*V.cholerae* Classical 154 & ϕ149
2.	Dr. R.K. Ghosh, IICB, Calcutta	*V.cholerae* ElTor Gr.V (Basu & Mukerjee)
3.	Dr. B.K. Lee, Director, National Institute of Health, Korea	*V.cholerae* ElTor Basu & Mukerjee - 5 phages; New phage - 5 phages & MAK 757
4.	Dr. S. Yamasaki, International Medical Centre of Japan, Japan	*V.cholerae* ElTor Basu & Mukerjee - 5 phages; New phage - 5 phages & MAK 757
5.	Dr. N. Mandal, Bose Institute, Calcutta	*V.cholerae* Classical 154 & ϕ149
6.	Dr. I. Sechler, Ministry of Health, Jerusalem	*V.cholerae* ElTor

		Basu & Mukerjee - 5 phages; New phage - 5 phages & MAK 757

14. Committee of the Institute:

Scientific Advisory Committee (SAC)

Prof. N.K. Ganguly

Director General, ICMR, Chairperson

Dr. V.I. Mathan

Divisional Director

Laboratory Science Division

Centre for Health & Population Research

Mohakhali, Dhaka - 1000, Bangladesh

Dr. M.K. Bhan

Professor

Department of Paediatrics

All India Institute of Medical Sciences

Ansari Nagar

New Delhi 110 029

Dr. Sandip K. Basu

Director

National Institute of Immunology

Aruna Asaf Ali Marg

New Delhi 110 067

Dr. D.C.S. Reddy

Prof. of Preventive & Social Medicine

Institute of Medical Sciences

Banaras Hindu University

Varanasi - 221 005

Dr. D. Mahalanabis

Director

Society for Applied Studies

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Flat No. 3/21

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Dr. Amit Ghose

Director

Institute of Microbial Technology

Sector 39-A

Chandigarh - 160 014

Dr. Sujoy Das

Director, Health Services

Department of Health and Family Welfare

Govt. of West Bengal

Writers Building

Calcutta - 700 001

Dr. Shyamal Kanti Bandopadhyay

Director of Medical Education

Govt. of West Bengal

Writers Building

Calcutta - 700 001

Dr. Ashish Datta

Vice-chancellor

Jawaharlal Nehru University

Aruna Asaf Ali Marg

New Delhi - 110 067

Prof. R.C. Mahajan

Emeritus Scientist

Department of Parasitology

Post Graduate Institute of Medical Education and Research

Chandigarh - 160 012

Dr. Lalit Kant

Senior Deputy Director General

Indian Council of Medical Research

Ansari Nagar

New Delhi - 110 029

Dr. K.P. Das

Superintendent

I.D. & B.G. Hospital

Beliaghata

Calcutta 700 010

Dr.(Mrs.) Ira Ray

Addl. Director General of Health Services

Directorate General of Health Services

Govt. of India

Nirman Bhawan

New Delhi - 110 011

Dr. S.C. Sehgal

Director

Regional Medical Research Centre

(Indian Council of Medical Research)

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Andaman and Nicobar Islands

Prof. K.B. Sharma

Centre for Infectious Diseases

Education & Research

B-7, Swasthya Vihar

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Dr. K.K. Datta

Director

National Institute of Communicable Diseases

22, Shamnath Marg, Post Box No. 1492

Delhi - 110 054

Dr.(Mrs.) Archana Ayyagari

Professor & Head

Department of Microbiology

Sanjay Gandhi Post Graduate Institute of Medical Sciences

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Lucknow - 226 014

Dr. D.A. Gadkari

Ex-Director

National Institute of Virology

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Pune - 411 004

Dr. Pradeep Seth

Professor and Head

Department of Microbiology

All India Institute of Medical Sciences

Ansari Nagar

New Delhi 110 029

Dr. Rashmi Arora

Deputy Director General (ECD)

Indian Council of Medical Research

Ansari Nagar

New Delhi - 110 029

Dr. S.K. Bhattacharya (Member Secretary)

Director, N.I.C.E.D., Calcutta

Library Committee

Dr.(Smt.) A. Dutta - Chair-Person

Dr. S. Chakrabarti - Member

Dr. P. Dutta - Member

Dr. T.N. Naik - Member

Dr. G.B. Nair - Member

Dr. M.K. Chakrabarti - Member

Mrs. Keya Roy - Member

Sri C.S. Dutta - Member

Sri S. Ghosh - Member

Purchase Committee

Dr.(Smt.) A. Datta - Chair-Person

Dr. S Chakraborty - Member

Dr. P. Dutta - Member

Dr. M.R. Saha - Member

Dr. R. Kumar - Member

Dr. G.B. Nair - Member

Dr. P.G. Sengupta - Member

Dr. T.N. Naik - Member

Dr. A.N. Ghosh - Member

Dr. M.K. Chakrabarti - Member

Dr. P. Das - Member

Sri C.S. Dutta - Member

Sri P.K. Ghoshal - Member

Official Language Implementation Committee

Dr. Tapas Biswas - Chairman

Dr.(Mrs.) Aparna Pandey- Member

Mr. C.S. Dutta - Member

Mrs. Keya Ray - Member

Mr. S. Ghosh - Member

Mr. C.R. Bose - Member

Mr. P. Sen - Member

Mr. Shyamal Banerjee - Member

Mr. Viswanath Besra - Member

Animal Ethics Committee

Dr. T.N. Naik - Chairman

Dr. R. Kumar - Member

Dr. M.K. Chakrabarti - Member

Dr. P. Das - Member

Dr. Ananga Mohan Chandra - Member

Dr. Ranatosh Chakrabarty - Member

Ms. Purnima Tulsidas - Representative

Member, (Nominated by the CPCSEA)

Dr. R.K. Sarkar - Member Secretary

Committee for Modernisation of the Animal House

Professor A.K. Roy - Chairman

Dr. A. Pal - Member

Dr. A.M. Chandra - Member

Dr. M.K. Chakrabarti - Member

Dr. R.K. Sarkar - Member Secretary

Institute's Ethical Committee

Justice Pinaki Ghosh - Chairman

Professor A.K. Choudhuri - Member

Professor S. Sikdar - Member

Dr. K.P. Das - Member

Dr. A.C. Ghosh - Member

Dr. Ananga Mohan Chandra - Member

Ms. Dipanwita Deb - Member

Dr. S.K. Bhattacharya - Member Secretary

Bio-safety Committee

Dr. S. Chakrabarti - Co-ordinator

Dr. P. Dutta - Bio-safety Officer

Dr. T.N. Naik - Member

Dr. G.B. Nair - Member

Dr. P. Das - Member

Dr. S. Sinha - Member

(Nominated by the DBT, Govt. of India)

Dr. R.K. Ghosh - External Expert (IICB, Calcutta)

SC/ST Cell

Dr. T.N. Naik - Liaison Officer

Dr. D.R. Saha - Member

Shri P. Bhadra - Member

Shri V. Besra - Member

Building Committee

Dr. Sandip Basu - Chairman

Dr. S.K. Bhattacharya - Project Manager & Co-ordinator

Dr. Amit Ghosh - Member

Dr. A.C. Ghosh - Member

Dr. Santu Banerjee - Member

Dr. Shyamal Kanti Bandhopadhayay - Member

Dr. K.P. Das - Member

Dr. S. Chakraborty - Member

Dr. P. Dutta - Member

Dr. T.N. Naik - Member

Dr. G.B. Nair - Member

Dr. M.K. Chakraborty - Member

Dr. M.K. Chakraborty - Member

Representative for ICMR/HQ - Member

Mr. P.K. Ghoshal - Member

Technical Committee

Dr.(Mrs.) A. Datta - Member Secretary

Dr. S. Chakraborty - Chairperson

Dr. T.N. Naik - Member

Dr. P. Das - Member

Dr. M.K. Chakraborty - Member

15. Scientific Conferences/Seminars/Meetings attended by the Scientists/Research Fellows

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
Dr. S.K. Bhattacharya	Participated in the Micronutrients, Maternal and Child Health, Indo-European symposium held at Goa, India during April 25-27, 1999	
	Attended the meeting of "Establishment of a Surveillance System" held at Nirman Bhaban, New Delhi on June 7, 1999	
	Attended the meeting of the Founder Members of the Society of the National Brain Research Centre and First meeting of the Governing Body of the National Brain Research Centre held at Department of Biotechnology, Ministry of Science & Technology, New Delhi on July 7, 1999	
	Attended the 11th Meeting of Directors/Officer in charge of ICMR Institutes/Centres held at India International Centre, New Delhi on August 4, 1999	
	Attended the 9th Meeting of the Scientific Advisory Group of ICMR held at ICMR Headquarter Office, New Delhi during August 5-6, 1999	
	Participated at the IXth International Congress of Bacteriology & Applied Microbiology and IXth International Congress of Mycology held at Sydney during August 16-20, 1999.	
Dr. S.K. Bhattacharya	Participated in the ICMR/WHO National Workshop on Health Research Management held at Chennai during September 14-16, 1999.	
	Attended the Meeting of the Food Microbiology Sub-committee of C.C.F.S as Chairman held at Nirman Bhaban, New Delhi on November 1, 1999.	

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	Acted as a member of the 13th Scientific Advisory Committee during the meeting of RMRC, N.E. Region, Dibrugarh held during November 5-6, 1999.	
	Attended the Expert Committee meeting for reviewing the applications/nominations for Sir Nilratan Sarkar Prize for the year 1998 held at Bose Institute on November 8, 1999.	
	Participated in the Regional Conference of WHO on Public Health in South East Asia in the 21st Century held at Calcutta during November 22-24, 1997	
	Participated in the 5th International Conference on Emerging Infectious Diseases in the Pacific Rim held at Chennai during January 7-9, 2000	
	Attended the Indo-US meeting on HIV/AIDS Prevention, Control and Research held at TRC, Chennai during January 11-12, 2000	
	Attended the meeting of the Executive Committee of the Council held at ICMR Hqrs., New Delhi on January 20, 2000	
	Attended the ICMR-INSERM meeting held at ICMR Hqrs on January 28, 2000	
Dr. S.K. Bhattacharya	Participated in the "Indo-US Workshop on Nutrition and Health of Women, Infants and Children" held at NIN, Hyderabad during February 10-12, 2000	
	Acted as a member of the Scientific Advisory Committee during the meeting of RMRC, Port Blair held on February 18, 2000	
	Attended and inaugurated the NACO - Workshop on STD and AIDS for Medical Officer organised by Institute of Serology, Calcutta on March 22, 2000	
Dr. P. Dutta	Delivered a talk at the 43rd Annual Conference of Indian Public Health Association held at AFMC, Pune	Role of hypo-osmolar oral rehydration solution (ORS) on outcome variables among patients

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	during April 15-18, 1999	suffering from persistent diarrhoea who are prone to develop clinical dehydration
	Attended workshop on `Leprosy' organised by National Institute of Cholera and Entric Diseases, in collaboration with West Bengal State Leprosy Society on November 16, 1999	
	Delifered a talk at the Seminar on perspective of environmental health : vector and water borne diseases held at Bose Institute, Calcutta, 4-5 December, 1999	Management and prevention of diarrhoeal diseases
	Attended XVII West Bengal State Conference of Indian Academy of Pediatrics at Howrah, 12 December, 1999	
Dr. P. Dutta	Organised UNICEF sponsored workshops on management of acute diarrhoea with special emphasis on cholera held at Dibrugarh and Guwahati during November 9-11 and 27-29, 1999 and at Malda during December 14-16, 1999 and March 22-24, 2000	Diarrhoeal disease control programme
	Attended ICMR sponsored workshop on "IEC training workshop on biomedical research" held at NIN, Hyderabad, during December 20-24, 1999.	
	Attended the workshop on "Environmental Epidemiology" Calcutta sponsored by Ministry of Environment and Forestry, Govt. of India and WHO held at Calcutta during February 21-25, 2000	
	Delivered an invited talk at the 44th All India Annual Conference of IPHA held at Agra during March 10-12, 2000	Double blind randomised clinical trial of hypo-osmolar oral rehydration salts solution in dehydrating

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
		acute diarrhoea in severely malnourished children
Dr. G.B. Nair	Organized and participated in the Brain Storming Meeting on *Helicobacter pylori* at the National Institute of Cholera and Enteric Diseases on April 1, 1999.	
	Invited as faculty member for training on PCR to the participants attending the "Hands on training workshop on laboratory diagnosis of leptospirosis" held at the Regional Medical Research Centre, Andamans and Nicobar Islands during May 6-8, 1999.	
Dr. G.B. Nair	Delivered an invited talk at the Central Agricultural Research Institute, Port Blair, Andamans and Nicobar Islands on May 7, 1999.	"Molecular epidemiology of cholera"
	Delivered DBT sponsored popular lectures at the Regional Research Laboratory (CSIR), Jorhat, Assam and Indian Institute of Chemical Biology (CSIR), Jadavpur, Calcutta on July 19 and 26, 1999.	"Molecular tracking of enteric pathogens"
	Participated in the Workshop on WHO collaborative centres held at India International Centre, New Delhi on August 6, 1999.	
	Participated in the IUMS Executive Board Meetings held at Sydney during August 13-15, 1999.	
	Convened the closed and open meetings of the ICSB Subcommittee on the Taxonomy of *Vibrionaceae* at Sydney during August 17-18, 1999.	
	Presented a poster at the IXth International Congress of Bacteriology and Applied Microbiology held at Sydney during August 16-20, 1999.	"Molecular epidemiology of *Vibrio cholerae* O139"

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	Delivered an invited talk at the Symposium on Research in Molecular Biology and Biotechnology in India. Challenges in the next Millennium held at Indian Institute of Chemical Biology during September 13-14, 1999.	"Pandemic clones of *Vibrio parahaemolyticus* : where does it come from and where is it going"
Dr. G.B. Nair	Participated in the ICMR/WHO National Workshop on Health Research Management organized by the Tuberculosis Research Centre, Chennei, September 14-16, 1999.	
	Participated in the 5th International Conference on Emerging Infectious Diseases in the Pacific Rim conducted by the U.S.-Japan Cooperative Medical Science Program held at Chennai during January 7-9, 2000.	
Dr. D. Sur	Delivered a talk at the XLIIIth All India Annual Conference of Indian Public Health Association held at Pune during April 15-18, 1999.	Impact of breast feeding on growth pattern and incidence of diarrhoea among Low Birth weight infants of an urban slum : An observational study
	Acted as Resource person in training programme on epidemiological surveillance of communicable diseases organised by World Bank funded State Health Systems Development Project of Govt. of West Bengal during July 20-21, 1999 .	
	Attended scientific programme on `AIDS & STD' on the occasion of Annual State Conference of Indian Public Health Association (WB State Branch) on August 15, 1999.	
	Acted as Adviser to International Consultation on `Action Research on Health Care Utilisation' organised by Centre for Research on Epidemiology of Disasters (CRED), Brussels and attended first meeting at New Delhi	

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	during September 26 to October 1, 1999.	
Dr. D. Sur	Attended workshop on `Leprosy' organised by National Institute of Cholera and Enteric Diseases, in collaboration with West Bengal State Leprosy Society on November 16, 1999.	
	Delivered a talk at the National Conference on Quality Assurance in Health Care at Park Hotel, Calcutta during November 20-21, 1999.	`Quality assurance in diarrhoea prevention and control programme'
	Acted as resource person for workshop on `Management of Acute Diarrhoea' held in Malda, organised by National Institute of Cholera and Enteric Diseases, Calcutta and Dept. of Health & Family Welfare, Govt. of West Bengal, sponsored by UNICEF, Calcutta during December 14-16, 1999.	
Dr. S. Chakrabarti	Participated at NIH/AVRC Workshop on "New concepts in HIV vaccine developments" held at Bethesda, USA during May 3-5, 1999	
	Participated at satellite vaccine meeting on "Current research and future plans for HIV vaccine trials in developing countries" held at John Hopkins University, Baltimore, USA, May 7, 1999.	
	Delivered a talk in Indo-US vaccine action program workshop on "Novel approaches to vaccine developments" held at NII, New Delhi, during October 26-27, 1999.	Optimization of promoter sequences for vaccinia virus expression system: A tool for vaccine research.
Dr. S. Chakrabarti	Attended a meeting to discuss the plans for establishment of a new vaccine development partnership in India held at International AIDS vaccine Initiative, New York, USA on November 8, 1999.	

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	Delivered a talk in the Annual meeting of Indian Association of Medical Microbiologists held at PGIMER, Chandigarh, during November 18-21, 1999.	Distribution of HIV-1 subtypes in eastern and north-eastern parts of India.
	Delivered a talk in the 5th international conference on emerging infectious diseases in the pacific rim held at Chennai, during January 7-9, 2000.	Recombinant pox virus as a candidate vaccine against HIV/AIDS.
	Participated at the Indo-US conference on HIV/AIDS prevention research at Tuberculosis Research Centre, Chennai, during January 11-12, 2000.	
Dr. T.N. Naik	Participated in the Dr. J. Das Memorial Symposium on Molecular Biology in Next Millennium at Indian Institute of Chemical Biology, Calcutta during September 13-14, 1999.	
	Delivered a talk at the training course on Laboratory Diagnosis of Animal Diseases and Zoonoses organized by Indian Veterinary Research Institute (Eastern Regional Centre), Calcutta on December 30, 1999.	Molecular approaches in the diagnosis of viral diseases in man and animals
Dr. P.G. Sengupta	Delivered a talk at the 43rd All India Annual Conference of IPHA held at AFMC, Pune during April 15-18, 1999	Hospital-based surveillance system for diarrhoeal diseases: some preliminary observations.
Dr. D.N. Gupta	Attended the scientific programme on AIDS and STD at the annual conference of IPHA, State Branch on August 15, 1999	
	Attended the workshop on Leprosy organised by NICED in collaboration with the WB State Leprosy Society on November 16, 1999	
Dr. S.K. Mondal	Delivered a talk at the 43rd All India Annual Conference of IPHA held at AFMC, Pune during April 15-18,	An education intervention to reduce diarrhoeal morbidity in a

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	1999	rural community of West Bengal.
Dr. P. Das	Delivered a talk at the symposium on Biological Research in the next Millennium organised by Society of Biological Chemists, Calcutta Branch held at Calcutta University on July 12, 1999	Role of ventral disc cytoskeletal proteins of *Giardia lamblia in* addition to target cells
	Delivered an invited talk at the XXIII National Congress Indian Association of Medical Microbiologists, held at PGI, Chandigarh on November 18, 1999.	Antigens of diagnostic and prophylaxis importance in Entamoeba histolytica.
	Delivered an invited talk at National Institute of Pharmaceutical Education and Research, Mohali, Punjab, on November 19, 1999.	Role of 29 kDa polypeptide in amoebiasis.
	Delivered a talk at the Seminar on Perspectives in environmental health : vector and water borne diseases, organized by National Environmental Science Academy West Bengal, Chapter, in collaboration with International College of Nutrition, Calcutta Chapter on December 5, 1999.	PCR detection of *Giardia lamblia* in stool : targeting intergenic spacer region of muticopy rRNA gene
Dr. P. Das	Delivered an invited talk at the Department of Zoology, Viswa Bharati University, West Bengal, on December 14, 1999.	Importance of monoclonal antibody in medical science.
	Delivered an invited talk at the Department of Zoology, Ballygunge Science College, Calcutta University, on January 7, 2000.	Hybridoma technology : some applications.
	Attended the second annual meeting of the joint coordinating committee of the collaborative project organised by JICA held at NICED, Calcutta on March 13, 2000	
	Attended the meeting of Immunology study group, West Bengal held at Bose Institute, Calcutta on March 15, 2000	

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
Dr. A.K. Sinha	Attended in CME on Haematology and workshop on Pediatric Oncology organized by Indian Academy of Pediatrics held at Burdwan during June 18-20, 1999.	
	Attended the 10th IAPM Conference of West Bengal Chapter held at R.G. Kar Medical College, Calcutta during October 9-10, 1999.	
	Delivered a talk at Golden Jubilee IAPM National Conference held at Sanjay Gandhi PGI, Lucknow, during November 22-25, 1999.	Role of cytokines (IFN- and IL-2) in host-immunity in shigellosis.
	Attended the first Annual Conference of Society of Haematology and Blood Transfusion held at N.R.S. Medical College, Calcutta, during November 27-28, 1999.	
Dr. M.K. Chakrabarti	Delivered a talk at UGC refresher course for college teachers in Physiology. Academic Staff College, Calcutta University, on April 19, 1999.	Enteric bacterial toxins and their mechanism of action.
	Organised Prof. N.M. Basu Memorial Oration and delivered a talk at 87th session of Indian Science Congress held at Pune during January 3-7, 2000	Age related differences in density of *Escherichia coli* heat stable toxin receptor, guanylate cyclase activity and intracellular calcium level in the brush border membranes of rabbit intestinal epithelial cells
	Delivered a talk at UGC Refresher course for college teachers in Zoology. Academic Staff College, Calcutta University, on February 4, 2000.	
	Attended the second annual meeting of the joint coordinating committee of the collaborative project organised by JICA held at NICED, Calcutta on March 13, 2000	Role of *Escherichia coli* as a diarrhoeagenic pathogen
Dr. M.K. Bhattacharya	Conducted UNICEF sponsored	

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	training programme on management of acute diarrhoea with special emphasis on cholera held at Guwahati during November 27-29, 1999.	
	Conducted UNICEF sponsored training programme on management of acute diarrhoea with special emphasis on cholera held at Dibrugarh during November 9-11, 1999.	
Dr. M.K. Bhattacharya	Attended meetings on research priorities, advances in design and analysis of epidemiological studies held at A.I.I.M.S., New Delhi during March 6-11, 2000.	
	Attended International Symposium on Micronutrient, Maternal and Child Health (INDO-EUROPEAN) held at Goa during April 25-27, 1999.	
	Attended meetings on research priorities, advances in design and analysis of epidemiological studies at A.I.I.M.S, New Delhi from March 6-11, 2000	
Dr. S. Dutta	Attended refresher course of W.H.O./IUIS on "Immunology, Vaccinology, Biotechnology applied to Infectious Diseases" held at Pune during November 24 to December 10, 1999.	
Dr. S. Ghosh	Delivered a talk at the 43rd All India Annual Conference of IPHA held at AFMC, Pune during April 15-18, 1999	Mothers education is a consistent determinant for diarrhoea: results of three prospective community based study.
Dr. D.R. Saha	Attended the 40th Annual Conference of Indian Society of Gastroenterology held at Science City, Calcutta during November 17-20, 1999.	
Dr. T. Ramamurthy	Participated in the 35th US-Japan cholera and other bacterial enteric	

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
	infections. Joint Panel Meeting held at Baltimore, USA during December 3-5, 1999.	
Dr. B. Manna	Delivered a talk at the 43rd All India Conference of IPHA held at Pune during April 15-18, 1999	Risk factors for life threatening dehydration
Dr. B.L. Sarkar	Delivered a talk at the National conference on plants, microbes and environment organised at the University of Burdwan on March 12, 2000	Cholera bacteriophages and its implication in cholera disease
Dr. T. Krishnan	Delivered a talk at the centenary year celebration of Haffkine Institute of Training Research & Testing held at Haffkine Institute, Mumbai, on July 6, 1999.	Rotavirus : molecular epidemiology and puzzling diversity.
	Presented a poster at the XI International Congress of Virology held at Sydney Convention Centre, Darling Harbour, Sydney during August 9-13, 1999.	Detection of rotavirus infections among adults and older children in Calcutta, India.
	Delivered a talk at the Seminar on Perspectives in Environmental Health: Vector and Water Borne Diseases organised by National Environmental Science Academy, West Bengal Chapter in collaboration with International College of Nutrition, Calcutta Chapter held at Bose Institute, Calcutta, during December 4-5, 1999.	Role of environmental factors in etiology of viral infections.
	Delivered an invited talk at the laboratory workshop on DNA profiling of forensic specimens using STRs markers held at Bureau Police Research and Development, Calcutta during March 21-31, 2000	Mono and multiplex PCR reactions and PCR inhibitors
Dr. A. Pal	Presented a poster at the 68th Annual Meeting of Society of Biological Chemists (India) held at Bangalore during December 27-29, 1999.	Rise in free intracellular calcium in HeLa cells infected with aggregarive *Klebsiella pneumoniae* strains isolated from cases of diarrhoea

Participants	Conferences/Seminars/Meetings	Title of Papers/Talk
Shri A. Sen	Presented a poster during the 68th annual meeting of the society of biological chemists and symposium on current trends in biology held at the Inidna Institute of Science, Bangalore during December 27-29, 1999	Amplification of various genes of the adult diarrhoea rotavirus isolated from India
Shri S. Das	Presented a poster during the 68th annual meeting of the society of biological chemists and symposium on current trends in biology held at the Inidna Institute of Science, Bangalore during December 27-29, 1999	Predominance of human group A rotavirus G serotype-4 in Calcutta

16. List of Publications

Original Publication

1. Ahamed J, Gangopadhyay J, Kundu M and Sinha AK (1999). Mechanisms of quinolone resistance in clinical isolates of *Shigella dysenteriae* (1999). Antimicrob Agents Chemother 43(9): 2333-2334.

2. Basu A, Garg P, Datta S, Chakraborty S, Bhattacharya T, Khan A, Ramamurthy T, Bhattacharya SK, Yamasaki S, Takeda Y and Nair GB 2000. *Vibrio cholerae* O139 in Calcutta, 1992-1998: incidence, antibiograms, and genotypes. Emerg Infect Dis 6: 139-147.

3. Basu A, Mukhopadhyay AK, Garg P, Chakraborty S, Ramamurthy T, Yamasaki S, Takeda Y and Nair GB (2000). Diversity in the arrangement of CTX prophages in classical strains of *Vibrio cholerae* O1. FEMS Microbiol Lett 182: 35-40.

4. Basu I, Mitra R, Saha PK, Ghosh AN, Bhattacharya J, Chakrabarti MK, Takeda Y and Nair GB (1999). Morphological and cytoskeletal changes caused by non-membrane damaging cytotoxin of *Vibrio cholerae* on Int 407 and HeLa cells. FEMS Microbiol Lett 179: 255-263.

5. Biswas D, Bagdasarian M and Kumar R (1999). *Shigella dysenteriae* type 1 carrying LPS biosynthesis genes of *Salmonella typhimurium* affects both invasive plasmid antigen H (IpaH) secretion and invasion. World J Microbiol Biotech 15: 693-698.

6. Bhattacharya J and Chakraborti MK (1999). Binding of *Escherichia coli* heat stable

toxin and rise of guanylyl cyclase activity in the brush border membranes of rabbit intestinal epithelial cells. J Diarrhoeal Dis Res 17: 28-33.

7. Bhattacharya MK and Khaled MA (1999). Higher body fat aggravates toxin induced infectious episodes. Metabolism 48(8): 946-948.

8. Chakrabarti AK, Ghosh AN, Nair GB, Niyogi SK, Bhattacharya SK and Sarkar BL (2000). Development and evaluation of a phage typing scheme for *Vibrio cholerae* O139. J Clin Microbiol 38: 44-49.

9. Chakrabarti MK, Bhattacharya J, Bhattacharya MK, Nair GB, Bhattacharya SK and Mahalanabis D (1999). Killed oral shigella vaccine made from *Shigella flexneri* 2a protects against challenge in the rabbit model of shigellosis. Acta Paediatr 88: 161-165.

10. Chakraborty S, Khanam J, Takeda Y and Nair GB (1999). Application of PCR for detection of toxigenic *Vibrio cholerae* O1 in water samples during an outbreak of cholera. Trans Roy Soc Trop Med Hyg 93: 1-2.

11. Dutta P, Mitra U, Dutta S, Manna B, Chatterjee MK, De A and Bhattacharya SK (2000). Hypo-osmolar oral rehydration salts solution in dehydrating persistent diarrhoea in children: double-blind, randomized, controlled clinical trial. Acta Pediatr 89: 411-416.

12. Dutta P, Mitra U, Saha DR, Niyogi SK, Manna B and Bhattacharya SK (1999). Mucoid presentation of acute enterocolitis in children : A hospital based case control study. Acta Pediatr 88: 822-826.

13. Dutta S, Pal S, Chakrabarti S, Dutta P and Manna B (1999). Use of PCR to identify enteroaggregative *Escherichia coli* as an important cause of acute diarrhoea among children living in Calcutta, India. J Med Microbiol 48: 1011-1016.

14. Faruque SM, Saha MN, Asadulghani, Bag PK, Bhadra PK, Bhattacharya SK, Sack RB, Takeda Y and Nair GB (2000). Genomic diversity among *Vibrio cholerae* O139 strains isolated in Bangladesh and India between 1992 and 1998. FEMS Microbiol Lett 184: 279-284.

15. Ghosh S, Mahapatra NR, Ramamurthy T and Banerjee PC (2000). Plasmid curing from an acisophilic bacterium of the genus Acidocella. FEMS Microbiol Lett 183: 271-274.

16. Guhathakurta B, Sasmal D, Pal S, Chakraborty S, Nair GB and Dutta A (1999). Comparative analysis of cytotoxin, hemolysin, hemagglutinin and exocellular enzymes among clinical and environmental isolates of *Vibrio cholerae* O139 and non-O1, non-O139, FEMS Microbiol Lett 179: 401-407.

17. Guthakurta B, Sasmal D, Ghosh AN, Kumar R, Saha P, Biswas D, Khetawal D and Datta A (1999). Adhesion and invasion of a mutant *Shigella flexneri* to an eukaryotic cell line in absence of the 220-kb virulence plasmid. FEMS Microbiol Lett 181: 267-275.

18. Kurazono H, Yamamoto S, Nakano M, Nair GB, Terai A, Chaicumpa W and Hayashi H (2000) Characterization of a putative virulence island in the chromosome of uropathogenic *Escherichia coli* possessing a gene encoding a uropathogenic - specific.

Microb Pathog 28: 183-189.

19. Matsumoto C, Okuda J, Ishibashi M, Iwanaga M, Garg P, Ramamurthy T, Wong H, Depaola A, Kim, Y, Albert MJ and Nishibuchi M 2000. Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and *toxRS* sequence analyses. J Clin Microbiol 38: 578-585.

20. Nair GB and Holmes B (1999). Minutes of the closed meeting, May 19, 1998, Atlanta, USA. International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Vibrionaceae*. Int J Sys Bacteriol 49: 1945-1947.

21. Nandy RK, Mukhopadhyay S, Ghosh AN and Ghose AC (1999). Antibodies to the truncated (short) form of O polysaccharides (TFOP) of *Vibrio cholerae* O139 lipopolysaccharides protect mice against experimental cholera induced by encapsulated O139 strains and such protection is mediated by inhibition of intestinal colonisation of vibrios. Vaccine 17: 2844-2852.

22. Niyogi SK, Dutta D, Bhattacharya MK and Bhattacharya SK (1999). Multi-drug resistant non-typhoidal *Salmonella* spp. associated with acute diarrhoeal disease. Indian J Med Res 110: 183-185.

23. Niyogi SK, Dutta P and Mitra U (2000). Trends in antimicrobial resistance of Shigella species isolated from children with acute diarrhoea. Indian Paediatr 37(3): 339-341.

24. Pal A, Ghosh S, Ramamurthy T, Yamasaki S, Tsukamoto T, Bhattacharya SK, Nair GB and Takeda Y (1999). Shiga-toxin producing *Escherichia coli* from healthy cattle in a semi-urban community in Calcutta, India. Indian J Med Res 110: 83-85.

25. Pal A, Saha PK, Nair GB, Yamazaki S, Takeda T, Takeda Y, Bhattacharya SK and Ramamurthy T (1999). Clonal analysis of nontoxigenic *Vibrio cholerae* O1 associated with an outbreak of Cholera. Indian J Med Res 109: 208-211.

26. Pal S, Chandra S, Chowdhury S, Sarkar D, Ghosh AN and Das Gupta C (1999). Complementary role of two fragments of domain V of 23S ribosomal RNA in protein folding. J Biol Chem 274: 32771-32777.

27. Saha MK, Chakraborty S, Panda S, Naik TN, Manna B, Chatterjee A, Detels R and Bhattacharya SK (2000). Prevalence of HCV & HBV infection amongst HIV seropositive intravenous drug users & their non-injecting wives in Manipur, India. Indian J Med Res 111: 37-39.

28. Sarkar BL, Roy MK, Chakrabarti AK and Niyogi SK (1999). Distribution of phage type of *Vibrio cholerae* O1 biotype ElTor in Indian scenario (1991-98). Indian J Med Res 204-207.

29. Sozhamannan S, Deng YK, Li M, Sulakvelidze A, Kaper JB, Johnson JA, Nair GB and Morris JG (1999). Cloning and sequencing of the genes downstream of the *wbf* gene cluster of *Vibrio cholerae* serogroup O139 and analysis of the junction genes in other serogroups. Infect Immun 67: 5033-5040.

30. Walia K, Ghosh S, Singh H, Nair GB, Ghosh A, Sahni G, Vohra H and Ganguly NK

(1999). Purification and characterization of novel toxin produced by *Vibrio cholerae* O1. Infect Immun 67: 5215-5222.

31. Yamasaki S, Garg S, Nair GB and Takeda Y (1999). Distribution of *Vibrio cholerae* O1 antigen biosynthesis genes among O139 and other non-O1 serogroups of *Vibrio cholerae*. FEMS Microbiol Lett 179: 115-121.

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Other publications

1. Biswas A, Biswas R, Manna B and Dutta K (1999). Risk factors of acute respiratory infections in underfives of urban slum community. Indian J Pub Hlth 43: 73-75.

2. Bhattacharya SK and Dutta P (1999). Vaccine for common enteric pathogens. Achievements and prospects. Child and Newborn 3: 107-114.

3. Das P, Debnath A. and Munoz ML (1999). Molecular mechanism of pathogenesis in amoebiasis. Indian J Gastroenterol 18: 161-166.

4. Dutta P (1999). Has treatment for childhood diarrhoea changed? Indian J Pub Hlth XXXXIII: 93.

5. Dutta P and Bhattacharya SK (1999). Should gastrointestinal infections in children be treated by fluoroquinolones? Child and Newborn 3: 82-86.

6. Dutta P (1999). Environment and diarrhoeal diseases : A public health prospective. Indian J Pub Hlth XXXXIII: 57.

7. Gupta DN, Sarkar BL, Bhattacharya MK, Sengupta PG and Bhattacharya SK (1999). An ElTor cholera outbreak in Malda district, West Bengal. J Commun Dis 31: 49-52.

8. Roy S, Biswas D and Kumar R (2000). Degradation of petroleum hydrocarbons by a *Pseudomonas* strain isolated from Haldia port. Proceedings of National conference on plants, microbes and environment held at Burdwan, West Bengal.

9. Saha MK, Dutta P and De SP (1999). Possibility of public health hazards in contamination of toxin though fishes reared by swage fed fishery. Indian J Pub Hlth XXXXIII: 71-72.

10. Sur D (1999). Community action for health. Your Health XXXXVII: 61-62.

Article/Chapter in Books

1. Bhattacharya SK, Deb AK and Nair GB (1999). Enteric pathogens. In: National Report (1994-1999) of the Indian National Committee of the International Union of Microbiological Societies (IUMS). Indian National Science Academy, New Delhi, p.227-246.

2. Dutta P (2000). Acute diarrhoea. In: Recent Advances in Pediatrics. Special column 6.

Gastroenterology, hepatology, nutrition. Ed. Gupta S. pp.71-92.

3. Garg P, Ramamurthy T, Ghosh A, Bhattacharya SK, Takeda Y and Nair GB (1999). Molecular shifts within clonal populations of *Vibrio cholerae* O1 and O139 and its impact on the persistence and spread of cholera. In: Molecular Intervention in Disease, Ed. S. Gupta and O.P. Sood. Proceedings of the Fifth Annual Ranbaxy Science Foundation Symposium, New Delhi, India. Pages 43-49.

4. Garg P, Basu A, Ramamurthy T, Sharma C, Maiti S, Ghosh A, Bhattacharya SK, Takeda Y and Nair GB 2000. Emerging clones of *Vibrio cholerae* and multi drug resistance. In. Multi-Drug Resistance in Emerging and Re-emerging Diseases. Ed. R.C. Mahajan and A. Therwath. Indian National Science Academy, New Delhi, India, pp.31-42.

5. Ghosh A, Thungapathra M, Sharma C, Gupta N, Ghosh RK, Mukhopadhyay A, Kole H, Nair GB (2000). Efforts towards the development of oral cholera vaccines in India against the backdrop of global endeavours. In: Diarrhoeal Diseases Research Perspectives. Ed: N. Appaji Rao and N.K. Ganguly, Indian National Science Academy, Narosa Publishing House, New Delhi; pages 1-16.

6. Garg P, Ramamurthy T, Ghosh A, Bhattacharya SK, Takeda Y and Nair GB (1999). Molecular shifts within clonal populations of *Vibrio cholerae* O1 and O139 and its impact on the persistence and spread of cholera. In: Molecular intervention in disease, Ed. S. Gupta and O.P. Sood. Proceedings of the Firth Annual Ranbaxy Science Foundation Symposium, New Delhi, India Pages 43-49.

7. Morris JG and Nair GB (1999). "Noncholera" *Vibrio* infections. In: Tropical Infectious Diseases: Principles, Pathogens and Practice. Ed: R.L. Guerrant, D.H. Walker and P.F. Weller. Published by Churchill Livingstone, Harcourt Brace and Company, Pages 336-344.

8. Takeda T, Yoshino K, Ramamurthy T and Nair GB (1999). Heat stable enterotoxins of *Vibrio* and *Yersinia* species. In: The Comprehensive Sourcebook of Bacterial Protein Toxins. Ed: Joseph E. Alouf and John H. Freer, Second Edition. Academic Press, Pages 545-556.

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Proceeding/Abstract

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