ABSTRACTS BOOK

ASCODD 2003
In Dhaka Bangladesh

10TH ASIAN CONFERENCE ON DIARRHOEAL DISEASES AND NUTRITION

7-9 DECEMBER 2003

Theme: "Improving Child Health and Nutrition"

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ABSTRACTS BOOK

10TH
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DIARRHOEAL DISEASES AND NUTRITION
7-9 December 2003

Bangladesh-China Friendship Conference Centre, Dhaka

"Improving Child Health and Nutrition"

ICDDR,B: Centre for Health and Population Research
Mohakhali, Dhaka 1212, Bangladesh

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Preface

On behalf of the Local Organizing Committee and ICDDR,B, I am pleased to welcome you to the 10th Asian Conference on Diarrhoeal Diseases and Nutrition. I hope that you will find the presentations informative and inspiring. We are indebted to the members of the Local Organizing Committee, which includes individuals from both national and UN organizations, for guidance and support in organizing this conference. I would also like to thank the Scientific Committee for its hard work in screening abstracts and preparing the programme, the Publications Committee for the production of the Abstracts Book and the Programme Book, and other committees which have helped in organizing this conference.

I would especially like to thank the Government of Bangladesh for agreeing to host this prestigious scientific event.

This conference will remind us of a unifying theme that brings us together: that many children, especially the poor, continue to suffer needlessly from ill health, and much can be done to help them. It would be particularly important to develop and test strategies to save lives and improve health, which are easily implementable at low cost. The recent series of publications appearing in the *Lancet* show how interventions can help these children, and this conference will focus on many such interventions. This meeting will go beyond the existing strategies and will explore new experimental interventions that will help these children in the future.

This conference will give us the opportunity to meet with old friends and make new ones. Often these friendships lead to exchange of ideas, concepts and innovations, and mutually beneficial collaborations. Please take the time to meet your colleagues from other countries in Asia and around the world.

David A. Sack, MD
Director, ICDDR,B
and
Chair, Organizing Committee

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DAY 1: SUNDAY, 7 DECEMBER 2003

09:00 am-09:45 am (Venue: Main Conference Hall)
Plenary 1: Diarrhoeal Disease

001* (359+)

Role of Micronutrients in the Case Management Strategy of Childhood Illnesses

Dilip Mahalanabis

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Role of micronutrients, namely zinc, vitamin A, and folate, as adjunct therapy of illness episodes in children in developing countries will be discussed in the ligh2t of health policy. This is based on a selective review and some of our attempts to statistically combine results of relevant studies to address policy issues. Many studies in this subcontinent and other developing countries reported the use of zinc supplements to treat acute diarrhoea along with oral rehydration therapy. These studies clearly show benefit in reducing the duration and severity of the episodes. In some trials in children with acute diarrhoea, when zinc was given daily for 7 to 14 days beyond recovery, diarrhoeal and respiratory morbidity was reduced over 4 to 8 weeks following recovery. The reported follow-up impact has substantial implication for health policy. Before zinc is considered in primary healthcare programme for treating acute diarrhoea, we need additional effectiveness trials on reduction of hospitalization or deaths due to diarrhoea, impact on oral rehydration solution (ORS) use and on use of antimicrobials and probiotics commonly prescribed in developing countries. While competition with antibiotics and probiotics is desirable that with ORS is not, large ongoing field trials could provide essential information on these policy issues. Concerning policy, zinc supplementation as a component of a micronutrient mixture is recommended in the rehabilitation of severely-malnourished children and in persistent diarrhoea. Present evidence suggests that use of zinc in diarrhoea may be an effective intervention to reduce hospitalization and child mortality if one could reach the most vulnerable children in the community. In an unpublished study, authors have found that zinc as adjunct therapy of pneumonia in infants and young children render benefit in boys but not in girls. Studies are needed to evaluate the role of zinc in the treatment of childhood pneumonia. In developing-country children, vitamin A supplementation during illness has a profound effect in reducing mortality from measles, a significant effect in reducing persistent episodes in children with acute diarrhoea but no benefit in the treatment of non-measles pneumonia. Several studies reported adverse effect of large-dose vitamin A in children with pneumonia and, it is, therefore, not recommended. Use of large-dose vitamin A is, however, recommended during measles episodes. Its use in acute diarrhoea is debatable but recommended in persistent diarrhoea and in severe malnutrition as a component of a micronutrient mixture. Large-dose vitamin A supplementation should be used with caution in young infants, as there are unresolved concerns about its safety, particularly bulging fontanelle observed in infants when co-administered at immunization. In two randomized controlled trials, folic acid has been evaluated in acute and persistent diarrhoea and found to have no beneficial effect. Folate is, therefore, not recommended as adjunct therapy of diarrhoea. Role of folate, however, in preventing severe disease and/or death from diarrhoea or pneumonia deserves further evaluation. The latter is based on an elegant animal study conducted at ICDDR,B.

09:00 am-10:00 am (Venue: Meeting Room 1)
Scientific Session 1: Environmental Health 1

002 (196)

Relationship Between Meteorological Elements and Diarrhoeal Diseases in Bangladesh

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Background: Previous study demonstrated the climate phenomenon accounted for over 70% of diarrhoeal disease variance. A study in Peru showed that the 1997-1998 El Nino event had an effect on infectious disease hospital admissions greater than explained by the regular seasonal variability in ambient temperature. **Objective:** Present quantitative evidence of the role of seasonal and interannual climate variability on the dynamics of an infectious disease. **Methodology:** Time scales of meteorological elements used were diurnal, intra-seasonal, seasonal and inter-annual variations (available for 1950-2001). Meteorological elements available were pressure, wind direction and speed, temperature, relative humidity, cloud amount, precipitation, maximum and minimum temperature, and sunshine hours routinely collected by the Bangladesh Meteorological Department. Diarrhoeal disease surveillance data from the Dhaka Hospital of ICDDR,B from 1980 to 2001 provided numbers of cases of cholera, rotavirus, and other diarrhoeal diseases. Lag-correlation and time-series regression models were used for assessing the effects of meteorological phenomena on the epidemiology of diarrhoeal diseases. **Results:** Two peaks of diarrhoeal diseases were found: the first peak was in April-May, and the second peak in August-October. The first peak lagged behind the rise in ambient temperature by about one month and was related with low temperature during the preceding winter. The second peak corresponded with higher overall rainfall, especially with the latter half of the rainy season. The effect of ambient temperature on incidence of diarrhoeal diseases was the greatest during the winter months. **Conclusion:** Ambient temperature and rainfall patterns were associated with incidence patterns of diarrhoeal diseases. Distinguishing characteristics were found between the first and the second diarrhoeal peaks. Further studies are required to confirm and measure the impact of meteorological patterns and events on epidemic diseases. **Acknowledgements:** The study was supported by the National Science Fund of Japan through the Disaster Prevention Research Institute, Kyoto University. The financial support of the United States Agency for International Development (USAID) is acknowledged for the Diarrhoeal Surveillance System in ICDDR,B. The authors are grateful to the Bangladesh Meteorological Department for their collaboration and provision of meteorological information.

Role of Chemotaxis in Association with *Vibrio cholerae* O1 with a Cyanobacterium, *Anabaena* sp.

M.S. Islam[1] (sislam@icddrb.org), M.M. Goldar[2], M.G. Morshed[2], H.B.M. Bakht[1], R.B. Sack[3], and D.A. Sack[1]

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Background: It has been demonstrated that the mucilaginous sheath of a blue-green algae, *Anabaena* sp., can provide a microenvironment for long-term survival of *Vibrio cholerae* O1 in the aquatic environment of Bangladesh. As mucinase is responsible for the degradation of mucilaginous sheath, its activity could be an essential tool for measuring chemotactic response of *V. cholerae* O1 towards mucilaginous sheath. **Objective:** Compare the chemotactic response of wild-type *V. cholerae* O1 (*hap*+, i.e. mucinase-positive) and its isogenic mutant *V. cholerae* O1 (*hap*-, i.e. mucinase-negative) to mucilaginous sheath of *Anabaena* sp. **Methodology:** The chemotactic response of *V. cholerae* O1 towards mucilaginous sheath of *Anabaena* sp. was investigated by the capillary tube method using a virulent strain of *V. cholerae* O1, ElTor, Ogawa (3083-T) and its isogenic mutant (*hap*-1-T) that lacks the hap gene, which codes for mucinase (HA/protease). Homogenates of *Anabaena* sp. and partially-purified mucin were used as chemoattractants. **Results:** Results showed 5.7% bacterial accumulation of wild-type *V. cholerae* O1 towards 4% homogenates of *Anabaena* sp., whereas its mutant (hap) showed 2.9% accumulation after 90 minutes. In porcine mucin, the wild-type *V. cholerae* O1 showed 4.4% accumulation towards 2% solution of mucin, but the mutant showed l% accumulation after 90 minutes. The higher percentage of attraction of wild-type *V. cholerae* O1 than the mutant (*hap*-) towards mucin and the homogenates of *Anabaena* sp. may be due to the activity of mucinase. **Conclusion:** The results indicate the important role of enzyme mucinase in the chemotactic motility of *V. cholerae* O1 towards *Anabaena* sp., which ultimately help *V. cholerae* O1 in the long-term survival in association with *Anabaena* sp. **Acknowledgements:** The research was funded by the National Institutes of Health (NIH) (grant no. 1R01A139129-01), USA and by ICDDR,B: Centre for Health and Population Research.

Role of Cyanobacteria in Persistence of *Vibrio cholerae* O139 in Microcosms

M.S. Islam[1] (sislam@icddrb.org), S. Mahmuda[2], M.G. Morshed[2], H.B.M. Bakht[1], M.N.H. Khan[1], R.B. Sack[3], and D.A. Sack[1]

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Background: It was demonstrated by both laboratory and field studies that cyanobacteria (blue-green algae), particularly *Anabaena* spp., can act as a long-term reservoir of *Vibrio cholerae* O1 in the aquatic environment. However, it is not known whether cyanobacteria also can act as a reservoir of new serotype of *V. cholerae* O139. **Objective:** Find out the role of various cyanobacteria as reservoirs of both culturable and viable but non-culturable (VBNC) *V. cholerae* O139 in microcosms. **Methodology:** An environmental isolate of *V. cholerae* O139 and 3 cyanobacteria (*Anabaena* sp., *Nostoc* sp., and *Hapalosiphon* sp.) were used. Survival of culturable *V. cholerae* O139 in microcosms was monitored using taurocholate-tellurite gelatin agar medium. VBNC *V. cholerae* O139 were detected using the fluorescent antibody technique. **Results:** *V. cholerae* O139 could be isolated up to 12 days in a culturable form in association with cyanobacteria but could not be isolated in the culturable form after 2 days from control water without cyanobacteria. VBNC *V. cholerae* O139 could be detected in association with cyanobacteria for up to 12 months. **Conclusion:** The results suggest that cyanobacteria can act as a long-term reservoir of *V. cholerae* O139 in the aquatic environment. **Acknowledgements:** The research was funded by the National Institutes of Health (NIH) (grant no. 1R01A139129-01), USA and by the ICDDR,B: Centre for Health and Population Research.

Arsenic Epidemiology in Matlab: Some Preliminary Observations

Mohammad Yunus[1] (myunus@icddrb.org), Mahfuzar Rahman[1], Samar Kumar Hore[1], Chandra Sekhar Das[1], Sultana Yeasmin[1], and Lars Åke Persson[1,2]

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Background: A major proportion of tubewells for drinking-water in Bangladesh are contaminated with arsenic. Health consequences of this exposure will be extensive. **Objective:** Investigate arsenic-associated skin lesion, arsenic in tubewell water, and association of arsenicosis with other chronic diseases. **Methodology:** Trained field staff screened all individuals aged >5 years in the ICDDR,B Matlab field area for arsenic-induced skin lesions and measured arsenic contamination in all functioning (n=12,334) tubewells by the Merck screening test kit method. Suspected cases of skin lesions were referred for confirmation and randomly selected 1,579 referents were invited for clinical examinations. **Results:** In total, 165,897 individuals were screened for arsenic-induced skin lesions and referred 1,598 suspected cases for confirmation. Study physicians confirmed 578 cases with a mean age of 41.2±13.7 years. The overall prevalence was 3.5/1,000. Males had significantly higher prevalence than females ((4.5 vs 2.7/1,000). The prevalence was significantly higher (4.75/1,000) among the population aged ³15 years compared to the age group of 10-14 years (0.16/1,000). However, no significant difference was observed among individuals aged 15-49 years and ³50 years. A significantly higher prevalence of diabetes mellitus (1.55% vs 0.56%), hypertension (3.97% vs 1.26%), and chronic respiratory problems (10.2% vs 2.4%) was observed among cases with skin lesions compared to the referents. The screening test results revealed that 70% of the tubewells had an arsenic concentration of 50 µg/L or above, 28% had 500 µg/L or above, and only 23% had no arsenic content. **Conclusion:** A major part of the population in Matlab is exposed to arsenic in drinking-water. In addition to skin lesions, risks are increased for chronic diseases, such as diabetes, hypertension, and chronic respiratory diseases. **Acknowledgements:** The study was supported by SIDA, World Health Organization, and United States Agency for International Development (USAID).

09:00 am-10:00 am (Venue: Meeting Room 3)
Special Session 1: IMCI

006 (360)

Child Health: Challenges and Future Directions

Hans Troedsson

Department of Child and Adolescent Health and Development,
World Health Organization, Geneva, Switzerland

Despite progress in the last decades, almost 11 million children still die every year. The number of children and adolescents who died last year was double the total number of adult's deaths from AIDS, tuberculosis, and malaria combined. Ninety-nine percent of these child deaths are in developing countries, and more than half of the deaths are caused by malnutrition and just five communicable diseases: pneumonia, diarrhoea, measles, malaria, and HIV/AIDS. The majority of these deaths are avoidable, as effective low-cost interventions are available that can prevent at least two-thirds of the deaths. Child health programmes need to move beyond addressing single diseases and instead address the overall health and well-being of the child. Integrated Management of Childhood Illness (IMCI) is one way forward. IMCI is a comprehensive strategy to reduce mortality and morbidity in children aged under 5 years and promoting their health, growth, and development. IMCI was developed by WHO and UNICEF in collaboration with countries and paediatricians around the world during the 1990, and was launched for implementation in 1996. It includes a number of complementary interventions to improve the performance of health workers, health systems, and family and community practices. Today, IMCI is at some stage of implementation in almost 100 countries. The implementation of IMCI has shown that technically sound case-management guidelines that improve the performance of health workers and provide increased quality case management with the potential to decrease mortality, could be impeded by insufficient health systems and limited availability of high impact key practices at community level.

007 (000*)

IMCI in Bangladesh—Present Status and Future Plans

Nazmun Nahar

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008 (000)

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009 (000)

Multi-country Evaluation of IMCI: An Overview

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10:15 am-12:15 pm (Venue: Main Conference Hall)
Scientific Session 2: Diarrhoeal Disease 1

010 (161)

A Randomized Controlled Trial of Hand-washing with Soap to Reduce Diarrhoea among Vulnerable Children

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Background: Washing hands with soap prevents diarrhoea, but children aged less than one year at the highest risk of death from diarrhoea are too young to wash their own hands. Previous studies have been too small to evaluate whether improving mothers' and other family members' hand-washing will reduce diarrhoea among the youngest children. **Objective:** Evaluate the effect of increased household hand-washing with soap on diarrhoea among children aged less than one year. **Methodology:** Thirty-six separate neighbourhoods within a squatter settlement in Karachi, Pakistan, were identified. Soap and hand-washing promotion were randomly assigned to 25 of the neighbourhoods (600 households, 3,253 children) and standard habits and practice to 11 neighbourhoods (306 households, 1,570 children). Field workers provided soap and encouraged residents of intervention households to wash their hands after defecation, and before preparing food, eating, and feeding a child. All households were visited weekly for one year, and symptoms of diarrhoea were recorded for each child. **Results:** Children in households receiving soap had a 52% lower incidence of diarrhoea than children in the control households (1.96 vs 4.06 episodes per 100 person-weeks, $p<0.001$) and a 26% lower incidence of diarrhoea episodes lasting longer than 14 days (0.12 vs 0.16 episodes per 100 person-weeks, $p=0.02$). Among children aged less than one year, those in households receiving soap had a 45% lower incidence of diarrhoea than children in control households (6.41 vs 11.57 episodes per 100 person-weeks, $p<.001$). **Conclusion:** In a setting where diarrhoea is a leading cause of child death, improvement in hand-washing at critical times reduces the incidence of diarrhoea among children at high risk of death from diarrhoea. **Acknowledgements:** The Procter & Gamble Company provided funding for the study.

Information Dissemination Impact, Over the Decade, on Awareness of Mothers Regarding Management of Diarrhoea

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Background: Diarrhoea is a leading cause of morbidity and mortality among children aged less than 5 years, claiming 3.2 million lives each year. Diarrhoeal deaths can be prevented by oral rehydration solution (ORS) whose use in India is presently 27%. It is imperative to assess the impact of education campaign on awareness among mothers regarding diarrhoea for taking targeted measures. **Objective:** Study the impact of information dissemination on the awareness of mothers of children aged less than 5 years regarding diarrhoea and its management. **Methodology:** This cross-sectional study was conducted at the Medical College Hospital, Bhopal, from October 2001 to October 2002. The study included 300 children aged less than 5 years presenting with acute diarrhoea and their mothers. A predesigned questionnaire was used for collecting data. Data were programmed and analyzed, and results were compared with those of a similar study done a decade ago under the same setting. **Results:** Over the decade, knowledge of ORS increased from 9% to 58% and to 100% among higher educated mothers. The practice of starvation reduced from 33.7% to 3.6%, and hand-washing with soap/ash increased from 18% to 80%. Less significant changes requiring extra focus seen in knowledge of cause of diarrhoea were considered as teething. Correct knowledge of reconstitution of ORS increased from 26% to 49%. Only 7.3% used it in proper amounts. 45% opined that both fluids and food should be given in diarrhoea. All mothers were unaware of giving extra feeds in convalescence. Food restriction during diarrhoea prevailed over the decade, unaffected by increase in literacy, and use of loperamide and opium increased to 21%. **Conclusion:** Ignorance of cause of diarrhoea hinders its prevention. Demonstration of use of ORS, banning antimotility drugs, and nutrition education need emphasis. Reinforcement and re-evaluation are needed for sustained effect. **Acknowledgements:** F. Qureshi. Study of maternal knowledge and attitude regarding feeding practices and oral rehydration therapy in diarrhoea. 1993.

Mothers' Perceptions and Categorizations of Diarrhoeal Episodes as Predictors of ORT Use in Rural and Urban Bangladesh

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Background: Because of its proven efficacy in the prevention and correction of diarrhoea-induced dehydration, oral rehydration therapy (ORT) is of central importance within diarrhoeal case management. In the late 1990s, current usage of ORT in developing countries accounting for 90% of deaths of children, aged less than 5 years, still remained at a mean of 20%, ranging from 4% to 50%. Current ORT use in Bangladesh is, however, much higher. An understanding of the predictors of ORT use in Bangladesh may, therefore, be relevant to the promotion of ORT use in other developing countries where current rates of ORT use remain much lower. **Objective:** Examine mothers' categorizations of diarrhoeal episodes and their perceptions of observable episode characteristics as predictors of ORT use. **Methodology:** Observations for this research are 2,410 two-week prevalent diarrhoeal episodes in children aged below 5 years identified in the 1987/1988 Bangladesh Diarrhoeal and Treatment Survey (BDMTS). Exploratory bivariate analyses were performed to examine differentials in ORT use by named types, profiles of observable characteristics of these named types, and differentials in ORT use by causal interpretations and mothers' perceptions of the episode as dangerous. Multivariate analyses were performed by logistic regression, with age group of the child included as a potential confounder. **Results:** There were pronounced differentials in ORT use by mothers' categorizations of child diarrhoeal episodes by named types, but these were, in turn, primarily based on observable characteristics, e.g. stool consistency, volume and frequency, and mothers' perceptions of the occurrence of dehydration symptoms. ORT use was also higher for episodes considered to be dangerous. Causal attributions were, by contrast, not significant as predictors of ORT use. In the multivariate analyses, episode severity characteristics that were directly related to the potentially dehydrating effect of the episode were strongly predictive of ORT use. Regardless of episode severity, predicted ORT use remained, however, much lower for diarrhoeas in very young infants (0 to 5 month(s)). **Conclusion:** Decisions on ORT use for diarrhoeal episodes in children, aged less than 5 years, in Bangladesh were primarily based on observable episode characteristics that were directly related to the likelihood that the episode would lead to dehydration. Further promotion of ORT for acute watery diarrhoea and of correct case management for other clinical types of diarrhoeal disease should build on mothers' ability to sensibly differentiate diarrhoeal episode characteristics. **Acknowledgements:** Fellowship support for the author during the study was given by the United States Agency for International Development (USAID) under its Child Survival Fellows Program with the Institute for International Programs of the Johns Hopkins University. The study was financed by bilateral USAID financing to the Social Marketing Project in Bangladesh and directly from the Child Survival Program of USAID/Washington.

Perceptions of Dysentery: Implications for the Introduction of a New Vaccine

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Background: Dysentery is a severe form of diarrhoeal disease and a major cause of morbidity and mortality. Paradoxically, virtually no studies have been conducted examining beliefs and behaviours associated with dysentery. **Objective:** Understand how the local explanatory model of diarrhoeal illness will influence the acceptance of a vaccine against dysentery. **Methodology:** Data collection occurred over an 11-month period from August 2001 to June 2002 in a low-income area of Dhaka city. The research strategy entailed a breadth of complementary methods developed to collect in-depth, descriptive information. These include key informant interviews, semi-structured interviews with community residents, community leaders and healthcare providers, free-listing and rating exercises, and detailed accounts of self-treatment and sequences of health-seeking behaviours from confirmed *Shigella* cases. **Results:** A local term for bloody dysentery is widely known, and residents describe a progression of symptoms, which closely mirrors the biomedical model of the disease. Due to the symbolic significance of blood loss and the fact that there is much uncertainty regarding treatment, bloody dysentery is perceived to be extremely serious. Causal interpretations most commonly relate to concepts associated with hot/cold beliefs, and remedies involve the consumption of foods that will create an internal balance. While from a biomedical standpoint many misconceptions about the purpose of vaccines were uncovered, it was also observed that vaccines garner a certain respect for their effectiveness. Because they are associated with injections, medicines, and treatment, vaccines often fall out of the general rubric of prevention, which is regarded as time, consuming, costly, and ineffective. **Conclusion:** The data suggest that vaccines present an attractive alternative to other measures designed to prevent dysentery. The results illuminate relevant information for the implementation of a new vaccine. **Acknowledgements:** The financial support of the International Vaccine Institute, South Korea, is acknowledged.

Effectiveness of a Dietary Treatment Algorithm for Home Management of Persistent Diarrhoea

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Background: Dietary treatment algorithm is effective in the management of severe persistent diarrhoea in the hospital settings. Since hospital treatment is not affordable to the common people, a similar home-based algorithmic approach could be useful for the management of children with non-severe persistent diarrhoea. **Objective:** Evaluate the effectiveness of a dietary treatment algorithm for home management of children with non-severe persistent diarrhoea, based on a successful hospital-based regimen. **Methodology:** In a randomized controlled trial, children were allocated to receive either the study diet-an initial milk-based (cow's milk, rice powder, and sugar, 68 kcal/L) diet for 7 days, followed by rice-based (rice powder, egg white, soy oil, and sugar, 68 kcal/L) diet, for children who failed to recover with the initial diet or the control diet (the usual home diet without any advice to change). Other treatments were similar. The children were followed up every third day up to 14 days. **Results:** Of 274 children with persistent diarrhoea, 138 received study diet (milk-based diet, followed by rice-based diet) and 136 received control diet. The rates of recovery within 7 days (milk-based diet vs control diet, 48% vs 47%) and within 14 days (milk-based diet, followed by rice-based diet vs control diet, 86% vs 87%) were similar. The rate of withdrawal due to complications was also similar in the two groups (study diet vs control diet, 12.5% vs 10.3%). **Conclusion:** Both the dietary treatment regimens were equally effective in the management of children with non-severe persistent diarrhoea. Thus, children with non-severe persistent diarrhoea can be successfully managed at home with supportive treatments (oral rehydration solution, vitamins, and minerals) in addition to the usual diets as recommended for acute diarrhoea. **Acknowledgements:** The study was funded by the Swiss Agency for Development and Cooperation (SDC).

Green Banana Reduces Clinical Severity in Acute Childhood Shigellosis: A Double-blind, Randomized, Controlled Trial

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Background: Green banana is used as a home treatment for intestinal disorders in many communities. Green banana is rich in non-digestible fibres (cellulose, hemicellulose, alpha glucans) which are fermented in the colon into short-chain fatty acids (acetate, propionate, butyrate), which stimulate colonic absorption and mucosal proliferation. **Objective:** Evaluate the therapeutic effects of green banana in the treatment of severe childhood shigellosis. **Methodology:** Seventy-four children, aged 6-24 months, with a history of acute dysentery due to *Shigella* spp., were treated with a rice-based diet (54 kcal/g) supplemented with (n=34) or without (n=39) cooked green banana (250 g/L) for 5 days. Volume and frequency of stool, presence of faecal blood and mucus, and other clinical and laboratory parameters were determined. **Results:** Green banana diet significantly ($p<0.05$) reduced faecal mucus; on day 5, 51% of the children in the banana group had no mucus compared to 36% in the control group. Faecal blood was completely cleared from 93% of the children on banana diet compared to 83% without banana ($p<0.05$). Faecal volume (mg/day, mean±SD) was consistently less in children given banana diet compared to the controls throughout the treatment: day 1: 197.9±122.5 vs 263.4±228.0; day 2: 143.6±117.0 vs 245.7±158.8; day 3: 147.2±88.4 vs 294.7±168.1; day 4: 219.3±126.4 vs 315.1±209.2, $p<0.05$. Numbers of stool per day were also significantly less ($p<0.05$) in the banana diet group compared to the controls. The overall clinical success rate was 85% in the banana group which was significantly ($p<0.05$) higher than 64% in the controls. **Conclusion:** It is concluded that green banana would be a useful and simple strategy for home management of dysenteric illness. Acknowledgements: The financial support of the United States Agency for International Development (USAID) is acknowledged.

Absorption of Water and Electrolytes from a Liposomal Oral Rehydration Solution: An *in vivo* Perfusion Study of Rat Small Intestine

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Background: Oral rehydration solution (ORS) can successfully rehydrate 90% of patients with dehydration from acute diarrhoea. However, oral rehydration therapy (ORT) with the present ORS formulation does not reduce the volume, frequency, or duration of diarrhoea. Delivering ORS in liposomes may add an additional mechanism of absorption to that already provided with glucose-mediated transport. Another practical improvement is the much-improved taste. Because the salts are incorporated into liposomes, the Aquis Liposomal-ORS tastes less salty and more acceptable to those who drink it. **Objective:** Study whether incorporation of ORS components into liposomes increases small intestinal absorption of water and electrolytes from ORS in an experimental animal model. **Methodology:** *In vivo* perfusion of the entire small intestine was performed among 73 adult rats divided into 3 groups-normal rats, rats exposed to cholera toxin (CT), and rats exposed to 5-fluorouracil (5-FU). Net movements of water and electrolytes were compared between Aquis Liposomal-ORS, Tapioka-based ORS (HS-ORS), and recent WHO-recommended hypo-osmolar ORS (S-ORS). **Results:** All the 3 ORS solutions resulted in significant absorption, but the Aquis Liposomal-ORS provided statistically significant increase of 31%, 45%, and 15% over HS-ORS in water absorption in the normal, CT-stimulated and 5-FU-treated rats respectively. Similar increases in sodium absorption were also noted with the Aquis Liposomal-ORS. There was essentially no difference in water and electrolyte absorption observed between S-ORS and HS-ORS. **Conclusion:** All the 3 ORS solutions are well-absorbed, but the Aquis Liposomal-ORS was associated with the highest level of water and electrolyte absorption. The results suggest that the liposomes are absorbed from both damaged and healthy mucosa and that the liposomes were responsible for increased absorption noted with the use of Aquis Liposomal-ORS.

Effect of Black Tea on Intestinal Fluid Absorption in an *ex vivo* Piglet Model of Diarrhoea

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Background: Tea was associated with antimicrobial properties. Moreover, chewing tea was associated with improved resistance to enteric infections. **Objective:** Test the effect of black tea on acute infectious diarrhoea, enterotoxigenic *Escherichia coli* (ETEC), or *Escherichia coli* heat-labile toxin (LT). **Methodology:** All experiments were conducted in accordance with the standards of the Ethics Committees of the Research Institutes. Isotonic, equiosmolar solutions of black tea-leaf extract were perfused (8 mL/h) into ETEC- or heat-labile toxin (LT)-infected small intestinal segments of ETEC receptor-positive anaesthetized piglets. After 8-hour perfusion, changes in net fluid and electrolyte absorption were determined per cm2 gut surface area. In addition, the capacity of black tea extract to inhibit LT-induced cytotoxicity was tested in a toxin-sensitive kidney Vero cell line *in vitro*. Significance was tested by ANOVA and was set at $p<0.05$. **Results:** ETEC significantly impaired (-55%) the intestinal net fluid absorption from 494±112 µL/cm^2 (control segments) to 171±112 µL/cm^2. Black tea significantly improved this reduction in net fluid absorption to 352±112 µL/cm^2 (-20%). Black tea also improved ETEC-induced reduced net sodium and chloride absorption from -43% to -23% and from -27% to -12% respectively. LT induced a small but significant impairment in net fluid (-27%, 322 µL/cm^2)-sodium and chloride absorption which all were improved by black tea. In addition, black tea significantly inhibited LT-induced cytotoxicity towards Vero cells *in vitro*. **Conclusion:** Consuming black tea may be beneficial in treating diarrhoea due to ETEC. Black tea probably not only acts against pathogen growth or adhesion, but also against enterotoxins or enterotoxin-mediated fluid secretion, although the exact mode of action remains to be elucidated.

10:15 am-12:15 pm (Venue: Meeting Room 1)
Scientific Session 3: Nutrition 1: Micronutrients

018 (002)

Magnitude of Zinc Deficiency among Non-pregnant and Pregnant Women in a Rural Block of Haryana State, India

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Background: Zinc deficiency is widespread in developing countries. Zinc deficiency during pregnancy is associated with growth retardation, congenital abnormalities, and low birth-weight. Limited data are available on zinc deficiency among non-pregnant and pregnant women from India. **Objective:** Assess the magnitude of zinc deficiency among non-pregnant and pregnant women in a rural community of Haryana State, India. **Methodology:** A community-based cross-sectional survey was conducted in 6 villages of a rural block of Faridabad district, Haryana State, India. All non-pregnant and pregnant (pregnancy duration-28 weeks and more) women aged 18 years and more residing in the villages were enrolled during November 2000-October 2001 for the study. Data were collected on sociodemographic profiles and obstetric parameters using a pre-tested semi-structured questionnaire. Blood from the antecubital vein was drawn to assess the serum zinc levels using atomic absorption spectrophotometer. Serum zinc level less than 70.0 µg/dL was considered to be an indicator of zinc deficiency. **Results:** In total, 288 non-pregnant and 283 pregnant women were enrolled for the study. Almost 41.5% and 73.5% of the non-pregnant and pregnant women respectively had zinc deficiency. Thirty-three percent and 65% of the non-pregnant and pregnant women respectively had dietary intake of calories less than 75% of that recommended for their age and physiological status. **Conclusion:** A high prevalence of zinc deficiency was found among non-pregnant and pregnant women in the district surveyed possibly due to poor dietary intake of food and thereby low dietary intake of zinc. There is a need for conducting multi-centric studies in different parts of the country to have more scientific evidence on the magnitude of zinc deficiency before zinc supplementation can be initiated as a public-health intervention programme to improve the pregnancy outcome. **Acknowledgements:** The financial support provided by the Director General, Indian Council of Medical Research, New Delhi, for the study is acknowledged. The infrastructure facilities provided by the Director, All India Institute of Medical Sciences, New Delhi, is also acknowledged.

Pulse Vitamin A Campaign in Assam, India and Vitamin A-related Adverse Effects around Cachar

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Background: On 11 November 2001, Government of Assam, under an UNICEF-sponsored programme, administered vitamin A solution to all children aged 1-5 year(s). This was the third round of bi-annual statewide vitamin A distribution in Assam, and contrary to the media report, no polio vaccine was administered on that day. The sudden spread of the news of death of a few children in remote tea-garden areas in Cachar district and news of children falling ill after taking vitamin A solution caused all the 3 districts of Barak Valley and other parts of Assam to go into an unprecedented panic, and the rest was a history with worldwide debate and a setback in vaccination. **Objective:** Evaluate the vitamin A-induced symptomatology and the reason for panic and opine on the remedial measures. **Methodology:** The total number of patients attending Outpatient and Casualty Departments of Silchar Medical College, Silchar, Assam, with vitamin A toxicity were noted. The patients attending private clinics (chambers) in and around Silchar following vitamin A administration were also analyzed. **Results:** Of 1,253 patients attending the Silchar Medical College, 773 (61.69%) were symptomatic, and 480 (38.30%) were asymptomatic. The nutritional status of patients (IAP classification) were: Gr I-25.13%, Gr II-31.6%, Gr III-9.09%, Gr IV-5.42%, normal-27.13%; symptom-wise-66.6% of Gr I, 82.07% of Gr II, 98.24% of Gr III, and 95.58% of Gr IV, and 17.9% of normal nutritional status were symptomatic. 1,120 patients attended the private clinics (chambers) and other hospitals. Of them, 116 (10.35%) were symptomatic cases. The total number of recorded cases of vitamin A-related symptoms was 2,373 (1253+1120). The main symptoms were vomiting (88.4%) and loose stool (33.07%); 16 patients were admitted with vomiting, loose stool, and dehydration. The patients attending the Medical College Hospital from nearby tea gardens, slum areas, and villages were more symptomatic (61.69%), and the symptomatic patients were more from Gr II-82.07%, Gr III-98.24%, and Gr IV-95.58% of protein-energy malnutrition (PEM) groups. Only 10.35% of patients attending the private chambers were symptomatic. They reported mostly out of fear or anxiety. In most cases of vitamin A toxicity, it was found to be due to administration of higher-than-normally-advocated doses. In many cases, the dose even exceeded 25,000 units/kg. Ignorance and lack of proper training among health workers and over-enthusiasm among poor illiterate mothers to give more vitamin for more health of their children probably led to this disaster. The reported death was of a tea-garden labourer's child who had PEM with acute gastroenteritis. A 5-mL measuring cup was used for delivery of medicine instead of a 2-mL spoon used previously. Personnel were not properly trained about the programme. Age specification was not maintained. Ignorance prevailed among health workers and doctors regarding toxicity of vitamin A and its management. **Conclusion:** Vitamin A is better given with routine vaccination. Pulse campaign is not necessary. Health education regarding diet and nutrition is more beneficial. **Acknowledgements:** The help of the authorities of the Government Hospital, the paediatric and general practitioners, and the paramedics is acknowledged.

Effects of Thankuni in Children with Persistent Malnutritional Diarrhoea

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Background: Elderly people of this region have a strong belief that thankuni leaf as a pulp or extract can control loose motion. The mechanism of action of thankuni leaf extract is not known but the elderly, especially grandmothers, use thankuni leaf extracts for their grandchildren who suffer from loose motion. **Objective:** Evaluate control of motion and fluid loss as affected by intake of thankuni extract. **Methodology:** In a prospective study, 25 children aged 1-2 year(s), having more than 5 loose motions/day were randomly advised to take 60 mL of thankuni extract (extracted from 50 leafs with stem). The children were suffering from persistent malnutritional diarrhoea. They were also fed khichuri made with 300 g of rice, 200 g of vegetables, two eggs, 150 g of fish, 150 g of musur dal, and 30 mL of soybean oil. The total amount of khichuri was divided into 3 meals, and after each meal, 60 mL of thankuni extract was given to ingest. They were also advised to drink oral saline (tasty saline) in between the meals, and if capable, to eat fruits, such as banana, mango, guava, star fruit, and shaddock. The study was conducted at the private chamber of the author during 3 January-3 June 2003. None was admitted to hospital. Urinary excretion and stool of each patient were examined routinely on the first and the fifth day. After fifth day, they were advised to eat normal diets. **Results:** On the second day, 5 patients showed controlled motion (2-3 motions a day). Eleven cases showed controlled motion on the third day, 7 cases on the fourth day, and 2 cases on the fifth day. Signs of dehydration were absent in 15 cases on the third day, 8 cases on the fourth day, and 2 cases on the fifth day. Motion and dehydration both were controlled within the fifth day of thankuni therapy. **Conclusion:** Treatment of diarrhoea with thankuni, a common herb in Bangladesh, is not yet established, but the observation on 25 cases in this study showed 100% cure within 5 days. So, ICDDR,B and other big children hospitals handling diarrhoea should give a clinical trial upon thankuni extract and should establish this low-cost easy treatment in Bangladesh and abroad.

Wealth and Health: Mother's Employment, Socioeconomic Status, and Nutritional Status of Children in Bangladesh

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Background: Poverty is one of the known factors of malnutrition among children. Employment of poor urban women reduces impoverishment, however if it adversely affects child nutrition is not clear. **Objective:** Explore the influence of mothers' employment and socioeconomic status (SES) on the nutritional status of children and on the knowledge and childcare behaviour of mothers. **Methodology:** A cross-sectional survey was conducted among 299 mothers of children aged 6-36 months in a diarrhoea clinic in Dhaka. Anthropometric measurements were used for determining nutritional status. Using average household monthly income, low SES was defined as monthly income of <5,000 taka (US$ 86) and high SES as >10,000 taka. Most (75%) children were aged less than 16 months; 64% were male; 71% were from urban area; and 88% had non-working mothers. **Results:** The table shows the percentages of working and non-working mothers with normal nutrition and working and non-working malnuourished mothers, along with the average income of these mothers.

Characteristics	Working mothers (n=35)	Non-working mothers (n=264)
Normal nutrition (%)	14	25
Malnourished (%)	54	42
Mothers' average income (mean±SD)	1674±1430	NA

63% of working mothers were from low SES group; 51% had no education; 40% had delay in seeking care due to work; 40% of their children were cared by older sibling. Children from higher SES had a 3-time odd of having normal nutritional status compared to those from lower/middle SES, although rate of morbidity and treatment expenditure were similar between the groups. **Conclusion:** Household income was one of the most important factors affecting children's nutrition status. However, poor mother's employment could have a negative influence on their children's nutritional status. Larger studies are required to confirm the findings and to revise policies.

Challenges in 'Routine Public Health Programmes' in India: Evaluation of Vitamin A and Iron Folic Acid Supplementation Programme(s)

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Background: Vitamin A and iron folic acid supplements are currently distributed as part of Reproductive and Child Health (RCH) Programme. Despite 3 decades of operations, the programme coverage remained sub-optimal, and vitamin A and iron folic acid deficiency persisted in the community. **Objective:** Carry out a process evaluation to determine the strengths and limitations in programme implementation and identify the determinants of client behaviour. **Methodology:** Data were collected using rapid assessment procedures in 15 states across India in the form of 1,530 in-depth interviews with policy-makers, programme planners, providers, facilitators, and clients, and 60 focus-group discussions with health workers and clients. **Results:** High variability in the implementation reflected lack of clear understanding about programme objectives, its delivery, and target groups among implementers and providers. This could be attributed to poor training imparted to the functionaries. Passiveness and lack of proactive efforts to implement programme were evidenced by statements like "*Those who come to us we give them. Clients not coming must be healthy*." There was no focus on 100% coverage. Supervision was incidental with no definite mechanism and was restricted to checking registers. ICDS was involved in distribution of supplements but supplies were routed through health department. This interaction lacked coordination and at places was the reason for conflict and poor performance. Inadequate social mobilization activities by the health sector led to lack of awareness among clients which was a reason for non-use of services by the community. **Conclusion:** The programme suffers from passiveness with lack of proactive efforts to reach clients and clear understanding of the implementation guidelines. Training of functionaries, regular and adequate availability of supplements, and a proper mechanism for monitoring, duelled with an intense social mobilization campaign, can help improve the programme. **Acknowledgements:** The financial support of the Micronutrient Initiative is acknowledged.

Concomitant Prevalence of Multiple Micronutrient Deficiencies among Pregnant and Non-pregnant Women in a Rural Block of Haryana State, India

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Background: The prevalence of low-birth-weight (LBW) babies in developing countries is in the range of 30-40%. The role of zinc, iron, folic acid and iodine deficiencies during pregnancy in the causation of LBW is well-established. Intervention trials have been initiated with supplementation of a cocktail of several micronutrients to pregnant women to reduce the incidence of LBW. However, data on the magnitude of concurrent prevalence of micronutrient deficiencies in developing countries are limited. **Objective:** Assess the concurrent prevalence of multiple micronutrient deficiencies among pregnant and non-pregnant women in a rural area of India. **Methodology:** A community-based cross-sectional study was conducted in a rural block in Faridabad district, Haryana State, India. The rural block had an incidence of 30% LBW. All villages in the rural block were enlisted, and 6 were randomly selected for the detailed study. About 283 pregnant women (PW) with pregnancy duration of 28 weeks and more, and 288 newly-married non-pregnant women (NPW) were included. Data were collected on sociodemographic profile, obstetric history, and dietary intake using standard methodology and a pre-tested questionnaire. Blood was collected from the antecubital vein to assess serum zinc, copper, magnesium, ferritin, folic acid, and thyroid-stimulating hormone levels. Serum zinc, copper and magnesium levels were estimated using atomic absorption spectrophotometer. Serum ferritin and folic acid were estimated using ELISA and radioimmunoassay method respectively. Serum thyroid-stimulating hormone level was estimated using the Abbot AxSYM system. Serum zinc, copper, magnesium, ferritin, and folic acid levels less than 70.0 µg/dL, 80.0 µg/dL, 1.80 mg/dL, 15 ng/mL, and 3 ng/mL respectively were considered to be indicative of deficiency for respective micronutrients. Thyroid-stimulating hormone levels of 4.670 and more were considered indicative of iodine deficiency. Dietary intake of micronutrients was assessed using the standard 1-day 24-hour dietary recall methodology. The study was conducted during November 2001-October 2002. **Results:** Data on dietary intake revealed a low dietary intake of all the micronutrients, except magnesium, among pregnant and non-pregnant women. 73.5%, 2.7%, 43.6%, 73.4%, 26.3%, and 6.4% of the pregnant women were deficient in zinc, copper, magnesium, iron, folic acid, and iodine respectively according to the serum levels. Similarly, 41.5%, 27.8%, 18.2%, 63.8%, 27.7%, and 4.8% of the non-pregnant women were deficient in zinc, copper, magnesium, iron, folic acid, and iodine respectively according to the serum levels. Among the pregnant women, concurrent prevalence of two micronutrient deficiencies were zinc and iron (54.9%), followed by magnesium, and iron (33.9%), zinc and magnesium (33.1%), iron and folic acid (21.4%), and zinc and folic acid (18.6%). Other deficiencies in combination of two were prevalent in the range of 1.6-13.9% of the pregnant women. The concurrent prevalence of three micronutrient deficiencies was zinc, magnesium, and iron (25.65%), followed by zinc, iron, and folic acid (16.0%). Other micronutrient deficiencies in combination of three were prevalent in the range of 1.2-12.2% of the pregnant women. The most common concurrent prevalence of four micronutrient deficiencies was zinc, magnesium, iron, and folic acid (9.3%). Deficiencies with other combinations were in the range of 0.8-1.9% of the pregnant women. The most common concurrent prevalence of five micronutrient deficiencies was zinc, magnesium, iron, folic acid, and iodine (0.8%). No pregnant woman had concomitant deficiencies of all the six micronutrients included in the study. Among the non-pregnant women, the concurrent prevalence of two micronutrient deficiencies were zinc and iron (29.6%), followed by deficiency of zinc and magnesium (25.7%), copper and iron (19.7%), iron and folic acid (16.5%), and zinc and copper (14.6%), and. The concurrent prevalence of other two micronutrients was in the range of 0.8-14% of the non-pregnant women. The concurrent prevalence of three micronutrient deficiencies was deficiency of zinc, copper, and iron (10.2%),

followed by zinc, iron, and folic acid (8.5%), and zinc, magnesium, and iron (7.8%). The concurrent prevalence of other three micronutrients was in the range of 0.4-6.3%. The most common concurrent prevalence of four micronutrient deficiencies revealed that the prevalence of deficiency of zinc, copper, magnesium, and iron was 3.9%. The concurrent prevalence of five micronutrient deficiencies revealed that zinc, copper, magnesium, iron, and folic acid was in 0.8%. No non-pregnant woman was had deficiency of all the six micronutrients included in the study. **Conclusion:** A high prevalence of deficiency of zinc, iron, and folic acid was found among pregnant and non-pregnant women. In non-pregnant women, the prevalence of copper deficiency was lower compared to pregnant women. The concurrent prevalence of two, three, four, and five micronutrients were common among the pregnant and non-pregnant women. The findings of the present study indicate a strong need of adding zinc to iron and folic acid tablets routinely distributed to pregnant women and lactating women in developing countries for prevention of anaemia. **Acknowledgements:** The financial support provided by the Director General, Indian Council of Medical Research, New Delhi, India, is acknowledged. The infrastructure facilities provided by the Director, All India Institute of Medical Sciences, New Delhi, are also acknowledged.

Wheat Flour Fortified with Micronutrients in Improving Vitamin A and Iron Status in School-age Children in Rural Bangladesh

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Background: Food fortification is among the most cost-effective and sustainable strategies to prevent or correct micronutrient deficiencies. **Objective:** Detect an impact of consuming chapatti made from fortified wheat flour by school-age children over a 6-month period on changes in their vitamin A and iron status. **Methodology:** In a double-blind control trial, 352 school-age children (6-15 years old) were enrolled to consume daily two chapattis of 100 g non-fortified or fortified wheat flour containing about 20-60% of the recommended daily allowance for multiple micronutrients for 6 months. The study was conducted in a rural area of Chittagong district in Bangladesh. Blood samples were collected prior to the start of feeding, 3 months later, and 6 months later when chapatti feeding was stopped. **Results:** The mean serum retinol level at 6 months was significantly higher in the fortified group (1.06 vs 0.94 µmol/L, $p<0.01$). Proportion of subclinical vitamin A deficiency (serum retinol <0.70 µmol/L) was significantly reduced in the fortified group from baseline (13.6% vs 15.4%) to 3 months (7.9% vs 16.2%, $p<0.05$) and 6 months (7.4% vs 22.5%, $p<0.05$). Similarly, proportion of suboptimal vitamin A deficiency (serum retinol <1.05 µmol/L) was significantly reduced in the fortified group from baseline (67% vs 63.6%) to 6 months (47.9% vs 60.6%, $p<0.05$). There was no demonstrable effect of consumption of fortified chapatti on iron status and on average haemoglobin levels at 3 or 6 months, nor was there any reduction in the proportion of children who had anaemia. **Conclusion:** This is the first study in Bangladesh that tested the efficacy of consuming fortified chapatti which clearly demonstrated a significant improvement in the vitamin A status of school-age children in rural Bangladesh. **Acknowledgements:** The financial support of MOST (USAID) is acknowledged.

Impact of Supplementation of Zinc, Zinc and Vitamin A, and Combination of Micronutrients and Vitamins on Acute Watery Diarrhoea in Mild to Moderately-malnourished Children

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Background: Importance of zinc supplementation has recently been recognized. However, very little is known about the therapeutic role of micronutrients and vitamin A in the treatment of acute diarrhoea. **Objective:** Evaluate the impact of supplementation of zinc, zinc and vitamin A, and combination of micronutrients and vitamins in malnourished children with diarrhoea. **Methodology:** In a double-blind, randomized, placebo-controlled, hospital-based clinical trial, malnourished children suffering from acute diarrhoea were studied. These children were randomized to allocate the specific numbered bottle of syrup of either elemental zinc (2RDA) and a single oral dose of placebo vitamin A (A), or elemental zinc (2RDA) and a single dose of vitamin A (B), or micronutrient combination (2RDA of all micronutrients) and a single oral dose vitamin A (C), or only placebo syrup and a single dose of placebo vitamin A (D) for 14 days. All the syrups were similar in taste and appearance and were supplied in identical bottles. All the patients received standard oral rehydration solution (ORS) and normal hospital diet, including breast-feeding. Patients were followed-up until recovery or up to 5 days. Input and output records were collected. Stool samples were collected for the detection of enteropathogens. **Results:** Forty-one children received therapy schedule of (A), 44 received (B), 39 received (C), and 43 received (D). All the 4 groups were comparable with regard to various initial characteristics. Outcome variables (duration of diarrhoea, percentage of recovery, stool output, intake of ORS, and intake of other fluids) of all the supplemented groups were statistically significantly different compared to that of the placebo group. However, no statistical significant differences in the above-mentioned variables were observed between the 3 supplemented groups. **Conclusion:** Zinc supplementation as an adjunct therapy to ORS has beneficial effects on the clinical course of dehydrating acute watery diarrhoea in malnourished children without any added beneficial effects of supplementation of vitamin A and micronutrients.

026 (003)

Health Risk Relating to Maternity among Adolescent Girls of Rural Bangladesh: A Case of Satarkul Union

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Background: Adolescence is a period of transition from childhood to adulthood when changes occur in their physique and the mind and in the social relationship. During this period, they need adequate nutritional food, but because of extreme penury they cannot have food they need. Early marriage is a social problem which affects adolescent health severely, while legislation in Bangladesh prohibits marriage for girls aged less than 18 years. **Objective:** Focus mainly on the health risk of adolescent girls of a village in Bangladesh. **Methodology:** The study was conducted in Satarkul union of Dhaka district in early 2001. In total, 250 households randomly selected were interviewed. For selecting a representative sample size, a standard method was adopted. Both quantitative and qualitative data were collected. Both descriptive and analytical statistics were used for data analysis. **Results:** The mean age of marriage was 16.3 years. Nearly 25% of 14 years old girls were the victims of early marriage, and the mortality rate among mothers aged less than 19 years was 7.3 per 1,000 livebirths, while teenage pregnancy was considered a high risk factor for maternal health and child death. A significantly higher percentage of under-age respondents was married off ($p<0.000$) compared to those of legal age. 93% of pregnant adolescents compared to non-adolescents suffered from various malnutrition which resulted in anaemia, abortion, pre-eclamptic toxaemia, recto-vaginal fistula, uterine prolapse, postpartum haemorrhage, etc. ($p<0.000$), and nearly 95% did not have complete knowledge about hygienic practice. Knowledge variation among different age groups were significantly high ($p<0.003$). **Conclusion:** Social and family pressure for early marriage, lack of knowledge about sex relationship and family planning, and poverty need to be addressed by government and non-government endeavours. **Acknowledgements:** The author acknowledges Munira Murshed for her contribution to the whole work process of this research and M. Shamsul Islam Khan, Head, DISC, ICDDR,B, for his valuable comments on the earlier version of this paper.

Relation Between Antenatal Visits and Pregnancy Outcome, Birth-weight, Parity, and Maternal Age in Rural Bangladesh

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Background: Maternal mortality is high in Bangladesh. Proper antenatal care through scheduled visits can identify the risks of pregnancy, thereby reducing maternal mortality. **Objective:** To test the hypothesis that three or more antenatal visits could improve pregnancy outcome and birth-weight, aiming at showing the relationship between number of antenatal visits and pregnancy outcomes (livebirth, stillbirth, premature birth, and abortion), birth-weight, parity, and maternal age in rural Bangladesh. **Methodology:** It was a cross-sectional study. Information about antenatal visits of 11,061 pregnant women was collected from 3 BRAC health programme areas (Naruli, Dublagasi, and Dinajpur Sadar) of Bangladesh during 1995-1997. Chi-square and odds ratio with 95% confidence interval in univariate analysis were calculated. **Results:** A significant relationship was found between the number of antenatal visits and pregnancy outcomes in all the areas in chi-square test ($p=0.03$). It was highly significant in Dublagasi ($p=0.00$). The number of antenatal visits was also significantly associated with maternal age ($p=0.02$). No association was found between the number of antenatal visits with birth-weight and parity. **Conclusion:** To reduce maternal mortality, efforts to increase scheduled visits during antenatal period must be strengthened. **Acknowledgements:** BRAC and Umea University of Sweden.

Effectiveness of a School-based Adolescent Reproductive Health Intervention in Rural Bangladesh

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Background: The adolescent population of Bangladesh has a generally poor understanding of sexual and reproductive health. This is associated with early marriage, adolescent pregnancy, and the increasing occurrence of high-risk sexual practices. **Objective:** Determine the effectiveness of school-based intervention which combined community sensitization with the distribution of 3 booklets addressing (1) puberty, (2) fertility and family planning, and (3) STDs/AIDS. **Methodology:** Secondary schools located in two rural sub-districts, Abhoynagar and Mirsarai, were designated as intervention or controls based upon the union in which they were located. All attending students in grade 8 and 9 were eligible. Using a multi-staged sampling procedure, 1,226 females and 1,163 male subjects were enrolled. Group meetings were held with parents, teachers, and local decision-makers. These groups participated in the development of the booklets. At approximately 3-month intervals, the 3 booklets were distributed to all students. Baseline and post-intervention (18 months) interviews assessing knowledge and practices were completed in the subject's home. The study was carried out from February 2001 to September 2002. **Results:** The pre- and post-intervention interviews were completed by 73% of the students. 76% of girls and 75% of boys reported reading all 3 booklets, and over 95% had read at least one. None of the control subjects had seen or read the booklets. Based upon both univariate and multivariate analyses, significant improvements in knowledge favouring students attending the intervention schools were found. Among girls, this included methods of modern contraception ($p<0.002$), what diseases can be transmitted sexually ($p<0.001$), and how to prevent them ($p<0.001$). Among boys, this included methods of modern contraception ($p<0.001$), knowledge about birth-control pills ($p<0.001$), their symptoms ($p<0.001$), what diseases can be transmitted sexually ($p<0.001$), and how to prevent them ($p<0.001$). With respect to practices, too few students reported high-risk behaviours or attended health services to permit a statistical analysis of group differences. **Conclusion:** An intervention that combines community sensitization with the distribution of booklets to school-attending teenagers can effectively improve knowledge about sexual and reproductive health. Changes in practices could not be demonstrated and suggest the need for longer-term follow-up and more discrete, confidential methods of reporting.
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Mortality of Children, Aged Less than 5 Years, in Rural Area of Bangladesh

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Background: Increased child immunization coverage and maternal health interventions may have contributed to substantial reduction in deaths among children aged under 5 years in the past decade, but it is still unacceptably high in Bangladesh. **Objective:** Assess causes, trends, and sociodemographic factors associated with mortality in children aged less than 5 years. **Methodology:** Data obtained from verbal autopsy of 1,238 reported deaths in children of <5 years old through the health and demographic surveillance system administered by the Health Systems and Infectious Diseases Division of ICDDR,B in Abhoynagar and Keshobpur sub-districts of Jessore district during 1983-2002 were analyzed. **Results:** Most (54%) deaths were in neonates, 29% were post-neonate infants, and 17% were aged 1-4 year(s). The major causes of neonatal deaths were prematurity (29%), maternal/pregnancy complications (15%), infections (11%), acute respiratory infection (10%), and birth asphyxia (9%). More than half of neonatal deaths occurred within 3 days of birth. Among post-neonatal infants, 41% of deaths were associated with pneumonia, followed by diarrhoea (19%) and malnutrition-related causes (14%). The major causes of death among 1-4-year(s) old children were drowning (23%), followed by diarrhoea, pneumonia, and malnutrition. Three percent of all deaths were from EPI vaccine-preventable diseases. Only 45% of deaths had medical consultation prior to death, of which 23% were from qualified practitioners. No significant difference was observed in relation to cause, age, and socioeconomic status by gender. **Conclusion:** Neonatal deaths contributed to major shares of mortality of children aged less than 5 years. Appropriate intervention is needed to reduce neonatal deaths. Pneumonia and diarrhoea remain key causes of death for infants and children. Strategies are needed to increase the use of appropriate medical services. Drowning has emerged as the leading cause of childhood deaths in this rural setting, and programmes should focus on reducing the potential for its occurrence. **Acknowledgements:** The funding support of the United States Agency for International Development (USAID) for maintaining the health and demographic surveillance system is acknowledged.

A Descriptive Analysis of Inter-pregnancy Intervals by Previous Pregnancy Outcome in Gaibandha, North Bangladesh

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Background: The process of pregnancy and childbirth is often physiologically taxing on maternal health. Shorter interbirth/interpregnancy intervals have been implicated as risk factors for adverse peripartum and perinatal outcomes, maternal morbidities, and even low birth-weight. Increased birth spacing has also been associated with decreased infant and child mortality. Understanding culture-specific birth-spacing patterns could help target family-planning interventions to high-risk groups. **Objective:** Explore the relationship between type of previous pregnancy outcome and pregnancy-spacing decisions in a contracepting multigravid population in North Bangladesh. **Methodology:** JiVitA Bangladesh is a Maternal Health and Nutrition Project, implemented by the Johns Hopkins University under the National Integrated Programme for Health and Population of Ministry of Health and Family Welfare, Government of Bangladesh. The JiVitA study is following 113,060 married women of reproductive age for incident pregnancies in 19 unions of rural Gaibandha and Rangpur districts. Since the study's onset in July 2001, 34,700 pregnant women have been enrolled into the JiVitA-1 trial study and visited weekly by locally-hired and trained female workers called Field Distributors. At the enrollment of a new pregnancy, an interview is conducted by a trained interviewer who assesses, along with current health and nutrition status, the woman's previous pregnancy-history which includes date of the last pregnancy outcome. For this analysis, only 'wanted' pregnancies were included to capture deliberate birth-spacing patterns. Interpregnancy interval was examined by type of previous pregnancy outcome. The impact of gender of previous livebirth(s) on the interpregnancy interval was also examined. **Results:** The overall median time between pregnancies was 37 months. After a livebirth, the median interpregnancy interval was 42 months. After a miscarriage or stillbirth, the median time to next pregnancy was merely 13 months. Couples with no living children from previous pregnancies had a median interval of only 12 months, whereas those with at least one surviving infant waited for a median time of 43 months. The median interpregnancy interval for parents of 3 or more children (37 months) is about 6 months shorter than for parents of one or two children (43 months). For parents with at least one living child, the interpregnancy interval was slightly longer (between 2 and 4 months) for parents of boys than for parents of the same number of girls. In a regression analysis of determinants of interpregnancy interval, the number of living children in the household had the largest effect on increase in interval, adjusting for the mother's and father's education. If the mother's age at previous pregnancy was less than 15, the median subsequent birth interval was ~10-20 months longer than the median women who had their previous pregnancy *after* age 15. After age 15, a slightly decreasing trend in interpregnancy interval can be seen with increased maternal age at last delivery. **Conclusion:** The CATALYST Consortium, a global reproductive health activity of the Center for Population, Health, and Nutrition of USAID, recommends a 3-5-year birth spacing for improved maternal and child health. The current median interval in rural Gaibandha, Bangladesh, is near this recommended cut-off, which may lower peripartum and perinatal health risks. However, certain high-risk groups still exist in this population, especially women who currently have no live children and those who are recuperating from a premature termination of pregnancy, such as abortion, miscarriage, or stillbirth. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID), the Bill and Melinda Gates Foundation, the Sight and Life Research Institute, and the Government of Bangladesh is acknowledged.

Mortality Due to Suicide and Homicide in Rural Bangladesh

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Background: While suicide and homicide are common causes of death in Bangladesh, characteristics of people who commit suicide or are victims of homicide are not well-described. Objective: Ascertain sociodemographic characteristics of suicide and homicide victims. **Methodology:** Trained female interviewers routinely visited 6,953 households quarterly to record births and deaths among 6,757 married women of reproductive age (MWRA) under a health and demographic surveillance system administered by the Health Systems and Infectious Diseases Division of ICDDR,B in Abhoynagar/Keshobpur sub-districts of Jessore district from 1983 to 2002. Verbal autopsy was collected for 3,468 deaths, of which 177 were reportedly due to suicide and homicide. **Results:** Suicide and homicide contributed 5% of all deaths-90% due to suicide and 10% due to homicide. Deaths due to suicide and homicide were higher among females (65%), of which 40% were aged below 19 years, 36% were in the 20-29-year age group and 10% in the 30-39-year age group, and 14% were aged above 40 years. Two-thirds of the females were married, 28% were unmarried, and 6% were separated, divorced, or widowed. Four percent of the females were pregnant at the time of death. Most (83%) females were housewives, and 11% were students. Among males, 62% were married, and 38% were unmarried. Of the males, 16% were aged below 19 years, 42% were aged 20-29 years, and 11% were in the 30-39-year age group, and 31% were aged above 40 years. Manual labour (43%), farming (22%), business (13%), and study (11%) were the main occupations of males. **Conclusion:** Deaths due to suicide and homicide occur frequently among young females and working males. To develop strategies for prevention, data on risk factors are urgently needed. **Acknowledgements:** The funding support of the United States Agency for International Development (USAID) for maintaining health and demographic surveillance system is acknowledged.

Does Sex Preference Really Affect Contraceptive Behaviour among Married Women in Rural Bangladesh?

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Background: Sex preference is thought to be one of the reasons for plateauing of fertility. **Objective:** Investigate whether sex preference really affects contraceptive behaviour among rural women in Bangladesh. **Methodology:** Data for the present study came from 4 rural thanas in Bangladesh. Of these, 3 thanas are located in ICDDR,B study area and one in non-ICDDR,B areas. Multi-stage sampling procedure was employed to select 500 currently-married women in each area. A structured questionnaire was administered for quantitative data collection. Qualitative data collection was also done through in-depth interviews among some selected groups of men and women in the study areas. **Results:** Results showed that two-fifths of the women in 3 areas, who had no sons but two daughters, did not want additional children, while more than four-fifths of women who had two sons but no daughters did not want additional children. Among them, about 21% were using temporary methods of family planning. Among women in all areas, who already achieved two sons and one daughter and/or one son and two daughters, almost none desired additional children, and most of them were using effective methods of family planning. Qualitative data also indicate that majority of men and women expressed their views that they want son due to old-age security, which was common in all the areas. **Conclusion:** In Bangladesh, son preference is still presence and, as such, it is argued by many population experts that further reduction of fertility is unlikely without considerable reduction in the desire of children or sex preference. Policy-makers, thus, need to find how to bring down son preference. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Comparison of Different Methods to Detect Proteinuria in Normal and Pre-eclamptic Pregnancy

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Background: Accurate measurement of protein in urine is central to the diagnosis, management, and prognosis of pre-eclampsia (a common and potentially dangerous complication in pregnancy). There are several methods of detection of proteinuria but controversies still exist regarding the most appropriate technique to be used. In most public hospitals in Bangladesh, a crude test, the heat coagulations test, is still used for detecting proteinuria. **Objective:** Compare the methods used for detection of proteinuria and explore the degree of limitation of the heat coagulation test. **Methodology:** Fifty-eight primigravida subjects, aged 17-35 years, with pre-eclampsia (PE) were studied along with 100 non-hypertensive, non-proteinuric pregnant women. PE was diagnosed by standard criteria, and proteinuria was measured by heat coagulation (HC), dipstick, sulphosalicylic acid test (SSA), protein-creatinine (UPr/Cr) ratio (cut-off value 30 mg protein/mmol creatinine) and albumin-creatinine (Alb/Cr) ratio (cut-off value of 25 mg albumin/mmol creatinine). The significance of difference between proportions and medians was measured by Mcnemar chi-square test and Mann-Whitney test respectively. **Results:** While all the 100 cases in controls were negative for HC (by definition), there was a significant difference between HC and other tests in the control group. Dipstick detected 2 (c^2=4.5, $p<0.05$), SSA detected 3 (c^2=5.33, $p<0.05$), UPr/Cr ratio 5 (c^2=7.2, $p<0.01$), and Alb/Cr ratio 8 (c^2=10.125, $p<0.01$) positive cases in the control group. In the case of PE dipstick, SSA and Alb/Cr ratio gave equivalent results. HC was positive in all the 58 cases, but dipstick and SSA detected 41 cases (c^2=15.06, $p<0.001$), and Alb/Cr ratio 42 (c^2=14.06, $p<0.001$) positive cases. The difference of HC test and SSA with UPr/Cr ratio was statistically significant (c^2=27.03, $p<0.001$; c^2=5.5, $p<0.02$). **Conclusion:** HC test should be avoided to measure proteinuria in the case of pregnancy due to considerable chance of false negativity and positivity. At the least, dipstick or SSA should be practised which seems to measure albumin accurately as evident from their equivalence with Alb/Cr ratio. But UPr/Cr ratio is a better choice probably due to its capacity to include non-albumin proteins. **Acknowledgements:** The financial support of the International Program in the Chemical Sciences, Uppsala University, Sweden, Diabetic Association of Bangladesh, and the Ministry of Science and Technology, Government of Bangladesh, in conducting the study is gratefully acknowledged.

10:15 am-12:15 pm (Venue: Meeting Room 3)
Scientific Session 5: Genetics 1

034 (001)

Molecular Evidence for Acquisition of VP6 and NSP4 Genes of Porcine Origin by Human Group A Rotaviruses

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Background: Acquisition of foreign genes and truncation of amino acids at carboxyl terminal of a protein sequence are some of the novel mechanisms by which a virus increases its virulence and such strains are usually associated with an epidemic. **Objective:** Ascertain the characteristics of a rotavirus strain thought to be of animal origin with G9P[19] characteristics which was associated with an outbreak of infantile gastroenteritis in Imphal, Manipur, a North Eastern State of India; the strain was molecularly characterized. **Methodology:** The viral RNA extracted from stool sample was reverse transcribed, and individual gene segments were amplified by PCR with gene-specific end primers. The full-length VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, and NSP5 genes were cloned into pCR2.1 vector prior to sequencing. The sequence analysis was carried out with different sequence analysis software. **Results:** All the genes sequenced, except VP7, were closely related to porcine rotaviruses (95-99% identity at amino acid level) and clustered with porcine strains in phylogenetic analysis. This included VP6 and NSP4- two crucial proteins thought to be involved in host range restriction and pathogenicity. This is the first report of a rotavirus with VP6 and NSP4 of porcine origin which caused diarrhoea in a human host. This isolate had subgroup I nature and belonged to NSP4 genotype B which is a characteristic of animal rotaviruses. **Conclusion:** Findings of this study suggest that reassortment may be occurring between human and other animal strains, and some reassortant viruses may be virulent to humans. This also points to the difficulties ahead for developing a successful vaccine to control rotavirus-associated diarrhoea in children. **Acknowledgements:** Indian Council of Medical Research, Government of India and Japan International Co-operation Agency (JICA), Government of Japan, funded the research.

Genetic Analysis of a Human Group B Rotavirus Detected in China in 2002

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Background: Group B rotavirus (GBR), which causes severe cholera-like diarrhoea in adults, has been detected in China since its first identification in 1982 as adult diarrhoea rotavirus (ADRV). However, among Chinese human GBR strains, viral gene sequence was analyzed only for ADRV strain. **Objective:** Analyze gene sequences of a recently-detected GBR in China to estimate mutation frequency of human GBRs and to know phylogenetic relatedness of the Chinese strain to GBRs detected recently in India and Bangladesh. **Methodology:** A GBR strain WH-1, which was detected in Hubei province, China, in 2002 was analyzed and derived from a male patient aged 30 years. Sequences of viral genes were determined directly from RT-PCR products by the dideoxy nucleotide chain termination method. **Results:** VP7 gene of WH-1 showed extremely high (98.6%) sequence identity to ADRV and showed also high sequence identities to recently-reported viruses CAL-1 in India (92.5%) and Bang373 in Bangladesh (92.4%). In contrast, identities to animal (bovine and murine) rotaviruses were considerably low (61-64%). Other gene segments of WH-1 encoding VP4, VP6, NSP1, NSP2, and NSP3 showed also higher sequence identities to those of ADRV (98-99%) than those of CAL-1 or Bang373 (90-95%). Mutation rate of GBR was estimated to be 5.9-9.9x10^{-4}/base/year, which was considered to be lower than that of group A rotaviruses. **Conclusion:** WH-1 is genetically more close to ADRV than CAL-1 or Bang373, although these viruses are grouped into a same cluster of human GBR. It was suggested that human GBR are genetically highly conserved with low mutation frequency.

Molecular Epidemiologic Analysis of Human Group B Rotaviruses Detected Recently in Bangladesh

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Background: Group B rotavirus (GBR) causes severe cholera-like diarrhoea in adults, and this virus has been detected exclusively in China since 1982. However, recently, GBRs were sporadically detected in adult diarrhoeic patients in India (1997) and Bangladesh (2000) through epidemiologic investigations. **Objective:** Analyze genetically GBRs detected in Bangladesh to estimate their relatedness to other GBR strains reported so far, i.e. ADRV strain in China, CAL-1 strain in India, and animal (bovine and murine) GBR strains. **Methodology:** Four GBR strains, Bang373, Bang544, Bang402, and Bang334, which were detected in adult diarrhoeal cases in Mymensingh, Bangladesh (2000-2001), were genetically analyzed. Sequences of viral genes were determined directly from RT-PCR products by the dideoxy nucleotide chain termination method. **Results:** Sequence identities of VP7 genes among 4 Bangladeshi GBRs and CAL-1 strain were extremely high (more than 98%). Strains Bang373 and Bang544 showed also high VP7 gene sequence identity to ADRV (92%), while identities to animal GBRs were considerably low (e.g. 62-63% to bovine GBR WD653). Other gene segments of Bang373 encoding VP2, VP4, VP6, and NSP1-NSP5 showed higher sequence identities to those of CAL-1 (98-99%) than those of ADRV (91-94%). **Conclusion:** Human GBRs detected in Bangladesh and Indian CAL-1 strain were considered to be virtually identical, but genetically distinct from Chinese GBR strain ADRV, suggesting that Bang373 and CAL-1 might not be brought recently from China.

Phylogenetic Analysis of VP7 Genes of Bangladeshi G1 Rotavirus Strains

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Background: Rotavirus VP7 protein, which induces G-type-specific neutralizing antibodies, appears to play an important role in protection against rotavirus-associated illnesses. The differences in nucleotide and amino acid sequences of the antigenic sites of VP7 divide serotypes into different monotypes or lineages which are associated with different degrees of affinity against different G-specific monoclonal antibodies. **Objective:** Compare the VP7 sequences of recent G1 rotavirus strains from Bangladesh with strains circulating all over the world. **Methodology:** The VP7 genes of 9 G1 rotaviruses collected during July 2002-September 2002 in ICDDR,B, Dhaka, Bangladesh, were sequenced using the dideoxynucleotide chain termination method at the core sequencing facility of the Rega Institute for Medical Research, Belgium. **Results:** A high degree of amino acid identity was found between the recent Bangladeshi and Indian strains. They were different from old Bangladeshi strains isolated in 1996-1997. Phylogenetic analysis of the strains from the global rotavirus database showed that G1 rotaviruses could be divided into 5 lineages. All Bangladeshi strains belonged to lineage I, and the recent strains clustered together with strains from India and Thailand. Comparing with the prototype Wa strain from the United States and the K8 Japanese strain (lineage III), some novel amino acid substitutions were found in recent Bangladeshi strains. **Conclusion:** The G1 RV strains from Bangladesh are very different from prototype G1 RV strains commonly used as vaccine strains. **Acknowledgements:** This work was partly funded by a grant from the Flemish Fund for Scientific Research.

stx$_2$-positive but Stx2-negative *Escherichia coli* O157: H7/- Strains Isolated in Thailand and Japan

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Background: Nine strains of *Escherichia coli* O157:H7/- that carried the stx2 gene but did not produce Stx2 at a detectable level were isolated in Thailand and Japan. **Objective:** Elucidate the mechanism responsible for non-detectable-level expression of the stx2 gene. **Methodology:** Stx2 production was examined using the Verotox-F Seiken kit (Denka Seiken). The nucleotide sequence was determined by a cycle-sequencing method. The *stx*$_2$ promoter strength was compared using a *lacZ* transcriptional fusion vector. To examine the effect of Q protein on *stx*$_2$ synthesis in Stx2-non-producing strains, the *q* gene of strain EDL933 or Thai-12, a representative Stx2-non-producing strain, was cloned into an expression vector and transformed into the test strain. **Results:** The *stx*$_2$ promoter of Thai-12 was very weak. In addition, the 1.8-kb nucleotide sequence upstream of the *stx*$_2$ gene, including the *q* gene, of Thai-12 had only 50% homology with the *stx*$_2$ phage of EDL933 but was highly homologous with *phi* 21 and lambda phages. The nucleotide sequences of the *q*-*stx*$_2$ region of 8 other Stx2-non-producing strains had >99.5% homology with that of Thai-12, except that one strain had an additional 1.3-kb insertion sequence. Stx2 could be produced in Thai-12 at a high and low level when the *q* gene of strain EDL933 and Thai-12, respectively, was introduced and expressed. The effects of the introduced *q* genes varied for other Stx2 non-producing strains. **Conclusion:** Modification of the *q*-*stx*$_2$ region by homologous recombination is mainly responsible for non-detectable-level expression of the *stx*$_2$ gene. Additional nucleotide sequence changes within this region, such as the *stx*$_2$ promoter region, also have minor effects. Acknowledgments: This research was supported in part by the funds from the Ohyama Health Foundation and Yuasa International Foundation, by 4th Advanced Scientific Technology Research Fund from Toyota Motor Corporation, and by a Grant-in Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

Effect of Single Amino Acid Mutation in PrM Region of Dengue 2 Virus Associated with Increased Pathogenicity

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Background: Previous genetic analyses indicated Dengue-2 (DEN) virus strain-specific differences that correlated well with disease severity. The effect of amino acid residues Ile at premembrane 16 (PrM-16-Ile) and PrM-81-Thr was among the genomic loci that were identified in DEN-2 strains, which caused the more severe disease in patients with dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). **Objective:** Study the effect of mutation at PrM16 and PrM 81 on DEN-2 virus strain to understand the possible role in the pathogenesis of DHF and DSS. **Methodology:** A site-directed mutagenesis approach was used for investigating these two PrM loci. A genome-length infectious cDNA clone (pD2/IC-30-A) of DEN 2 16681 virus, containing PrM-16-Arg and PrM-81-Thr, was used for constructing a viral mutant (Mut16) with Ile at position 16 in the PrM protein. The Mut16 virus was studied to determine its infectivity, replication, and cytokine induction in human myelomonocytic K562 cells, peripheral blood mononuclear cells (PBMCs), and immature myeloid dendritic cells (DCs). **Results:** The results indicated that, at 48 hours post-infection, the Mut16 virus**,** possessing PrM**-**16-Ile and PrM**-**81**-**Thr, showed significantly higher level (68.7%) of infectivity in K562 cells compared to the parental clone-derived D2/IC-30P-A virus (3.14%) and an engineered Mut16-revertant (PrM-16 Ile-to-Arg) virus (3.04%). Furthermore, the Mut16 virus infected 49.7% of DCs at 24 hours of post-infection compared to 2.37% and 6.17% of cells infected with D2/IC-30P-A and Mut16-revertant viruses respectively. Mut16 virus induced significantly higher levels of certain cytokines, including tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), and interleukin-12 (IL-12) compared to D2/IC-30P-A and Mut16-revertant viruses. **Conclusion:** The presence of amino acid Ile at PrM-16 of DEN-2 virus, perhaps in conjunction with PrM-81-Thr, may play a crucial role in the pathogenesis of DHF/DSS associated with DEN-2 viral infection.

Multiplex PCR to Detect Toxin Genes of *Vibrio cholerae*

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Background: In Malaysia, cases of cholera occurring every year are mainly caused by enteropathogenic *Vibrio cholerae* 01 serotype. Until now, laboratory diagnosis of cholera still takes more than 24 hours to complete. **Objective:** Innovate a multiplex PCR for detection of cholera toxin genes (ctxA, ctxB, ace, and zot). **Methodology:** Thirty clinical isolates of *V. cholerae* (1 Hikojima, 14 Inaba, and 15 Ogawa) were obtained from the Institute for Medical Research, Malaysia, from 2001 to March 2003. Genomic DNA of all bacterial strains was extracted using the pure gene bacterial genomic DNA extraction protocol. Multiplex PCR was developed to detect all 4 toxin genes of *V. cholerae* (ctxA, ctxB, ace, and zot) in a single event. Specificity of the technique was tested by amplifying the genomic DNA of *V. cholerae* toxin genes against genomic DNA from *Salmonella typhimurium*, *S. enteriditis,* and *Escherichia coli* species, which were previously reported to produce cholera-like toxins. Later, all toxin genes of *V. cholerae* were sequenced for further analysis. **Results:** All the 30 clinical isolates of *V. cholerae* showed the presence of all the 4 toxin genes examined, but none of the organism included negative control showed the presence of the 4 toxin genes. This indicated that this multiplex PCR innovated for the detection of *V. cholerae* toxin genes is 100% specific and sensitive to *V. cholerae*. Sequencing results of all toxin genes showed that no specific mutations occurred in all 30 clinical isolates of *V. cholerae*. **Conclusion:** Multiplex PCR was successfully developed to detect cholera toxin genes in clinical isolates of *V. cholerae*. **Acknowledgements:** The financial support of the National Biotechnology Directorate is acknowledged.

Characterization of ElTor Vibriophage S5 by Electron Microscopy

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Background: Characterization of vibriophages is important for understanding the host chromosome and phage taxonomy and also in the development of a potential vaccine against cholera. **Objective:** Characterize choleraphage S5 genome by electron microscopy. **Methodology:** Purified vibriophage S5 was negatively stained to study the phage morphology. S5 DNA was visualized using Kleinschmidt's technique. A partial denaturation map was prepared to characterize the genome using alkali and formaldehyde. A restriction digestion pattern of S5 DNA was also obtained. **Results:** The lytic phage S5 has a hexagonal head with a short non-contractile tail. It, thus, belongs to the Podoviridae family according to ICTV (International Committee on Taxonomy of Viruses). Restriction endonucleases Hind III, Hpa II, EcoRV, and Hae III cleave S5 DNA. This confirms that S5 phage has double-stranded DNA. Partial denaturation map of S5 DNA indicates that the DNA is linear and non-permuted. Major denaturation sites near either end of the DNA moleculessuggest the presence of terminal redundancy. There is also a major denaturation site at 83% from the left end of the molecules, which makes the molecules asymmetric. **Conclusion:** The phage S5 has a hexagonal head with a short non-contractile tail and belongs to the family Podoviridae. S5 DNA is linear and double-stranded. The partial denaturation map indicates that S5 DNA is non-permuted and that the DNA possibly has terminal redundancy. Restriction endonucleases EcoRV, Hind III, Hpa II, Hae III cut S5 DNA. **Acknowledgements:** The project is funded by the Department of Science and Technology, Government of India.

3:00 pm-3:45 pm (Venue: Main Conference Hall)
Keynote Lecture 1

042 (361)

Nutrition and Diarrhoea: Fifty Years Experience

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In the 1950s, neither the textbooks nor the clinicians and researchers recognized a relationship between nutrition and diarrhoea and other infectious diseases. The field and metabolic studies of the Institute of Nutrition of Central America and Panama (INCAP) in the 1950s demonstrated that malnutrition and diarrhoeal disease were synergistic. These studies stimulated the review of available evidence that resulted in the 1968 WHO monograph on "*Interactions of Nutrition and Infection"*. This publication extensively documented both role of infections in precipitating clinical malnutrition and impact of malnutrition on morbidity and mortality from infection. The demonstrated high frequency of diarrhoea in developing-country infants and young children led to intensive studies in many countries of its effect on growth and development. They also linked the frequency and severity of diarrhoea to nutritional status. The metabolic effects of diarrhoeal and other infections were extensively investigated at Fort Dietrich, Maryland and hormonal and cytokine mechanisms identified. By 1981, research reviewed at a Bellagio Conference led to the book "*Diarrhea and Malnutrition: Interactions, Mechanisms and Interventions"*. Studies at ICDDR,B demonstrated the severe nutritional consequences of rotavirus and enteropathogenic *Escherichia coli* and a relationship of cholera purging to nutritional status. The modern explosion in knowledge of immune mechanisms has led to better understanding of how malnutrition lowers resistance to diarrhoea and other infections. Today, the synergistic relationship between nutrition and infection must be a major consideration in comprehensive public-health interventions to prevent infection and correct malnutrition.

4:00 pm-4:45 pm (Venue: Main Conference Hall)
Plenary 2: Vaccine

043 (362)

Recent Developments for Diarrhoeal Disease Vaccines for Young Children in Developing Countries

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Diarrhoeal diseases remain one of the principal causes of morbidity and mortality among children in the developing world. A recent review indicates that the median episodes of diarrhoea per child-year remain at 3.2, although mortality had decreased slightly to 4.9 children per 1,000 per year in developing countries (Kosek *et al. Bull World Health Organ* 2003). This decrease was most pronounced in infants aged less than 12 months. However, diarrhoeal disease still accounts for a median of 21% of all childhood deaths in developing countries and is associated with 2.5 million deaths per year in children aged less than 5 years. While diarrhoeal management strategies are crucial in this reduction in diarrhoeal disease, the development of vaccines against rotavirus, enterotoxigenic *Escherichia coli*, and shigellae is a major programme of World Health Organization. Rotavirus is recognized as the single most important enteric pathogen associated with high infant mortality due to diarrhoea and dehydration. A recent review estimated that approximately half a million young children die annually due to rotavirus infection (Parashar *et al. Emerg Infect Dis* 2003). However, since the demise of RotaShield□ in the USA, several second-generation rotavirus vaccine candidates have entered clinical trials. Two candidates developed by the multinational companies, Merck (a bovine reassortant vaccine) and GSK Biologicals (a monovalent human rotavirus vaccine), are in large-scale safety and efficacy trials. Both the candidates have shown good immunogenicity by serum IgA responses and good protective efficacy in smaller phase II and II trials. Other rotavirus vaccine candidates are also in the pipeline with other candidates, including another bovine reassortant candidate (UK strain), and a monovalent human neonatal rotavirus strain (RV3). Human-bovine reassortant strains are under pre-clinical development in India. Enterotoxigenic *E. coli* (ETEC) vaccine development is not as advanced, although the burden of disease of ETEC is considered to be considerable in the developing world. Preliminary epidemiological data and clinical trials indicated that there was a need for a protective response against specific commonly-occurring colonization factors (CFs). Vaccine development has included live, toxin-negative candidates or live vectors expressing CFs. However, the most evaluated approach has been to generate inactivated candidates with the B-subunit of cholera toxin coupled with 6 common CFs. Although early results showed that the CTB-CF vaccine gave protection against ETEC-associated diarrhoea in phase II and III trials, more recent studies have been disappointing. Further vaccine candidates are under clinical development. *Shigella*-associated diarrhoeal disease is associated with significant morbidity and mortality world-wide in both adults and children (Kotloff *et al. Bull World Health Organ* 1999) with an estimated 740,000 deaths in children aged less than 5 years per year. However, the complexities of vaccine development, which needs multiple bacterial antigens from the different species and serotypes of *Shigella*, have made vaccine development difficult. Although no *Shigella* vaccine is currently available, several candidates are under development, and some have completed phase I and II trials. Deletion mutations in the *Shigella* genome has been utilized to generate live-attenuated strains, while a different approach has been to examine conjugate vaccines. Trials are still required to test these two approaches and to compare the potential of monovalent or multivalent vaccine strains. Vaccine developments for the three major enteropathogens of infants and young children have proven complex and challenging, but several candidates are in the pipeline. Vigorous research efforts are required to bring this programme closer to fruition.

4:00 pm-5:45 pm (Venue: Meeting Room 1)
Scientific Session 6: Nutrition 2: Community Nutrition

044 (024)

Nutritional Status and Associated Factors among Urban Adolescent Girls in Bangladesh

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Background: Adolescence is a period of significance marked by accelerated growth and physiological maturation. The expression of growth potentiality depends on various factors. In Bangladesh, urban counterparts are almost to be neglected for such study. **Objective:** Study few selected factors and their association with nutritional status of adolescent girls from urban region of Dhaka city, Bangladesh. **Methodology:** 1,000 girls, aged 10-19 years, studying at 6 different schools and colleges, Dhaka city were selected. Information on date of birth, age at menarche, monthly family income, parent's educational status, possession of expensive household articles, immunization coverage, nutrition knowledge, etc. was collected using a structured questionnaire. Parent-child relationship was assessed using a 5-point PCR scale. Height and weight were measured using standard techniques. 24-hour recall method was used for obtaining data on nutrient intakes. Nutritional status was assessed by height-for-age, weight-for-height, and weight-for-age using the National Center for Health Statistics standards. Suitable statistical analysis was employed using statistical package SPSS (10.0 version). **Results:** According to height-for-age and weight-for-height, 52.7%, 17.6%, 13.5%, and 16.2% of girls were normal, stunted, wasted, and stunted and wasted respectively. According to weight-for-age, only 18.9% were normal; others exhibited various grades of malnutrition, wherein 6.0% had severe grade of malnutrition. Among the factors studied, calorie intake ($p<0.001$), age at menarche ($p<0.005$), and overall socioeconomic and educational status ($p<0.05$) exhibited a significant association with nutritional status. Better immunization and nutritional knowledge status were also the influential factors for better nutritional status during adolescence. **Conclusion:** Certain factors do bear influence on nutritional status among urban adolescent girls. **Acknowledgements:** The authors acknowledge to the Indian Council for Cultural Relations, Government of India, for awarding the fellowship for this research. The support of heads and students of the institutions selected for the present study is also acknowledged for their cooperation and help.

Absence of Balanced Diet and Its Link with Childhood Disability in Rural Bangladesh

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Background: Nutritional factors in the early years of life affect later child development. In Bangladesh, where 60% of children suffer from malnutrition, mental retardation has been shown to be significantly higher in those from poorer families. There is no information about the linkage between daily diets of children and their developmental status. **Objective:** Study the correlation among nutritional status, food intake, and developmental disabilities in rural children. **Methodology:** The study was part of an epidemiological survey of childhood disability being conducted in Kishoreganj district. Four thousand children, aged 2-9 years, who were screened for childhood disability in a door-to-door survey, comprised the study population. Every child was also subjected to a nutritional survey with a 24-hour food recall. Within two weeks of the survey, all children who were positive for disability and 10% screened as negative controls were assessed by a team of professionals comprising paediatricians and psychologists. Motor, vision, hearing, speech, cognition, seizures, and behavioural impairments and disabilities were diagnosed. Correlations were made between neurodevelopmental problems and nutritional findings. **Results:** Fifteen percent of children were positive for probable disability, and 29%, 29%, and 43% were moderately stunted, wasted, and underweight respectively. Another 26%, 6%, and 26% were severely stunted, wasted, and severely underweight respectively. The 24-hour food recall analysis revealed that lower-income households were consuming significantly less eggs ($p<0.0001$), pulses ($p<0.008$), fresh fish ($p<0.01$), milk ($p<0.05$), and oil ($p<.05$) compared to the more affluent households. Children, on average, were consuming 300 kcal less than requirement, while mothers' calorie-consumption was adequate. However, proportions of macronutrients for both mothers and children were carbohydrate-dependent, i.e. 89% carbohydrates, 8% protein, and 3% fats. Correlation with developmental variables revealed that children with wasting had significantly poorer behaviour scores and language achievements; underweight children had poor behaviour scores; and stunted children had poor personal-social development. Analysis of data is continuing. **Conclusion:** Children of growing age, who comprised the study population, are being deprived of adequate quantity and quality of food. This is resulting in 'chronic hunger' which is being expressed as poor behaviour scores. Problems in prosocial behaviour and expressive language are rendering these children at risk for poor school performance and psychosocial difficulties in later life. Given the magnitude of malnutrition in Bangladesh, the study has long-term developmental implications for the vast majority of children. Both mothers and children in Bangladesh need to improve quality of food consumption to overcome these difficulties. **Acknowledgements:** The paper is based upon a study being funded by the Centers for Disease Control and Prevention, Atlanta, GA, USA, to the Bangladesh Protibondhi Foundation.

Does Programme Quality Affect Nutritional Status? A Process Evaluation of an Integrated Nutrition Project in Vietnam

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Background: The link between programme quality and improved health remains largely unexplored. **Objective:** Test the hypothesis that children participating in high-quality nutrition education and rehabilitation programmes (NERPs) are more likely to be well-nourished than children attending NERP sessions of inferior quality. NERP sessions last two weeks, are held in health volunteers' homes, and promote healthy, locally-identified feeding, caring, and healthcare-seeking behaviours using the 'positive deviance' approach. **Methodology:** This prospective, randomized, community intervention trial took place during December 1999-December 2000 in a rural province west of Hanoi. It included 153 children, aged 5-25 months, living in Save the Children intervention communes, 86 of whom participated in at least one NERP session. Weight-for-age (WAZ), weight-for-height (WHZ), and height-for-age (HAZ) were assessed at study months 0 and 1 through 12. Measures of programme quality included NERP size, attendance, message delivery, and hygiene and were categorized into terciles to reflect low-, medium-, or high-quality programme implementation. Mixed multivariate regression analysis was used for determining short-term and long-term changes in nutritional status. **Results:** Programme implementation was less than ideal. Five of 7 measures of programme quality were associated with short-term improvements in WAZ and WHZ, but no measure was associated with long-term improvements in nutritional status before or after adjusting for covariates. **Conclusion:** Rigorously assessing programme quality will help programme implementers improve future efforts aimed at ameliorating child malnutrition. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Effectiveness of Community-based Interventions on Reduction of Malnutrition in Urban Slum Communities

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Background: The rate of urbanization in Bangladesh has contributed to unacceptably high levels of malnutrition affecting slum-based city dwellers. Concern is experimenting interventions that effectively reduce malnutrition among slum-based women and children so that malnutrition ceases to be a public-health problem. **Objective:** Test the effectiveness of specific community-based nutrition interventions to reduce malnutrition among urban slum dwellers. **Methodology:** An initial baseline survey was conducted in 2000. Subsequently, specific interventions were designed and implemented in 3 cities (Dhaka, Khulna, and Chittagong), covering a population of around 84,894; of these, 3,574 were children aged less than two years, 4,495 pregnant and 5,312 lactating women, 10,595 adolescent girls, and 1,092 newly-married couples. Interventions are: (1) demonstrative feeding and counselling for severely-malnourished children, pregnant and lactating mothers; (2) growth-monitoring promotion and advice for mothers of moderately-malnourished or growth-faltering children; (3) nutrition and reproductive health education for adolescent girls and newly-married couples; (4) development of a referral linkage to health services for severely-malnourished children; and (5) promotion of better caring and feeding practices at household level. In 2003, key outcomes were measured through a mid-term survey. **Results:** Comparison of baseline with mid-term data for children aged 0-23 month(s) revealed significant reduction in severe under-weight (<60% W/A) from 12% to 5%. Moderate under-weight (60-74% W/A) has also been reduced from 42% at baseline to 32% but remained a serious problem. Maternal wasting (BMI <18.5) also remained a serious problem among 38.4% of non-pregnant women aged 15-49 years compared to 43% at baseline. **Conclusion:** A vertical nutrition-programme approach has been effective in reducing severe malnutrition but less so with moderate malnutrition. Additional interventions to improve household food security and involve multisectoral players should be incorporated to maximize the impact of nutrition interventions. **Acknowledgements:** The funding support of the Department of Foreign Affairs, Ireland, is acknowledged.

Towards New International Growth References for Children Aged Less than 5 Years

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Background: The current international growth references (WHO-NCHS) do not accurately reflect the growth pattern of breastfed children. **Objective:** Develop new growth references for infants and young children through the WHO Multicentre Growth Reference Study (MGRS). **Methodology:** The design combines a longitudinal study from birth to 24 months with a cross-sectional study of children aged 18-71 months. The pooled sample from the 6 participating countries (Brazil, Ghana, India, Norway, Oman, and the USA) is about 8,500 children. Study sub-populations had socioeconomic conditions favourable to growth, low mobility, >20% of mothers following feeding recommendations, and access to breast-feeding support. Individual inclusion criteria consisted of no health or environmental constraints on growth, adherence to WHO/UNICEF feeding recommendations regarding breast-feeding, no maternal smoking, single term birth, and absence of significant morbidity. In the longitudinal study, mothers and newborns were screened and enrolled at birth and visited at home 21 times on week 1, 2, 4, and 6, monthly from 2 to 12 months, and bimonthly in the second year. In addition to data collected on anthropometry and motor development, information was gathered on socioeconomic, demographic and environmental characteristics, perinatal factors, morbidity, and feeding practices. **Results:** The timelines of this study foresee the release of the new growth charts not earlier than in 2005. **Conclusion:** The prescriptive approach taken by MGRS is expected to provide a single international reference that represents the best description of physiological growth for all children aged less than 5 years, and to establish the breastfed infant as the normative model for growth and development.

Assessing Nutritional and Health Status of Children and Women in the Chars of Bangladesh

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Background: The riverine chars in Bangladesh are highly vulnerable to riverbank erosion and flooding, which makes living both hazardous and insecure. More information about the nutritional and health status of their population is needed to identify appropriate interventions. **Objective:** Provide quantitative information on the nutritional and health status of char communities. **Methodology:** Data were collected by the Helen Keller International and the Institute of Public Health Nutrition's Nutritional Surveillance Project from 3,974 households living in chars in Kazipur, Nagessawari and Rajibpur sub-districts during December 2001-September 2002. Data were compared with nationally-representative rural data collected during the same period. **Results:** Char households were 13 times more likely to suffer from riverbank erosion and flooding than households in rural Bangladesh during the monsoon months, August-September 2002. The prevalence of child and maternal diarrhoea (12.2%, 1.8%) was higher than in rural Bangladesh (7.0%, 0.6%); and animal source foods were consumed less frequently. Under-weight among children aged less than 5 years (52-58%) and chronic energy deficiency in mothers (42-43%) were more common in the chars than in rural Bangladesh (48-53%, 36-38%). The prevalence of maternal nightblindness during the last pregnancy (4.0%) was more than double the prevalence in rural Bangladesh (1.8%). **Conclusion:** The nutritional and health situation of children and women in the chars is worse than in rural Bangladesh. Interventions should aim to support the livelihoods of char households to make best use of available resources and cope better with the hazardous environment. Homestead food production is an appropriate food-based strategy which has been shown to improve household food security sustainably to improve dietary diversity and to contribute to the prevention of micronutrient deficiencies. **Acknowledgements:** The contribution of data collectors and the households who participated in the survey is very much appreciated. The financial support of the United States Agency for International Development (USAID) is gratefully acknowledged.

4:45 pm-5:30 pm (Venue: Main Conference Hall)
Plenary 3: Health Systems

050 (363)

Health and Poverty

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The paper analyses the possible links among economic growth, poverty, and health, using panel data for Indian states. The findings indicate that, although growth tends to reduce poverty, significant improvements in health status are also necessary for poverty to decrease. Also, growth and health status are positively correlated and have a two-way relationship, suggesting that better health status enhances growth by improving productivity, and higher growth allows better human capital formation. Health expenditure is an important determinant of both higher growth and better health status and is, therefore, a key tool available to policy-makers. Among some of the other exogenous variables, literacy and industrialization seem to improve both health outcome and growth on the one hand and reduce poverty on the other.

4:45 pm-5:45 (Venue: Meeting Room 2)
Scientific Session 7: Infectious Diseases 1

051 (117)

HIV and HBV Infection among Sex Workers of Kolkata City, India

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Background: Currently, about 30,000 commercial sex workers (CSWs) are estimated to be operating in Kolkata, India. Sexually transmitted infection (STI)-intervention activities have been carried out in all brothels of Kolkata city for control and prevention of HIV/AIDS. **Objective:** Understand the prevalence of HIV and HBV infection among brothel-based sex workers and identify the possible lacunae of current STI-intervention programme. **Methodology:** A community-based cross-sectional study was initiated, and 168 sex workers were selected randomly as study subjects. The sample size was estimated to be 160. They were interviewed by two trained sociologists using a structured questionnaire. Blood samples were collected to test HIV and HBV. The tests were carried out by unlinked and anonymous method. **Results:** The prevalence of both HIV and HBV was 8.9% (CI 5.0-14.2). The prevalence of HIV/HBV was 16.6% (CI 11.3-23.1). HIV-2 was detected in only one of the 168 sex workers. A higher HBV infection was observed among younger sex workers. There was a wide gap between reported and evaluated condom use by the sex workers. Risk of HIV/HBV infection was more with sex workers, who had sex outside the brothel and sex with clients having visible penile ulcer/discharge. No statistical association was observed among HIV/HBV infection, duration of sex work, and number of clients entertained daily. **Conclusion:** STI-intervention programme needs to be strengthened by incorporating the above-identified factors. The role of hepatitis B vaccine needs to be considered for sex workers, particularly for young sex workers, to prevent/control HBV infection and to reduce disease progression of HIV in case of co-infection.

Street Child Sex Workers: Behavioural Risk Factors for HIV/AIDS and Sexually Transmitted Infections

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Background: A considerable proportion of street-based sex workers are under age (i.e. young and adolescent), and the number is increasing day by day. Child sex workers are highly demanded segment by clients and are often subjected to sexual coercion. Street-based sex workers are very marginalized, socially stigmatized, and highly mobile, and have often been presented as a major source of sexually transmitted infections (STIs) and HIV infections. **Objective:** Assess the vulnerability of street child sex workers towards HIV/AIDS and STIs. **Methodology:** Data for this analysis were taken from a study titled 'Mapping and situation analysis of the floating sex workers in four cities and towns in Bangladesh' conducted by HIV/AIDS and STD Alliance Bangladesh (HASAB) in 2002, where 770 sex workers were interviewed following a time-location-duration sampling scheme. Study areas were Chittagong, Barisal, Comilla, and Ishwardi. Both qualitative and quantitative data-collection methods were applied. The above study revealed that one-sixth of sex workers were aged less than 18 years. The current analysis of the study considered only this segment of sex workers (i.e. n=118). **Results:** Three-fourths (77%) of the child sex workers did not know valid ways to avoid STIs and HIV. More than half of the respondents reported their experience of STI symptoms, such as vaginal discharge, dysparonia, etc. during last 6 months. Each sex worker served, on average, 3 clients in each business day, and 47% of the sex workers had had group sex during last month. About 90% of the sex workers did not use condom regularly. **Conclusion:** The low level of knowledge, high prevalence of STI symptoms, and low use of condom by street child sex workers are solid catalysts in rapid spread of HIV and STIs. An HIV/AIDS/STI intervention with child right issues needs to be considered in future. **Acknowledgements:** Family Health International for financial support and four local NGOs in four sites—YPSA in Chittagong, SAS in Barisal, DRISTI in Comilla, and Nijpath in Ishwardi–which supported during data-collection period are acknowledged.

Occult Hepatitis B Virus Infection in Eastern India and Associated Naturally-occurring Surface Gene Mutants in 'AntiHBc only' Individuals

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Background: Occult HBV infection is characterized by the presence of HBV genome in hepatitis B surface antigen (HBsAg)-negative individuals. The presence of occult HBV infection has been detected in all geographical areas but it is not known how widespread these variants are. **Objective:** Determine the prevalence of occult HBV infection in eastern India and evaluate its significance and possible mechanisms. **Methodology:** Investigated were 2,551 samples collected from chronic hepatitis patients, blood donors and their family members, and samples from community-based serosurveys, for 'antiHBc' only samples by commercial ELISA. The samples identified as 'antiHBc only' were investigated for HBV DNA by a sensitive qualitative PCR. Viral loads were measured in samples with detectable HBV DNA, and the DNA sequences were analyzed by direct sequencing in an automated DNA sequencer. **Results:** Of 143 antiHBc-positive samples, HBV DNA was detected in 11 (7.7%) samples by nested PCR, all of which had a viral load of <400 copies per mL. The S gene region could be amplified in 11 samples. Phylogenetic analysis of HBV surface gene of amplified DNA showed the presence of genotype D and C only. The DNA isolated from one antiHBc only sample showed the presence of G to R mutation at codon 145, which can also infect vaccinated individuals. Moreover, it has previously been reported that this mutant gives false-negative HBsAg results. **Conclusion:** HBV DNA can be detected among 'antiHBc only' samples. The presence of vaccine escape mutant is of concern due to possibility of its spread by blood donation.

Seroprevalence of Hepatitis B Surface Antigen and Hepatitis C Virus Antibody among Newly-commissioned Officers, Gentlemen Cadets, and New Recruits of the Armed Forces

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Background: Hepatitis B virus (HBV) infection is a major health problem worldwide. Approximately, 30% of the world's population or about 2 billion persons have serologic evidence of HBV infection, and around 350 million are chronic carriers of the virus, 60 million of whom will die from liver cancer and another 45 million from cirrhosis. The carrier rate worldwide varies from as low as 0.1% to 0.2%. The prevalence of HBV infection in Bangladesh is high. Hepatitis C virus (HCV) infection is also extensive throughout the world. The World Health Organization (WHO) estimated in 1997 that about 3% of the world population have been infected with HCV. There are more than 170 million chronic carries of HCV worldwide who are at risk of developing liver cancer, liver cirrhosis, or both. **Objective:** Find out the seroprevalence of HBsAg and anti-HCV among newly-commissioned officers, gentlemen cadets, and new recruits of the armed forces. **Methodology:** The study was carried out in the Armed Forces Institute of Pathology (AFIP), Dhaka and two peripheral Combnied Military Hospitals (CMH) from 1 July 2001-30 June 2003. In total, 5,858 blood samples from the newly-commissioned officers (n=186) and gentlemen cadets (n=561) were collected. The sera were separated, and all were tested for HBsAg by ELISA. Of the 5,858 serum samples, 4,212 were also tested for anti-HCV using ELISA test kits. **Results:** HbsAg was positive in 214 (3.65%) persons. Statistically significant differences in HBsAg positivity ($p<0.01$) were observed between newly-commissioned officers and new recruits (1.61% vs 3.91%) and also between cadets and new recruits (1.96% vs 3.91%). Of 4,212 persons screened, none was positive for anti-HCV. **Conclusion:** Having known the seroprevalence of HBsAg and anti-HCV in the study groups from different socioeconomic backgrounds, appropriate measures aiming at preventing these infections in the armed forces personnel can be adopted.

DAY 2: MONDAY, 8 DECEMBER 2003

8:30 am-9:15 am (Venue: Meeting Room 1)
Plenary 4: Infectious Diseases

055 (364)

Emerging and Re-emerging Enteric Pathogens and Expanding Resistance to Antibiotics

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Current estimates of the global burden of diarrhoeal diseases based on studies published during 1992-2000 indicate a median of 3.2 episodes of diarrhoea per child-year, and estimates of mortality reveal that 4.9 children per 1,000 per year died as a result of diarrhoeal disease in the first years of life, resulting in about 2.5 million deaths per year. Therefore, diarrhoeal diseases continue to remain a formidable disease. The explosive introduction of enterohaemorrhagic *Escherichia coli* (EHEC) in Japan in 1996 is just an example of how devastating an emergent diarrhoeal aetiology can be, and its introduction has completely changed the view of infectious diarrhoeas in Japan. Recent studies have also shown the widespread occurrence of Shiga toxin-producing *E. coli* (STEC) in Calcutta, India, especially in bovine sources and the potential of these STECs to emerge as important aetiology of diarrhoeas. Among the bacterial enteric pathogens, *Vibrio cholerae* and *Shigella* species are in the forefront in several developing countries because of their propensity to be associated with epidemics and pandemics of disease. The sudden appearance and spread of the O139 serogroup of *V. cholerae* and the introduction of several new untypable variants of shigellae show that these enteric pathogens are in a perpetual state of change mediated by events like horizontal gene transfer. Another recent inexplicable event was the explosive spread of some serotypes of *V. parahaemolyticus*, starting with the O3:K6 serogroup into several South Asian countries and the United States. There are at least 4 serotypes of *V. parahaemolyticus*, which have now acquired the pandemic potential. Recent studies in Bangladesh have shown that enterotoxigenic *E. coli* (ETEC) is almost as common as *V. cholerae* O1 in causing acute watery diarrhoea and the prevalence of ETEC ranged from 16% to 20% with the highest incidence among children. The problem of expanding antimicrobial resistance in enteric pathogens also has caused major treatment problems. In the last two decades, reports from several cholera endemic countries of toxigenic *V. cholerae* strains resistant to antibiotics, including tetracycline, ampicillin, kanamycin, streptomysin, sulfonamides, trimethoprim, and gentamicin, are appearing with increasing frequency. Two recent outbreaks caused by ciprofloxacin-resistant strains *Shigella dysenteriae* type 1 in Calcutta and neighbouring areas have brought to fore the issue of antimicrobial resistance among enteric pathogens and the confusion this can cause to clinicians.

8:30 am-11:30 am (Venue: Meeting Room 2)
Scientific Session 8: Nutrition 3: Infant Feeding and Maternal Nutrition

056 (014)

Prevalence of Lactose Intolerance among Healthy Kuwaiti and Asian Adult Volunteers

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Background: Lactose intolerance is a common condition among the Asian population. Very few reports are available on the prevalence of lactose intolerance among the Arab population. **Objective:** Compare the prevalence of lactose intolerance between apparently healthy adult Kuwaiti and Asian volunteers following a challenge of lactose drink and investigate if a concentrated milk drink containing equivalent amount of lactose can be used as an alternative for the breath hydrogen test (BHT). **Methodology:** Seventy Kuwaiti and 79 matched Asian volunteers were randomly selected for the study. The study was conducted at the Amiri Hospital, Kuwait. After an overnight fasting, 40 g of lactose powder in 250 mL of water was given to the subjects for drinking. Twenty mL of expired air samples were collected before the drink and after every 30 minutes for the next 2 hours. Breath hydrogen levels were determined using Quintron Microlyzer. Hydrogen level of 10 ppm or more above the basal level was taken as positive. After one week, milk powder dissolved in 250 mL of water containing equivalent amount of 40 g of lactose was given to the Asians for drinking. Breath hydrogen levels were again determined from the expired air samples. **Results:** Forty-seven percent of the Kuwaitis and 58% of the Asians were positive for the breath hydrogen level after one hour of ingestion of lactose. Fifty-eight percent of the Asians were also positive after challenge with concentrated milk. **Conclusion:** The prevalence of lactose intolerance in the Kuwaiti and Asian healthy volunteers is similar after lactose ingestion. A natural milk-drink may be used also for the determination of breath hydrogen level with valid results.

Studies on Fatty Acid Composition of Edible Oil

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Background: Edible oil is an energy-dense food nutrient providing 9 kcal/g. Oil, rich in long chain mono- and polyunsaturated fatty acids, especially essential fatty acids (linoleic and linolenic), is of primary importance for its digestion and absorption. **Objective:** Find out a suitable variety of locally-available edible oil for general mass to combat malnutrition and to control cardiovascular diseases and gastrointestinal disorders. **Methodology:** Fatty acid analysis of 5 varieties of locally-consumed edible oils was carried out on a gas chromatograph (GC) equipped with a flame ionization detector (FID) and stainless steel-packed column. Nitrogen was used as a carrier gas. The study was conducted at the Oilseed and Lipid Technology Section-I, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, during July 2002-June 2003. **Results:** Sunflower oil contained the highest percentage of long chain mono- and polyunsaturated fatty acids (91.49±3.51%) compared to soybean oil (81.14±2.98%), mustard oil (86.80±6.28), palm oil (53.30±0.72%), and coconut oil (7.12±1.02%). **Conclusion:** Sunflower oil with the highest percentage of mono- and polyunsaturated fatty acids appears to be the most suitable edible oil for consumption, especially from the standpoint of cardiovascular disease, gastrointestinal disorders, and nutritional rehabilitation of post-diarrhoeal patients. **Acknowledgements:** This work was done by Gas Chromatograph procured under 'Food Packaging' Project (ADP Project) financed by the Government of Bangladesh.

Practice of Breastmilk Substitutes Use among Urban Lactating Mothers Attending Some Private Healthcare Facilities in Dhaka City

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Background: Extensive research documents diverse and compelling advantages to infants, mothers, families, and society from breast-feeding and the use of human milk for infant feeding. These include health, nutritional, immunologic, developmental, psychological, social, economic and environmental benefits. Regardless of these benefits, many women are still using breastmilk substitutes (BMSs), resulting in low prevalence of breast-feeding. Physician apathy, misinformation, and disruptive hospital policies are sometimes responsible for introduction of BMSs. However, the role of healthcare facilities in this regard is not yet well-documented. **Objective:** Assess the status of BMS use and also explore the reasons for their use among lactating mothers attending private healthcare facilities. **Methodology:** A cross-sectional study was conducted among 116 lactating mothers with infants, aged 0-6 month(s), who attended some private healthcare facilities in Mirpur of Dhaka city from April to June 2003. Purposive sampling method was followed. Data were collected by face-to-face interview using a semi-structured questionnaire. For collecting qualitative information, mothers were asked open-ended questions. Analyses were done using SPSS programme, version 11.5. Chi-square test was used for binomial data analysis. **Results:** The mean age of the mothers was 25.15 (Â±4.94) years. The large majority (85.34%) of them had formal schooling, with 46.46% having education up to secondary level. Of them, 90.5% were housewives, 60.3% lived in nuclear family, and 27.6% had a monthly family income of Tk 6,000-10,000. 63.8% used BMSs to feed their children and of them, 90.6% gave infant formula, and 9.4% gave other substitutes. Healthcare providers, such as doctors and nurses, mostly influenced the decision about the feeding of BMSs. As many as 87.8% of respondents fed BMSs according to advice of others. Of them, doctors and nurses influenced, respectively, 76.5% and 4.4% of the mothers. Insufficiency of breastmilk was the main reason (63.5%) for introducing BMSs. The rate of exclusive breast-feeding was only 17.2%, irrespective of prelacteals. Regarding the source of getting BMSs, it was reported that 40.5% of the mothers bought them from medical stores inside the healthcare facilities. No significant differences were observed in feeding BMSs among mothers who had received antenatal care and who did not. But antenatal care was associated positively with colostrum feeding (p=0.021). It was also evident that prelacteal feeding was associated with increased use of BMSs by mothers (p=0.019). **Conclusion:** Despite the demonstrated benefits of breast-feeding and a nation-wide breast-feeding campaign, exclusive breast-feeding practice was used by less than one-third of urban mothers attending private healthcare facilities with a very high percentage using BMSs. **Acknowledgements:** The support of Department of Nutrition, National Institute of Preventive and Social Medicine, Dhaka, is acknowledged.

Effect of Baby-friendly Hospital Initiative on Breast-feeding Practices in Sindh

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Background: Since infant mortality in Pakistan is very high, baby-friendly hospital initiative was started in Pakistan in 1992 after training of 10,500 healthcare providers in 10 years. The effect of antenatal counselling on the practices of breast-feeding was evaluated. Baby-friendly Hospital in Sindh is a role model programme with a total of 75 in whole Pakistan and 53 in urban and rural areas of Sindh. **Objective:** Find the change in the attitude of mothers after receiving correct knowledge and development of skills for optimum breast-feeding practices. **Methodology:** A WHO/UNICEF questionnaire used for assessment/re-assessment was evaluated, particularly from antenatal clinics where mothers are interviewed for knowledge, practices, and changes in their attitude. This was a cross-sectional, interventional study after antenatal counselling for at least 3 visits in the same baby-friendly hospitals. **Results:** Findings revealed that the pregnant mothers responded to the WHO/UNICEF questionnaire used to assess the baby-friendly hospital. Two hundred thirty-six women were included in the study. Qualitative analysis showed that 196 respondents were from urban and 40 from rural areas of Sindh. During antenatal care, 89% in urban and 11% in rural areas received counselling by healthcare providers. 96.2% in urban baby-friendly hospitals and 3.8% in rural area listed more than two benefits of breast-feeding. 94.5% of women were given help by healthcare providers, and 4.9% of women in rural area were helped by family members. The skill development for position and attachment was 95.1% in urban and 80% in rural areas. Importance of rooming in was known by 95.2% in urban and 4.8% in rural Sindh. The importance of demand feeding was 98% in urban and 2% in rural areas. The skill development in breast-feeding practices in urban area was 96.5%, and in rural area, it was 3.5%. The group talk about infant-feeding formula was not discussed in 98.7%. **Conclusion:** The KAP level can be significantly high by provision of antenatal counselling for at least 3 visits in a single pregnancy. The mothers are best motivated during the antenatal visits, and their previous bad experiences/taboos can be resolved. **Acknowledgements:** A joint programme of Government of Sindh was funded by UNICEF.

060 (077)

Exploring Feasibility of Social Marketing of Micronutrient-enriched Complementary Food Packets Similar to BINP Food Supplements

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Background: The Bangladesh Integrated Nutrition Programme (BINP) introduced a supplementary food (SF) which targeted growth faltering and severely-malnourished children aged less than 2 years. SF was a blend of roasted rice, roasted lentil, molasses, and edible oil produced locally. **Objective:** Gather qualitative information on the feasibility of marketing a micronutrient-enriched complementary food based on BINP SF that would target the same population after BINP withdraws. **Methodology:** Information was collected through interviews and focus-group discussions with project staff, Community Nutrition Promoters (CNPs), Community Nutrition Organizers (CNOs), Women's Group members, and project beneficiaries. **Results:** Women's Group members and beneficiaries understood the importance and value of feeding children supplement/complementary food and expressed interest in complementary food. However, since BINP SF has always been distributed at no cost, they were uncertain about the market potential of a commercial complementary food. They doubted whether husbands would purchase it. CNPs/CNOs were positive but had reservations, emphasizing the need for behaviour change communication (BCC), around consumer habits. They cautioned that adopting new consumer habits might take time and wondered how much the profit would be. Regarding willingness and ability to buy, both project beneficiaries and CNPs/CNOs opined that cost per packet should be no more than 2 taka per day. **Conclusion:** The findings of the study indicate that marketing a complementary food even in rural areas might work if social marketing were supported by strong BCC and if a group similar to the BINP Women's Group were utilized. Before considering a large-scale commercial production, a more-structured quantitative market analysis, exploring demand, availability of funds, and willingness to pay should be conducted among BINP SF beneficiaries, non-beneficiaries in BINP areas, and low-income populations in non-BINP areas.

Energy and Protein Adequacy in Urban Adolescent Girls: Assessment Using EAR-cut Point Method

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Background: To assess dietary adequacy, nutrient intake data are normally compared to standard intake for the respective population referred as recommended dietary allowance (RDA), and the level of intakes is expressed as percent adequacy. Food and Nutrition Board, Institute of Medicine (IOM), USA, has recently introduced a new approach called "Estimated Average Requirement (EAR)-Cut point method" to estimate adequacy of intakes. **Objective:** Assess energy and protein adequacy among adolescent girls from urban region of Dhaka city, Bangladesh, using the EAR-cut point method-probability approach. **Methodology:** In total, 310 girls, aged 10-19 years, studying at 6 different schools and colleges in Dhaka city, were selected. Dietary intake was obtained by 24-hour recall method, and calorie and protein intakes were analyzed using a ready reckoner developed for Bangladeshi cooked foods. To apply the EAR-cut point (the median value of the normally-distributed reference intakes) method, calorie and protein requirements per unit body weight (ICMR) were used as reference intakes to be distributed against actual intakes. Age-adjusted standardized predicted intake values were calculated to eliminate the effect of age factor. Statistical analysis regression coefficient, probability distribution, and other suitable analysis were employed using statistical package SPSS (10.0 version). **Results:** Using EAR-cut point, calorie and protein inadequacy was estimated to be 44.7% and 26.0% respectively. In comparison between EAR-cut point and conventional method (% RDA), higher level of calorie and protein inadequacy was observed in the conventional method (54.5% and 65.2% respectively considering cut-off level of 90%) than that in the EAR-cut point method. **Conclusion:** For assessing dietary adequacy, use of RDA as standard reference may be an over-estimation of requirements. Studies are needed to be done to recommend a more realistic standard for comparison. **Acknowledgements:** The authors acknowledge to the Indian Council for Cultural Relations, Government of India, for awarding the fellowship for this research. The heads and students of the institutions selected for the present study are also acknowledged for their cooperation and help.

Use of Diet Diversity for Improving Nutrient Adequacy and Nutritional Status in Children and Women in Rural Bangladesh

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Background: Few studies have quantified the importance of diet diversity and use in improving nutrient adequacy and nutritional status. **Objective:** Quantify the associations between food variety and diet diversity, adequacy of energy and selected nutrients, and nutritional outcome parameters for children and women in rural Bangladesh and evaluate the importance of food groups. **Methodology:** Repeated measurements of food intakes were taken by 24-hour recall, anthropometry, and haemoglobin. Three survey rounds in 1995-1996 were included in each of 3 rural areas. Children aged 24-59 months (n=170) and non-pregnant women aged 18-45 years (n=688) were included. Food variety score (FVS) and diet diversity score (DDS) were defined per individual per 24-hour recall as number of foods eaten and number of food groups eaten respectively. **Results:** Correlations between mean nutrient adequacy ratio (MAR) and DDS were (*r*±95% CI) 0.45±0.07 for children and 0.40±0.04 for women ($p<0.001$). For MAR and FVS, correlations were 0.43 for both children and women ($p<0.001$). Individuals with low FVS and DDS had increased risks of low MUAC: (OR 95% CI) 1.58 (1.04-2.38) and 1.76 (1.16-2.68) for children respectively and 1.08 (0.95-1.23) and 1.20 (1.05-1.38) for women respectively. Children with low DDS had increased risks of low blood haemoglobin and weight-for-height: 1.73 (1.15-2.61) and 2.22 (1.33-3.69) respectively. Repeated measures ANCOVA showed that the intake of one additional leafy vegetable would increase MAR with up to (least square mean±95% CI) 9.7±2.7% in children and 7.7±1.2% in women. **Conclusion:** FVS and DDS were positively associated with nutrient adequacy and MUAC in children and women. Leafy vegetables were the most important food group for increasing nutrient adequacy. **Acknowledgements:** The financial support of the Danish Council for Research in Developing Countries and the United States Agency for International Development (USAID) is acknowledged. Data were made available by Dr. Howarth Bouis, International Food Policy Research Institute, Washington DC, USA.

Effects of Energy Density of Complementary Foods on Total Energy Intakes by Healthy, Breastfed Children in Bangladesh: A Preliminary Study

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Background: Information is needed on the independent effects of energy density and feeding frequency of complementary foods (CF) on energy intake from CF and breastmilk to develop practical feeding recommendations for healthy breastfed children. **Objective:** Evaluate the effects of varied energy-dense diets on total energy intake (CF+BM) by healthy, breastfed children. **Methodology:** Ten children, aged 9-18 months, with weight-for-length and height-for-age z-scores >-1.5 and <1.5 were studied. Children were fed CF of two energy densities (0.4 kcal/g, as low density [LD], and 1.5 kcal/g, as high density [HD]) 3 times daily along with *ad libitum* breast-feeding (BF). The diets were fed during three 8-d study periods in random sequence (HD-LD-HD or LD-HD-LD). CF intakes were measured by weighing bowls before and after meals and breastmilk intakes by test-weighing. **Results:** The mean (±SD) intakes of CF and breastmilk during day 5-7 of the last 2 diet periods are shown by level of density of CF in the table.

Parameter	High density	Low density	p value
Complementary food (g/d)	439±111	752±252	<0.0001
Complementary food energy (kcal/kg/d)	75.2±18.8	35.2±10.9	<0.0001
Breastmilk (g/d)	192±115	234±121	0.03
Total energy (kcal/kg/d)	88.3±19.5	52.0±9.8	<0.0001

Conclusion: It is concluded that, despite greater intake of LD CF, energy intake from HD CF increased at this level of feeding frequency. Breastmilk intake decreased slightly when HD CF was provided, but total energy intake (CF+breastmilk) was substantially greater with the HD diet. Additional studies are needed with a broader range of feeding frequencies and energy densities of CF. **Acknowledgements:** The financial support of the Bill and Melinda Gates-Government of Bangladesh Fund of ICDDR, B and Fogarty International Center, National Institutes of Health, and University of California, Davis, is acknowledged.

Relation of Maternal-Infant Feeding Interaction with Stressors and Distress in Bangladesh

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Background: Previous research suggests that stress may affect maternal-infant interaction and child health and development in a poor population. Given the importance of maternal-infant interaction in shaping the well-being of children, understanding which factors lower the quality of interaction is needed. **Objective:** Examine (1) the relation of stressors and distress with quality of maternal-infant feeding interaction, and (2) the suitability of a measure of feeding interaction not previously used in a developing country. **Methodology:** As part of the MINIMat nutrition intervention study in Bangladesh, 120 mother-infant pairs were observed in home at 3.5 months to assess the quality of feeding interaction using the NCAST feeding scale. This scale is based on a checklist of 76 items that form 4 maternal and 2 infant subscales, and 18 contingency items. Internal consistency, test-retest reliability, and criterion validity were assessed. Questionnaires that had been developed and field-tested were administered to mothers to assess stressors (food insecurity and interpersonal conflict) and distress. Correlation and analysis of variance were used. **Results:** Quality of feeding interaction was slightly lower than reported in the USA. Lower quality of feeding interaction was related to higher food insecurity, greater interpersonal conflict, and increased distress. Internal consistency, reliability, and validity of the feeding scale were similar to that previously found elsewhere. **Conclusion:** The differences in quality of feeding interaction with some stressors, particularly interpersonal conflict, were much larger than the differences across populations or differences due to physical deformities. This study demonstrates the importance of assessing feeding interaction, the potential impact of stressors and distress in lowering the quality of feeding interaction, and the suitability of the NCAST feeding scale in a developing country. **Acknowledgements:** Financial support was provided by the American Institute of Bangladesh Studies, the Cornell Einaudi Center for International Studies, and the Cornell Program in International Nutrition. Support was also provided by the Child Health Unit, Public Health Sciences Division, ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh.

Influence of Care Practices on Growth and Morbidity Patterns of Young Infants from Lower Socioeconomic Status—Some Nutritional Aspects

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Background: Quantity and quality of breastmilk consumed and morbidity rates influence early growth faltering which subsequently manifests in malnutrition. The influence of maternal care-giving practices in early growth faltering is an area which is yet to be explored. **Objective:** Study the influence of maternal care-giving practices on breast-feeding patterns and determine its influence on morbidity patterns of young infants aged 0-6 month(s). **Methodology:** In a longitudinal cohort study, 87 infants (both boys and girls) from urban slum (n=42) and rural area (n=45) were followed from birth till 6 months of age. Monthly home visits were carried out, and required data were collected on breast-feeding practices, patterns of breastmilk consumption, maternal dietary practices, care-giving practices, and the infant's morbidity patterns. Further, data were also gathered on the infant's anthropometry (weight, length, head circumference, chest circumference, mid-upper arm circumference [MUAC], and maternal nutritional status body mass index [BMI] and haemoglobin [Hb] levels). All these factors were studied in detail to determine their influence on the infants' morbidity rates/patterns. The study was carried out from April 2002 to May 2003. **Results:** The results indicated that maternal care-giving practices (including breast-feeding practices) had an influence on morbidity of young infants ($p<0.05$). The dietary patterns of mothers indicated that the majority (71%) of rural mothers and 40% of urban poor mothers consumed restricted diets, especially devoid of green-leafy vegetables and fruits. The mean height of the respondents was 151.1±5.35 cm, weight 43.4±6.89 kg, BMI 19.03±3.04 cm, and Hb levels 10.5±0.99g/dL—that too when the majority of these mothers were advised to consume iron-folate tablets during pregnancy. Of these, 48.9% reported that their milk was insufficient to meet the need of their infants' hunger and, thus, supplemented their diet with animal milk/tea/water, etc. Most mothers practised 'Switch nursing' depriving the infants of hind milk—rich in vitamin A which could perhaps be the cause of frequent sickness in the majority of infants before the age of 6 months. The anthropometric measurement of infants indicated that the rural infants demonstrated a better gain in all the anthropometric parameters than the urban poor infants—the gain being slightly lesser in the first two months of infancy than in 4-6 months. **Conclusion:** Nutritional status of mother and her care-giving abilities influences her breast-feeding patterns/decisions which subsequently influences the infants' morbidity rates. **Acknowledgements:** The financial support of the Indian Council of Medical Research is gratefully acknowledged.

Effects of Fructo-oligosaccharide as a Prebiotic Ingredient in Baby Formula

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Background: Certain indigestible oligosaccharides may benefit gastrointestinal tract via fermentation and proliferation of desirable bacterial species. **Objective:** Investigate the effects of fructo-oligosaccharide on faecal microflora and evacuation habits of formula-fed infants. **Methodology:** The subjects were 14 infants (male 10, female 4) aged, on average, 12.4 weeks. They were all bottle-fed before the weaning period with good health condition. This experiment was cross-over design: all subjects were fed powdered milk mixed with FOS (0.25 g/kg/d) 3 times a day for 3 weeks, followed by 3 weeks without FOS or vive-versa. Faecal samples were collected at the start of the trial, after 3 weeks, and after 6 weeks. The total number of anaerobic bacteria, *Bifidobacteria, Lactobacillus*, and *Bacteroides,* was measured by plate counting of the faecal samples respectively. **Results:** No difference in growth was observed between the period of FOS treatment and the period without FOS. A slight but not significant decrease of faecal pH was found during FOS treatment, from pH 6.5 to 6.3. After stopping FOS treatment, faecal pH value was back to its starting value. The content of total anaerobic bacteria and *Bacteroides* did not differ among the 3 sampling times. A significant increase in *Bifidobacterium* sp. was found, when its total number before and during FOS treatment was compared ($p<0.05$). This number slightly decreased when FOS consumption was discontinued. For *Lactobacillus* sp., a similar pattern was found: a significant increase during FOS treatment ($p<0.01$) and a decrease to the basal level when the prebiotic consumption was stopped. Defecation frequency increased slightly compared to control, whereas faecal consistency became softer after 3 weeks of FOS treatment. **Conclusion:** Fructo-oligosaccharide has a prebiotic effect in Korean babies at a dosage of 0.25 g/kg/day. **Acknowledgements:** The financial support of the Sensus Operation in Netherlands is acknowledged.

Estimated Nutrient Intakes and Adequacies Change When Newer Food-composition Values are Applied

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Background: Accuracy of food-composition values affects the reliability of estimated nutrient intakes and adequacies. **Objective:** Compare intakes and adequacies of vitamin A, C, iron, and calcium, calculated by use of 3 sets of pertinent food-composition tables. **Methodology:** Food consumption of 81 children aged 24-71 months and 182 women aged 18-45 years in rural Bangladesh in 1996 was observed using 24 hours food weighing. Three sets of composition values were used: one published by Helen Keller International (HKI), an international database (World Food Dietary Assessment System (WFDAS)), and the HKI table modified with newer values obtained by chemical analyses of selected foods (M-HKI). Thirteen foods were selected on the basis of frequency of consumption and contribution of nutrients. **Results:** Differences in estimated intakes and adequacies were found for vitamin A, C, iron, and calcium ($p<0.001$). The largest relative differences were seen for vitamin A and iron. The use of HKI and WFDAS resulted in similar estimates of vitamin A intake (least square mean±SE: 234±73 RE/child/d and 627±52 RE/woman/d). The use of M-HKI resulted in lower estimates: 118±73 RE/child/d and 347±52 RE/woman/d. The use of HKI resulted in the highest estimated iron intakes (15±1.2 mg/child/d and 32±0.8 mg/woman/d), significantly different to estimates obtained by use of WFDAS (3.7±1.2 mg/child/d and 7.4±0.8 mg/woman/d) and M-HKI (7.7±1.2 mg/child/d and 15.0±0.8 mg/woman/d). The differences in estimated nutrient intakes were reflected in similar differences in estimated nutrient adequacies. **Conclusion:** Up to date chemical analyses of foods selected on the basis of a high frequency of consumption and contribution of nutrients are a useful approach to improve the quality of food-composition tables. **Acknowledgements:** The financial support of the Danish Council for Research in Developing Countries and the Bilateral Programme for Enhancement of Research Capacity in Developing Countries, Ministry of Foreign Affairs, Denmark, is acknowledged.

8:30 am-9:15 am (Venue: Meeting Room 3)
Special Session 2: Safe Water

068 (365)

Improving Health by Improving Water Quality: Distributed Technologies for Household Water Treatment and Storage

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Over 1.1 billion persons lack access to safe drinking-water, and an estimated 2 million children die from waterborne diseases each year. Efforts to prevent these deaths have largely focused on improvements in water sources, sanitation facilities, and hygiene practices. While these are essential long-term goals, recent studies have suggested that health gains can be greatly accelerated through improvements in water quality achievable by point-of-use water treatment and safe storage. Four point-of-use treatment methods have been field-tested: chlorination; flocculation plus chlorination; solar disinfection; and filtration. Each has proven safe, acceptable, inexpensive, and effective at reducing episodes of diarrhoeal diseases among users by approximately 50%. This presentation will review current scientific data on point-of-use water treatment and safe storage methods and consider why observed health gains far surpass those predicted by the dominant water and sanitation paradigm. Emerging technologies and new implementation strategies for improving water management at the point-of-use and the major challenges this approach faces in the future will be discussed. The role that the recently-created International Network to Promote Household Water Treatment and Safe Storage can play in meeting these challenges will also be described.

9:30 am-11:30 am (Venue: Meeting Room 1)
Scientific Session 9: Infectious Diseases 2

069 (073)

Sequence-based Typing of Dengue Viruses from an Outbreak of Dengue Fever in Dhaka, Bangladesh

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Background: Outbreaks of dengue fever (DF) and dengue haemorrhagic fever (DHF) have been occurring in Dhaka, Bangladesh, since 2000. The source of the virus is likely to be of South East Asian region where dengue is endemic. **Objective:** Characterize dengue virus strains from Dhaka by nucleotide sequencing and comparison with strains from different countries to determine its possible source. **Methodology:** During November 2000-December 2000 and during July 2001-October 2001, 18 serum samples were found to contain dengue (DEN) virus by reverse transcriptase-polymerase chain reaction (RT-PCR). These samples were shipped on dry ice to the Queensland University of Technology, Australia, where the viruses were initially cultured in the C6/36 mosquito cell line and typed by RT-PCR or immunofluorescence assay. Nucleotide sequencing of E-protein gene was done on 6 isolates that were recovered. **Results:** DEN 3 virus was recovered from 9 of the samples. Nucleotide sequencing of the E-protein gene was carried out with 6 of these. Phylogenetic analysis showed that DEN 3 strains of Bangladesh were very similar and clustered together with strains from Thailand. **Conclusion:** Outbreaks of DF and DHF in Bangladesh in 2000 and 2001 were due to DEN 3 viruses, which were likely to have originated in Thailand. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and the financial and technical support of the Lee Foundation and the School of Life Sciences, Queensland University of Technology, Australia, is acknowledged.

Clinical Characteristics of Visceral Leishmaniasis in an Endemic Community in Bangladesh

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Background: In Bangladesh, 20 million people live in areas with active transmission of visceral leishmaniasis (VL). In January 2002, community-based surveillance for VL was initiated in 3 sections of a village in Fulbaria, Mymensingh district. **Objective:** This study presents preliminary data on clinical characteristics of a series of recent cases of visceral leishmaniasis from the study site. **Methodology:** An initial household survey was conducted to identify past and current cases of VL in a population of 2,348 persons, and active surveillance was begun in January 2002. A current case of VL was defined by clinical findings consistent with VL and confirmation by a specific laboratory test (rK39 dipstick). All patients were referred to Fulbaria Upazila Health Complex for treatment with sodium antimony gluconate (SAG) according to the national guideline. **Results:** During 15 January 2002-31 August 2003, 56 active cases of VL were identified. Males (54%) and females (46%) were similarly affected. The mean age was 18 years (range 3-45 years). The median duration of fever before diagnosis was 3 months. Fever, weight loss, anorexia, abdominal pain, darkening of the skin, and cough were the most commonly reported (96.4%, 98.2%, 92.7%, 83.6%, 85.5%, and 70.9% respectively). Spleen and liver enlargement were observed in 88.7% and 81.1% respectively. Six (10.7 %) patients died; 5 of the deaths occurred after completing the recommended course of 20 doses of SAG. The sixth patient died during the course of therapy. Five patients who died were reported to have defervescence followed by recurrence of high fever, while one had severe haemorrhagic complications and another had tuberculosis with hepatitis. **Conclusion:** The case-fatality rate among patients described in this report was high, particularly in light of the fact that it included 5 patients who had recently completed a course of SAG therapy. These deaths were likely due to complications of VL, such as secondary bacterial infection and haemorrhage, but also raise the possibilities of drug-associated adverse events or emergence of drug resistance. **Acknowledgements:** The authors are grateful to Dr. Madon Gopal Dalta and Dr. S.A. Alam, Fulbaria Upazila Health Complex, and Prof. Kamal Chowdhury, Dr. Ibrahim Khalil, Dr. Utpol Chowdhury, and Dr. Momen, Mymensingh Medical College Hospital, for their collaboration on patient treatment. The financial support of the Centers for Disease Control and Prevention, Atlanta, GA, USA, is acknowledged.

Efficacy of Mefloquine in a Bangladesh Area with Reported Multidrug-resistant Falciparum Malaria

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Background: Malaria constitutes a significant public-health problem in Bangladesh. In recent years, multidrug-resistant falciparum malaria emerged as a major public-health problem with both first- and second-line drugs showing unacceptable proportions of treatment failures. Mefloquine, a proposed second-line drug for treatment failure cases, has shown a clinical failure rate of 11% and parasitological failure rate of 27% in the study area. A higher dosage (25 mg/kg), claimed to have higher cure rate, requires evaluation. **Objective:** Describe the cure rate of mefloquine in an area of Bangladesh with reported resistance. **Methodology:** In this open, controlled chemotherapeutic trial, study subjects included symptomatic patients, aged 12 years or older, with asexual *Plasmodium falciparum* density between 500 and 250,000/cmm and not taken antimalarials over last one week. Mefloquine (25 mg/kg), divided into 2 doses 6 hours apart, was allocated to 70 cases. Oral quinine sulphate was used in 7 cases (10 mg/kg 8 hourly for 7 days), and 5 healthy volunteers recruited as positive and negative controls. Subjects were housed in a reasonably reinfection-free environment for 28 days. Drug administration was supervised, and subjects were followed clinically and with blood slides in the hospital. **Results:** Eighty-three (71+7+5) cases were enrolled, but one case was lost to follow-up. The drugs were well-tolerated in all the cases, and there was no clinical failure in the mefloquine group. One patient in the quinine group had late clinical failure. Five patients in the mefloquine group showed R III type of parasitological response (D2 parasitaemia >25% of D0), and in all of them radical cure was achieved. **Conclusion:** The study area can be considered to have preserved sensitivity to mefloquine in the 25 mg/kg dosage. **Acknowledgements:** The study received financial support from the Tropical Disease Research, World Health Organization, Geneva, Switzerland.

Management of Malaria from Hospital to Community: Bangladesh Experience

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Background: The malarious areas of Bangladesh are along the eastern border with 35 million people at risk with >500 deaths per year. Predominant falciparum species, perennial transmission, mostly adults, cerebral malaria, multiorgan involvement, delayed hospitalization, poor terrain, transport, and diagnostic facility are important characteristics of severe malaria in Bangladesh. The case-fatality rate (CFR) in cerebral malaria remained high despite revised control strategy. **Objective:** Assess the survival benefit of early administration of rectal artesunate in patients with no immediate access to parenteral treatment. **Methodology:** A randomized, double-blind, placebo controlled trial, covering a population of 750,000 in a forest and forest fringe area of Bangladesh, was undertaken. Adults and children above crawling age becoming non per os with fever diagnosed as malaria by criteria were randomized to receive either artesunate suppository or placebo after witnessed informed consent was obtained. Patients in both the groups were referred to a nearest hospital to receive usual treatment of malaria and were evaluated between 7 and 30 days post-enrollment for assessment of outcome. **Results:** In total, 1,940 patients aged 1-80 (median 15) year(s) were recruited with non per os symptoms: unable to eat/drink (92.3%), convulsion (22.7%), coma (9.4%), and irrelevant behaviour (26.8%). Positive smear was found in 67% of the patients with 53% falciparum species. 813 patients were alive with new difficulties, and 33 patients (1.7%) died (children–3.30%, adult–1.17%). No serious neurological sequelae was detected among the survivors. In general, suppository was acceptable to the community. Some problems encountered in this community-based study included molding and softening/bursting of suppository. **Conclusion:** The community-based efficacy trial is feasible in rural tropical area following the GCP guidelines. Interim analysis found no reason for unblinding. The overall low CFR in community-based intervention is encouraging for reducing malaria mortality. **Acknowledgements:** The financial support of the Tropical Disease Research, World Health Organization, is acknowledged.

Malaria Due to *Plasmodium falciparum* in Chakaria, Cox's Bazaar District

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Background: Malaria is a major public-health problem in South Asia, made worse by the emergence of multiple drug resistance. Ten million people are estimated to be living in areas with high risk for malaria transmission in Bangladesh. **Objective:** Characterize the epidemiology of malaria in a high-incidence area. **Methodology:** A surveillance was conducted in Kakara union within Chakaria upazila of Cox's Bazaar district. All 12,590 people living within 3 km from a hill tract area were assigned unique identification numbers, and each family was visited every two weeks and monitored for fever. There is one Village Health Post (VHP) and 3 trained paramedics within the study area. In addition to VHP, malaria can also be diagnosed at two newly-established 'malaria' posts where paramedics are trained to use a rapid diagnostic test to detect *Plasmodium falciparum* (Paracheck® 'dipstick', Orchid Biomedical Systems, India). 'Clinical malaria' was defined as an illness with at least one of the following criteria: parasitaemia, reported history of fever, and prescription of ≥1 antimalarial drug by a physician or paramedic. Patients meeting the case definition for clinical malaria had blood evaluated by the Paracheck® dipstick. **Results:** During June-September 2002, 1,543 clinical malaria cases were identified. Of these, 575 came either to VHP or malaria post and were evaluated with the dipstick test, and 95 (16.5%) were confirmed to have *P. falciparum* infection. All cases were treated with a 3-day course of quinine and one dose of sulphadoxine/primethamine. One-half (47.4%) of the cases with confirmed *P. falciparum* infection were children aged less than 18 years (10.5% were <5 years old). Three cases died. *P. falciparum*-confirmed cases occurred most commonly in July. Assuming a 16.5% *P. falciparum* rate among those with 'clinical malaria', the estimated incidence of *P. falciparum* malaria was 2,025 per 100,000 population for the 3-month period (which is the 'peak malaria-transmission season'). **Conclusion:** The findings of this community-based incidence study suggest that the burden of *P. falciparum* malaria is substantial within endemic areas in Bangladesh. Children appear to be heavily affected. There may be a need to examine healthcare for people living in endemic communities to determine whether malaria is recognized and optimally managed. **Acknowledgements:** The authors are grateful to team members of Chakaria Community Health Project. Special thanks are to field research officers who facilitated community participation for the study. Chakaria upazila Health and Family Planning Officer and Director of Christian Memorial Hospital are acknowledged for their support on malaria diagnosis. The financial support of the Department for International Development (DFID), United Kingdom, is acknowledged.

Positivity Rate of Peripheral Smear and Immunochromatographic Test in Clinically-suspected Severe Malaria

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Background: Globally, there is an annual toll of about 300-500 million new malaria cases with 1.5-2.7 million deaths from malaria. For diagnosis of malarial parasite, the simplest and surest test is the peripheral smear study. Microscopic examination remains the most widely-used and accurate method for the diagnosis of malaria, although its implementation is often limited because of cost and logistics required. A simplified newer method Paracheck Pf test based on immunological detection of the trophozite derived (*Plasmodium falciparum*) histidine-rich protein, which is highly sensitive and specific. **Objective:** Determine the positivity rate of microscopic peripheral smear and newly-developed immunochromatographic test in clinically-suspected severe malaria. **Methodology:** All cases admitted to one adult medical unit of Chittagong Medical College Hospital fulfilling the inclusion criteria were enrolled. Informed consent was taken either from patients or from eligible attendants. Two separate individuals performed the peripheral microscopy and Paracheck Pf test. None had any idea about the result of the other test. **Results:** In total, 106 cases of clinically-diagnosed severe malaria cases were studied. The mean (□S.D) age of patients was 27.48 (□11.37) years. Of them, 78.3% were males, and 21.7% were females. The main occupation groups affected were day labourers (19.8%), housewives (16.1%), and agricultural labourers (15.1%). 34% of patients had residence in malarial endemic area, whereas 52.8% had history of travel to malaria endemic area. 65.1% of the patients had history of taking antimalarial drug before hospitalization. The positivity rate of peripheral microscopy and Paracheck Pf test among the cases was 70% and 90.6% respectively. **Conclusion:** The reliable quality-controlled malaria microscopy is difficult to provide in many rural areas. In such a situation, rapid diagnostic test, such as Paracheck Pf test, could be of use. **Acknowledgements:** The support of Malaria Research Group, Bangladesh, is acknowledged.

Epidemiological and Clinical Profile of Dengue in Dhaka

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Background: Dengue and dengue haemorrhagic fever emerged in Bangladesh in 2000. A surveillance conducted at major hospitals in Dhaka has provided the opportunity to characterize clinical and epidemiologic characteristics. **Objective:** Define the epidemiological and clinical characteristics of dengue in Dhaka. **Methodology:** 833 suspected dengue cases were identified through systematic surveillance from July to December 2002 at the Dhaka Medical College Hospital and Holy Family Red Crescent Hospital. Clinical information and serum specimens were collected from all the suspected cases. Serum IgG and IgM was detected by capture (MAC) ELISA. **Results:** MAC ELISA confirmed 624 (75%) of the 833 cases as dengue. The mean (□SD) age of dengue cases was higher than non-dengue cases (28.5□11 years; range 3-70 years vs 25.3□11 years; range 1-65 year(s); p=<0.001). Most (76%) confirmed cases were males. The peak incidence was in August [296 cases (47.4%)] and in September [147 cases (23.6%)]. Dengue patients had a higher (≥6,000 taka) monthly income (75.9% vs 67.9%, p=0.02) and >10 years of schooling (53.5% vs 36.8%, p=<0.001) than patients without laboratory confirmation of dengue infection. Fever of >5 days before hospitalization was more common for those with income <6,000 taka per month (RR=1.9, 95% CI 1.3-2.7, p=<0.001) and <11 years of schooling (RR=1.6, 95% CI 1.2-2.3, p=0.005) compared to cases with higher income and more education. Dengue haemorrhagic fever was present in 245 (40.6%) of 603 cases and dengue shock syndrome in 23 (3.8%) of 597 cases. Three (0.48%) confirmed dengue cases died. Common complaints on admission for confirmed dengue patients were fever (100%), headache (88.0%), muscle pain (80.5%), vomiting (76.9%), joint pain (63.3%), bleeding manifestation (59.9%), rash (55.9%), and retroorbital pain (50.3%). Dengue patients were more likely than non-dengue patients to have skin rash, pruritus, gum bleeding, severe weakness, petechial rash, and subconjunctival haemorrhage. Tourniquet test was positive in 80% of the dengue patients compared to 47% without dengue (p=<0.001). **Conclusion:** Poor and less-educated people with dengue took longer to be hospitalized than those with higher incomes and more education. The most common clinical findings of confirmed dengue were fever, headache, musculoskeletal pain, retroorbital pain, rash, pruritus, vomiting, severe weakness, and bleeding manifestations. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and Canadian International Development Agency (CIDA) is acknowledged.

Use of Ultrasonography to Characterize Dengue Illness

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Background: Pleural effusions or ascites in patients with dengue are considered evidence for dengue haemorrhagic fever. **Objective:** Use ultrasonography as a tool to demonstrate presence of fluid in serous cavities in patients with dengue illness who have unrecognized dengue haemorrhagic fever. **Methodology:** Ultrasonography was used for documenting presence of pleural or ascitic fluid in patients with suspected dengue identified through systematic surveillance at the Dhaka Medical College Hospital and Holy Family Red Crescent Hospital in Dhaka from July to December 2002. Epidemiologic and clinical information was collected systematically from each consenting suspected case. Serum specimens were assessed for dengue IgG and IgM antibodies by capture ELISA; 40 antibody units were considered positive for dengue. IgM/IgG ratio of $\geq$1.78 was defined as primary (first time) infection and IgM/IgG ratio <1.78 was defined as secondary infection (previous exposure). **Results:** Ultrasonography was performed on 805 patients suspected to have dengue, of whom 603 (75%) had dengue antibodies identified. One hundred fifty seven (26%) were primary dengue cases and 446 (74%) were secondary cases. Pleural or ascitic fluid was detected in 50.9% of dengue cases. Higher proportions of pleural effusion (42.5% vs 12.4%, p=<0.001), ascites (40.3% vs 18.3%, p=<0.001), and gall bladder wall thickening (34.3% vs 19.3%, p=<0.001) were found among laboratory-confirmed dengue patients compared to patients without laboratory confirmation. Patients with secondary infection were more likely to have evidence of pleural effusion (50.7% vs 19.1%, p=<0.001), ascites (46.9% vs 21.7%, p=<0.001), gall bladder wall thickening (41.7% vs 13.4%, p=<0.001), and hepatomegaly (35.0% vs 25.5%, p=0.029) compared to cases with primary infection. **Conclusion:** Accumulation of fluid in serous cavities is more common in patients with secondary infection compared to patients with primary infection, which is consistent with the hypothesis that previous exposure to dengue viruses increases the risk for dengue haemorrhagic fever. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and the Canadian International Development Agency (CIDA) is acknowledged.

9:30 am-11:30 am (Venue: Meeting Room 3)
Scientific Session 10: Health Systems 2

077 (054)

Impact of Family Health Awareness Campaign on Clients' Awareness about RTI/STD/HIV-AIDS

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Background: Family Health Awareness Campaign (FHAC) was launched by the National AIDS Control Organization (NACO), Ministry of Health, Government of India, to create awareness in the community regarding RTI/STD/HIV-AIDS through home visits and village-based camps and encourage clients suspected to have RTI/STD to seek early treatment. **Objective:** Understand the process involved in programme management and social mobilization, identify the determinants of client behaviour, and assess its impact on awareness about RTI/STD/HIV-AIDS. **Methodology:** Data were collected using qualitative research methods from 23 districts spread across 16 states of India through 1,704 in-depth interviews with policy-makers, providers, implementers, facilitators, and clients, and 69 focus-group discussions with health workers and clients. **Results:** FHAC has been widely accepted (5+), and clients opined that it should be continued in future as well. Although RTI/STD/HIV-AIDS was considered a health issue, people discussed only with close confidants and visited private clinics for treatment. Only a few (1+) respondents mentioned shyness/embarrassment as a reason for non-use of the programme services. The social mobilization strategies were grossly inadequate and, thus, most (4+) clients were unaware of the existence of such a programme. Inconvenient timing was another reason (3+) for non-use of services. Participation of adolescents was less than expected, shyness and embarrassment being the most important factor (3+). Clients (4+) felt that camps were a useful opportunity for exposing adolescents to the important issues of RTI/STDS/HIV-AIDS. **Conclusion:** The FHAC programme succeeded in reducing the stigma and shyness among clients while discussing about RTI/STD/HIV-AIDS with health workers. However, intensified social mobilization campaign and organizing client-friendly camps would ensure improved reach of the programme. **Acknowledgements:** The financial support of the International Clinical Epidemiology Network (INCLEN) and the United States Agency for International Development (USAID) is acknowledged.

Linking Cultural Beliefs in Bhut/Jinn with Healthcare-seeking Behaviour

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Background: The formal Bangladesh healthcare system (government/NGO providers) has had much secularizing input over the years which can down-play some significant cultural barriers to seeking care. This report highlights one area where strengthening one positive cultural belief may help overcome another more negative belief. **Objective:** Explore how family attribution of bhut/jinn/dushi as cause of death among children aged less than 5 years reveals a need for health staff to respectfully address these to improve infant care. **Methodology:** Verbal autopsies document family and health background, circumstances (in narrative) and biomedical causes (by questionnaire) of deaths of children aged less than 5 years. Verbal autopsies collected from October 2001 to February 2003) were analyzed for statements that bhut/jinn/dushi contributed to death. A sample of the population was surveyed in the LAMB Hospital and community clinics to determine belief prevalence and content. Focus-group discussions (FGDs) further explored whether a healthcare service provider could mitigate this influence. **Results:** Of 111 verbal autopsies analyzed, 48% of deaths were perinatal. Twelve (10.8 %) implicated bhut, 10 of 12 occurring within 1 month old, and 7 within one week. Outside treatment sought in 9 of 12 was predominantly of a traditional nature (7 homeopathic or kabiraji, 2 of 12 allopathic). High medical risk of newborns was consistent with the cultural belief of vulnerability to bhut at that age. FGDs demonstrated that a substantial proportion of people acknowledged the superiority of a Creator God (Allah/Isshor) over bhut/jinn and also uncovered a community-identified link between medicine (daowa) and prayer (dowa). **Conclusion:** Spiritual beliefs affect help-seeking behaviour of rural Bangladeshis. Attribution of death to bhut was linked to not accessing qualified healthcare, especially for newborns. Results suggest that emphasizing the link between medicine and prayer could help overcome barriers to newborns and their mothers accessing care during the critical perinatal period. Further investigation to develop culturally-relevant interventions with perceived spiritual benefits by health workers could reduce child mortality by utilizing rather than minimizing significant “superstitions of uneducated persons”. **Acknowledgements:** The village health volunteers who entrust us with their knowledge of their own communities.

Healthcare Use Patterns for Acute Respiratory Infection and Diarrhoea/Dysentery among Infants and Children in Rural Bangladesh

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Background: Acute respiratory infection (ARI) and diarrhoea/dysentery are the major causes of morbidity and mortality among children in Bangladesh. Appropriateness of therapy can dramatically impact outcome. **Objective:** Assess care-seeking behaviour during episodes of ARI and diarrhoea/dysentery. **Methodology:** Data on ARI and diarrhoea/dysentery were collected from mothers of 441 children (£5 years of age) by trained female interviewers using one-week recall period during April 2000-December 2002 under a surveillance system administered by the Health Systems and Infectious Diseases Division of ICDDR,B at Abhoynagar/Keshobpur upazila of Jessore district. **Results:** Of the 441 children (£5 years of age), 34% were infants, 31% were aged 1-2 year(s), and 35% aged more than 2 years. Most (82%) children had at least one diarrhoeal or dysentery episode, and a quarter of them had at least one ARI episode during this period. The use of oral rehydration solution (ORS) was higher during diarrhoeal than dysentery episodes. A qualified practitioner was consulted in 10% of diarrhoeal or dysentery episodes, and in the remaining cases, either self-care was given, or an unqualified practitioner was consulted. The majority (53.6%) of ARI episodes occurred among infants. In 36% of ARI episodes, a qualified practitioner was consulted. No significant associations were found among care-seeking and education of parents, immunization status, parity of mothers, and gender. **Conclusion:** Despite the immense growth of the health service-delivery infrastructure in the public sector, unqualified practitioners still dominate healthcare-seeking behaviour during illnesses of children in Bangladesh. Difference in care-seeking behaviour occurred during diarrhoeal versus ARI episodes. Higher consultation with practitioners perhaps seems to be influenced by perceived severity of illnesses by caretakers. Appropriate strategies are needed to increase timely use of qualified practitioners for optimal treatment and prevention of complications due to ARI and diarrhoea/dysentery in children (£5 years of age). **Acknowledgements:** The funding support of the United States Agency for International Development (USAID) for maintaining the health and demographic surveillance system is acknowledged.

Improving Survival, Growth, and Development of Urban Slum Children of Agra District Through Cluster Community Approach

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Background: Uttar Pradesh, a state in India, has high infant mortality rate of 87 per 1,000 livebirths, and many of those who survive fail to gain their full development potential, both physical and intellectual. Lower literacy, ignorance, lack of responsive care, disorganized health infrastructure, and failure of existing health services to reach the deprived and needful are the major underlying causes. **Objective:** Promote 6 key care practices (viz. care of women during pregnancy and lactation, feeding behaviour, psychosocial care, food-preparation practices, hygiene and sanitation, and home management of childhood illnesses) for improving survival, growth, and development of children. **Methodology:** The programme is implemented by the Department of Social & Preventive Medicine, S.N. Medical College, Agra, which has been identified as State Nutrition Resource Centre for Community Nutrition, along with the involvement of Integrated Child Development Services (ICDS), health and development sectors. Eighty of nearly 250 urban slums in Agra were selected in 3 phases, and 7 women—comprising two socially-active women, one Anganwadi Worker (AWW), an ICDS functionary, one traditional birth attendant, one health worker (female), and two women members of neighbourhood committee—were identified from each slum, who were then trained for 3 days on 6 key care practices. Further on, in each slum, one AWW and two socially-active community women were identified as peer educators for communicating the key messages in their respective communities. This team covers 300-350 households in their slum and contributes to improving the existing practices and creating demand for health services. A management information system has also been developed for assessing the coverage and is supported by monthly follow-ups of peer educators. **Results:** Glimpses of success were visible after one year of implementation and were reflected from the effective delivery of health services to the beneficiaries of these slums. The community started realizing the importance of early childhood care and responsive care, its implications for future development, and the need of psychosocial childcare. **Conclusion:** Cluster community approach, i.e. participation of community members to decide and fulfill their own health needs, has been an effective tool to minimize the communication gap among different caste community clusters of urban slums. This can be utilized for improving the health status of children and women in the community. **Acknowledgements:** The support of the United Nations Children's Fund (UNICEF) is acknowledged.

Families of Migrant Workers: Patterns of Health Services Utilization and Coverage

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Background: The proportion of families with an absent husband/father working abroad or in other parts of Bangladesh (referred to a migrant worker) is steadily increasing. In Mirsarai, a rural sub-district in Chittagong and a field site of ICDDR,B, it is estimated that migrant workers are found in one of every 4 households. No published data relating to the health impact on families of migrant workers in Bangladesh could be found. **Objective:** Determine the impact of migrant workers on health services utilization and coverage of family members left at home. **Methodology:** Data were extracted from 5,991 ever-married women of reproductive age for one year (April 2001-March 2002) from the Centre's ongoing surveillance system in 7 unions of Mirsarai. In this study, the outcome variables are visits and services received from health facilities (community clinics, satellite clinics, EPI outreach, union and sub-district health centres, and hospitals). Services included child health, immunization, general healthcare, contraception, antenatal care, and TT coverage from any service-delivery points. Families of migrant workers were compared with non-migrant workers. **Results:** About 25% of husbands/fathers were living away from home (17% working within the country and 8% abroad). Significant differences in the patterns of health services use among families of migrant workers were identified. They were less likely to have visited a health facility (29% vs 43%, $p<0.01$). There were important differences in the services received from any service-delivery point, in particular family planning which accounted for 25% of the contacts among women with husbands living at home vs 5% among wives of migrant workers ($p<0.01$). Families of migrant workers were more likely to utilize general healthcare services (53% vs 34%, $p<0.01$). Despite these differences, parity was significantly lower in wives of migrant workers (mean number of children 2.1 vs 2.9, $p<0.01$). Children of migrant workers had better EPI coverage (67% vs 56% fully immunized, $p<0.01$). Use of current modern contraception was significantly lower among wives of migrant workers (11% vs 53%, $p<0.01$). There were no differences in pregnancy outcomes or TT coverage. **Conclusion:** Important differences in patterns of health services utilization and coverage were identified among families of migrant workers. The low contraception-prevalence rate among wives of migrant workers does not appear to be associated with higher parity, however this may be explained by other practice patterns not measured, such as induced abortion. In general, when compared to others, families of migrant workers are as well covered or better in terms of child immunization and general healthcare services.

Screening for Unmet Health and Family-planning Service Needs in Primary Healthcare Clinics in Bangladesh

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Background: In primary healthcare (PHC) clinics, clients tend to visit for one particular service, and the providers concentrate on that. Other needs remain undetected unless clients are asked about them, and an opportunity can be missed to provide services needed. Use of a simple screening tool could help identify unmet needs and provide more services per visit. **Objective:** Develop and test a screening tool to identify unmet health and family-planning needs of clients at the PHC clinics and evaluate effectiveness of the tool. **Methodology:** The study design was quasi-experimental comparing changes in selected indicators in two government and two non-government (NGO) intervention and comparison clinics, before and after the intervention from April 2002 to March 2003. In total, 1,510 observations on client-provider interaction and 1,222 exit interviews were conducted in 4 areas during evaluation of the study. Referred clients were followed up, and use of the screening tool was monitored. **Results:** Checking for two or more additional needs increased significantly, and 13 additional needs were identified per hundred services requested in the government area and 30 per 100 in the NGO area. The proportion of additional needs met improved from 4.6% to 14.0% and from 24.3% to 80.3%, in the government and NGO clinics respectively. The total number of additional services provided per 100 requested increased from 3 to 10 in the government area and from 12 to 28 in the NGO area. These improvements were statistically significant and significantly greater than in the respective comparison areas. On follow-up, more than 80% of referred cases received services. **Conclusion:** The results suggest that introducing a screening tool in the PHC clinics could significantly increase the coverage of health and family-planning services in Bangladesh. **Acknowledgements:** The study was conducted in collaboration with the Ministry of Health and Family Welfare, Government of Bangladesh, and the NGO Service Delivery Programme (NSDP), at union-level government clinics in Mirsarai union of Chittagong district and NGO clinics in Sherpur district. The study was funded by the United States Agency for International Development (USAID), Bangladesh.

Delivery of Community-based Nutrition Services through Community Clinics: Does it Make Any Cost Savings?

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Background: The community-based nutrition services of the National Nutrition Project (NNP) are designed to be delivered by NGOs through community nutrition centres (CNCs) under a fixed cost basis. It was, however, not determined whether there would be any cost savings if the services are delivered through Community Clinics (CCs). **Objective:** Estimate the difference between the cost of delivering community-based nutrition services of NNP through CCs and NGOs-led CNCs. **Methodology:** In a cost-minimization analysis, conducted during June-October 2002, the cost of delivering community-based nutrition services through the government-led CCs was estimated using the standard process of costing. The full cost of providing community nutrition services through community clinics that includes both capital and recurrent costs has been estimated from the perspective of providers. All capital costs were annualized using an assumed economic life and standard discount rate. For the reason of high variability, the cost of land was excluded from the exercise. The shared costs, if any, were apportioned indirectly using allocation factors. Finally, the average cost of community nutrition services per community clinic per year was determined which was, then, compared with the average cost of community nutrition services per CNC per year by NGOs, the information of which was synthesized from the project document of NNP. **Results:** The average cost of community nutrition services (without food and supplementation cost) per community clinic per year was only Tk 3,174.99 compared to Tk 54,702.00 for a NGO-led CNC. **Conclusion:** Significant amount of cost-savings can be attributed to public purse if the community nutrition services are delivered and implemented by the government through CCs. **Acknowledgements:** The support of the National Nutrition Project, Ministry of Health and Family Welfare, Government of Bangladesh, and the faculty members of Institute of Health Economics, University of Dhaka, is acknowledged.

Growing the Sheltering Tree: Protecting Children's Right to Health in Emergencies

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Background: Every child has the right to grow up in a healthy environment to live, learn, and play in healthy places. However, frequent natural disasters and emergencies in the country are increasingly posing substantive threats to the vision of a safer and healthier world for the children in Bangladesh. **Objective:** Undertake a content analysis of the existing national policies, legislations, treaties, and child rights instruments in Bangladesh to find out their appropriateness for promotion and protection of children's health in emergencies. **Methodology:** A systematic review of all national policies, treaties, and child rights instruments in Bangladesh and all international legal instruments, humanitarian laws, treaties, conventions, and UN resolutions on children's right to health in emergencies and other difficult situations, to which Bangladesh ratified, was done using a checklist. **Results:** Although Bangladesh ratified the International Convention on Child Rights (CRC) and its two Optional Protocols back in 2001 and, moreover, Bangladesh adopted a National Children Policy in 1994 which has one of the major goals of assisting children in difficult circumstances as in emergencies, the efforts are not sufficient to put into practice appropriate administrative and legislative measures that are required for full implementation of children's rights to health in difficult circumstances. **Conclusion:** Emergencies, by their very nature, are complex events, and children in such situations are to be treated as subjects of rights and not as objects of charity. It is the conjoined responsibility of national government, civil society, and all professional bodies dealing with children's rights to ensure that children's rights to sustainable health are also protected in emergencies. **Acknowledgements:** The support of the World Health Organization, Bangladesh, is acknowledged.

1:30 pm-2:15 pm (Venue: Meeting Room 1)
Keynote Lecture 2

085 (366)

Concentric Circles: A Twenty-First Century Context for Climate and Health

Rita R. Colwell

Director
National Science Foundation, USA

A global context indisputably frames human health issues in the 21st century, created by worldwide movement of people and goods, a recognition that earth processes operate on a global scale, and a dynamic, international scientific enterprise. Health issues are no longer just a matter between patient and physician alone—if they ever were—but now encompass an individual's complex relationship with the global environment. With such a perspective, the study of infectious disease today must draw insights from a series of contexts, each nesting like a concentric circle within the next—from nanoscience to genomics, and from mathematics, ecology, and social science to agricultural land use, climatology, and remote sensing. The connections between cholera, an ancient, water-borne disease, and the environment, provide a paradigm for this perspective. Fully dimensional understanding of an infectious disease, whether cholera, hantavirus, West Nile virus, dengue fever, or malaria, now reaches from countries to continents and beyond, and from medicine to viewpoints across science and engineering.

2:30 pm-4:30 pm (Venue: Meeting Room 1)
Scientific Session 11: Diarrhoeal Disease 2

086 (183)

Setting up Rotavirus Surveillance: Hong Kong's Experience

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Background: A global initiative is underway to fast-track rotavirus vaccine development and introduction in developing countries. Good surveillance data are necessary to enable opinion leaders and local experts to create awareness of, and demand for, a future rotavirus vaccine. **Objective:** Describe Hong Kong's experience in setting up rotavirus surveillance. **Methodology:** Hong Kong has undertaken a range of studies on rotavirus. Initial surveillance studies (both active and passive) involved only single hospitals without denominator data. In 1997, the Clinical Management System (CMS) with routine ICD coding was introduced into all Hong Kong government hospitals. However, these data appeared to underestimate the incidence of rotavirus. WHO's generic protocol for hospital-based rotavirus surveillance is now being used by 9 countries participating in the Asian Rotavirus Surveillance Network (ARSN). **Results:** Review of Hong Kong's earlier rotavirus prevalence studies indicated that 24-35% of paediatric hospital admissions for diarrhoea were due to rotavirus. CMS data (1997-1999) showed that 11.5% of all paediatric (<5 years) medical hospitalizations had a primary diagnosis of diarrhoea, and 10.9% of these were coded as rotavirus. As part of ARSN, active surveillance at 4 of 12 government hospitals (December 2000-March 2003) showed that 79% (6,934/8,772) of diarrhoea admissions (age <5 years) had a stool tested for rotavirus and 33% of these were positive. Comparison of active ARSN surveillance data with passive CMS data confirmed that the latter system underreported rotavirus infection. **Conclusion:** Simple prevalence studies requiring few resources can lay the foundation for future more detailed studies. Hong Kong's experience with the WHO protocol suggests that the proportion of diarrhoea admissions due to rotavirus has remained similar to that found in the earlier prevalence studies. **Acknowledgements:** The financial support was received from the Hong Kong Research Grants Council, World Health Organization, and Merck & Co. Inc.

Enteric Pathogens in Children Aged Less Than 5 Years in Hanoi, Vietnam

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Background: Rotavirus, diarrhoeagenic *Escherichia coli*, and *Shigella* are considered to be some of the most common enteropathogens causing diarrhoea in developing countries. The role of these pathogens has not been studied in details in Vietnam. **Objective:** Determine the prevalence of rotavirus, diarrhoeagenic *E. coli*, and other bacteria in faecal samples from Vietnamese children and the antibiotic susceptibility of isolated bacterial pathogens. **Methodology:** In total, 884 children aged less than 5 years (627 with diarrhoea, 257 age-matched controls) in Hanoi, Vietnam, were studied from March 2001 to April 2002. The study was carried out at the Hanoi Medical University, Hanoi, Vietnam and Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden. Multiplex PCR assay was developed for identification of diarrhoeagenic *E. coli*. The routine diagnostic methods were used for other bacteria. Group A rotavirus was identified by ELISA. The isolated bacteria were determined for their antibiotic susceptibility by the NCCLS protocol. **Results:** A multiplex PCR was successfully developed to identify diarrhoeagenic *E. coli* strains from faecal sample in a single reaction. Of 836 processed faecal samples, 172 strains of diarrhoeagenic *E. coli* were identified with the significantly higher prevalence in the diarrhoea group compared to the healthy ones ($p<0.001$). In addition, 19 *Shigella* strains were isolated. Imipenem, ciprofloxacin, nalidixic acid, cefotaxime, and cefuroxime were active against *E. coli* and *Shigella* strains, while high frequencies of resistance to ampicillin, chloramphenicol, and trimethoprim-sulphamethoxazole were shown. Of 884 samples, group A rotavirus was identified in 45% of children with diarrhoea, and 3.5% in the healthy ones showed significant difference. Within the diarrhoea group, the highest prevalence was seen in children aged 13-24 months, males more than females. More than 1/5 of rotavirus-infected children had association with diarrhoeagenic *E. coli* or *Shigella* spp. **Conclusion:** Group A rotavirus, diarrhoeagenic *E. coli*, and *Shigella* were the major causes of diarrhoea in children aged less than 5 years in Hanoi, Vietnam. Most isolated bacteria were resistant to ampicillin, chloramphenicol, and trimethoprim-sulphamethoxazole. **Acknowledgements:** The study was supported by SIDA/SAREC.

Giardiasis in New Zealand: A Descriptive Analysis

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Background: *Giardia* is a leading cause of human gastrointestinal illnesses globally with an incidence rate of 46.6/100,000 in New Zealand and appears to be one of the highest among developed countries. Environmental and person-to-person risks are suspected. **Objective:** Describe the epidemiology of giardiasis and explore the environmental links in New Zealand. **Methodology:** The study utilized anonymous information from 7,818 notified cases throughout New Zealand between July 1996 and June 2000. A weighted average of drinking-water grades was estimated using the Community Drinking Register, and Pearson's coefficient was used for measuring correlation between water grade and infection. Daily-recorded climate data were plotted against daily case notifications, and significant daily giardiasis notification was predicted using the Poisson regression model. Data are presented by age, gender, ethnicity, and area using statistical and spatial methods. **Results:** Most cases were in the 1-4-year age group, followed by 25-44 years, and were Europeans. Ethnicity for 18% of the cases was not known, affecting demographic calculations. Infection rates were high for a number of Health Districts compared to the national average. Over 50% of the population received Aa† -quality drinking-water. No correlation between infection and water grades {treatment-plant ($r=-0.12$) and reticulation-system ($r=-0.11$)} was found. A significant correlation with the mean daily maximum ($r=0.05$) and minimum temperature ($r=0.06$) was observed. Poisson regression identified minimum temperature ($chi^2=5.40$, $p<0.05$) and relative humidity ($chi^2=5.37$, $p<0.05$) as predictors of the number of daily infections due to *Giardia*. **Conclusion:** This study has identified vulnerable groups and major data gaps. Time-trend analysis suggests a seasonal pattern. The weighted average drinking-water grading is a novel measure and may not be truly representative of local supplies. Modelling of climate data showed an association with giardiasis but was inconclusive. **Acknowledgements:** ESR (Institute of Environmental Science and Research Limited, New Zealand): Health; and NIWA (National Institute of Water and Atmospheric Research, New Zealand) for sharing data.

Importance of Human Caliciviruses as a Cause of Childhood Diarrhoea in Bangladesh

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Background: Human caliciviruses (HuCVs) are a common cause of outbreaks of non-bacterial gastroenteritis worldwide in persons of all age groups. However, the significance of HuCVs as enteric pathogens in Bangladesh has not been well-established. **Objective:** Determine the prevalence and genogroups of noroviruses in children hospitalized with diarrhoea in Dhaka, Bangladesh. **Methodology:** Tests were conducted for HuCV infections in paired sera and faecal specimens of 211 Bangladeshi children, aged less than 5 years, hospitalized with diarrhoea at ICDDR,B, who were negative for other enteropathogens. HuCV infection was defined as either a 4-fold rise in IgG antibody titre to baculovirus-expressed capsid antigens in paired sera by enzyme immunoassay (EIA) or the presence of HuCV RNA in faecal specimens by reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR results were later confirmed by nucleotide sequencing. Astroviruses were also detected by RT-PCR. **Results:** HuCVs were detected in 32.7% of the children by any of the methods employed. By EIA, 54 (25.6%) children showed seroconversion with at least one NLV antigen. The seroconversion rate was the highest with GII/4 antigen [rBLV (15.6%)] and the lowest with GII/3 antigen [rTV (10.4%)]. Besides these, 46 patients (21.8%) showed seroconversion with both Hawaii (rHV) and Burwash-landing (rBLV) viruses. By RT-PCR, HuCVs were detected in 27 (12.8%) children, of which 21 (9.9%) were noroviruses, and 7 (3.3%) were sapoviruses. Viral RNA was detected by RT-PCR in stools of more children who had been seroconverted [22.2% (12/54)] compared to non-seroconverters [9.6% (15/157)] (p=0.031). In addition to HuCV, astroviruses were detected in 11.4% (24/211) of the children. **Conclusion:** Human caliciviruses may be an important enteric pathogen in Bangladeshi children. **Acknowledgements:** This study was funded by the United States Agency for International Development (USAID) (grant no. HRN-A-00-96-90005-02).

090 (017)

Incidence, Pathogenicity, and Attributable Risk of Enteropathogens in a Birth Cohort Study in Guinea-Bissau

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Background: Diarrhoeal diseases remain a major cause of childhood mortality and morbidity in developing countries. **Objective:** Describe the natural history and relative importance of various diarrhoeagenic agents and estimate the protection against re-infection and disease that natural infections may confer. **Methodology:** Two hundred children were recruited shortly after birth, and stool specimens were collected weekly, irrespective of whether the children had diarrhoea or not, up to two years of age. All children were recruited during the first year of the study and were followed for a median of 18.4 months. To measure pathogenicity, the odds ratio for diarrhoea, adjusted for age, sex and for co-infections with other enteropathogens were determined by logistic regression. **Results:** Standard estimation of the population attributable risk indicated that rotavirus, enterotoxigenic *Escherichia coli* that produced only heat-stable toxin (ST), *Isospora* spp., *Cryptosporidium parvum,* Shiga-like toxin (Stx)-producing *E. coli* (STEC), and *Shigella* spp./enteroinvasive *E. coli* were the most important contributors to diarrhoea in this population. Stx2- but not Stx1-producing STEC was pathogenic. Enteroaggregative *E. coli*, diffusely adherent *E. coli,* and attaching and effacing *E. coli*, which were the most commonly-isolated micro-organisms, were not associated with diarrhoea. With the exception of rotavirus, primary infections did not confer protection against reinfection with the same organism. Protection against diarrhoea from reinfection was conferred by primary infections with rotavirus, enterotoxigenic *E. coli* that produced only heat-labile toxin (LT) and attaching and effacing *E. coli*. **Conclusion:** The high pathogenicity of *Isospora* spp. and of Stx2- but not Stx1-producing STEC should be further explored. **Acknowledgements:** The financial support was provided by the European Commission-DG Research, FP3-International Cooperation STD programme (TC*-CT94-0311), Danish Council for Development Research (104.Dan.8/717, 9501931 and 91010), DANIDA, Danish Medical Research Council, Danish National Research Foundation, L. Meltzers Høyskolefond, and Research Council of Norway.

Changing Pattern of *Shigella* Species and Its Antibiotic Susceptibility in Rural Bangladesh: A 25-year Review

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Background: Shigellosis continues to be a major public-health problem in Bangladesh. The emergence of multiple antibiotic resistance in shigellosis is a great concern to physicians in choosing an appropriate antibiotic. **Objective:** Describe the changing patterns of isolation of *Shigella* species, and its antibiogram and implication for treatment. **Methodology:** Rectal swabs/stool specimens were cultured from all patients of the ICDDRB project area reported to the Matlab Diarrhoea Hospital. *Shigella* isolation and antibiotic sensitivity test were performed following standard methods. Species isolation was calculated as percentage of total *Shigella* isolated in the year. **Results:** In total, 7,545 *Shigella* strains were isolated during 1978-2002. Marked year-to-year variations were observed in the isolation of *Shigella dysenteriae* type 1 ranging from 0% to 59.7% with two peaks—one during 1983-1985 (48-50.5%) and the other during 1993-1995 (45.2-59.7%). No *S. dysenteriae* type 1 strains were isolated during 2001-2002. Isolation of *S. flexneri* strains ranged from 36.1% to 79.7% with the highest proportion in 2002. The other *Shigella* strains were also present and ranged from 4.2% to 40.3%. By 1987, >90% of *S. dysenteriae* type 1 and 53% and 22% of *S. flexneri* became resistant to ampicillin and SXT respectively. In 1987, almost all *Shigella* strains were sensitive to nalidixic acid, but by 2002, >90% of *S. dysenteriae* type 1 and 59% *S. flexneri* became resistant to nalidixic acid. In 1993, 61% of *S. flexneri* strains were resistant to pivmecillinam. But all the *S. flexneri* strains isolated in 2002 were sensitive to pivmecillinam. All the *Shigella* strains were sensitive to ciprofloxacin. **Conclusion:** All types of *Shigella* species were prevalent in rural Matlab. *S. flexneri* was the most predominant strain, followed by *S. dysenteriae* type 1 during 1978-1999. Resistance of *Shigella* strains to common antibiotics continues to be a major problem. Monitoring of antimicrobial susceptibility should be continued. **Acknowledgements:** The support of ICDDR,B and the United States Agency for International Development (USAID) for surveillance is acknowledged.

Green Banana-associated Increase in Faecal Short-Chain Fatty Acids in Children with Shigellosis

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Background: Short-chain fatty acids (SCFA) provide energy for colonocytes, stimulate colonic fluid and electrolyte absorption, and possess anti-inflammatory properties. Green banana is a cheap source of amylase resistant-starch for treating diarrhoea and is, therefore, a potential source of SCFA generated by colonic bacterial fermentation. **Objective:** Determine the therapeutic effects of green banana on severe childhood shigellosis by specifically determining faecal SCFA and myeloperoxidase (MPO) activity in faeces of children with shigellosis. **Methodology:** In a double-blind, randomized, controlled trial, 74 children (both boys and girls), aged 6-24 months, with *Shigella* spp., were given a rice-based diet (54 kcal/g) containing either 250 g/L cooked banana or rice-diet alone for 5 days. Faecal SCFA (acetate, propionate, and butyrate) and MPO activity were determined on admission, day 3, and day 5 using high-performance liquid chromatography and spectrophotometric assay respectively. The study was conducted at the Physiology Laboratory, Clinical Sciences Division and the Nutritional Biochemistry Laboratory, Laboratory Sciences Division of ICDDR,B during January 2002-October 2002. **Results:** Treatment with green banana diet for 5 days significantly ($p<0.05$) increased faecal concentrations (mM/kg) of acetate (green banana 62.65□18.10 vs control 41.14□28.25), butyrate (97.45□5.19 vs 18.31□12.8), and propionate (31.53□9.45 vs 23.31□18.72) compared to the group treated with rice-diet alone. The MPO activity (U/g) was significantly ($p<0.05$) reduced in faeces of the treatment group (99.59□21.10 vs 197.88□30.12) compared to the control group. On admission, there were no significant differences in faecal SCFA concentrations and MPO activity between the two groups. Good correlation was found between SCFA and MPO activities and clinical outcomes. **Conclusion:** Green banana increases the production of SCFA and reduces the severity of colonic inflammation as indicated by a marked reduction in MPO activity in faeces. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Introducing a Safe Water System in Delhi, India: An Operations Research Project

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Background: Home water treatment with dilute sodium hypochlorite—a simple, inexpensive intervention—eliminates microbial contamination and reduces the risk of diarrhoea by approximately 50% (range 30-85%). Social marketing and community-based behaviour change approaches have both been used successfully in other countries for making the intervention available at household level. **Objective:** Assess the retention of information, education, and communication manages on Safewat in Delhi, India. **Methodology:** Population Services International (PSI) packaged sodium hypochlorite as a product, Safewat, and with the technical guidance from CDC, WHO, and UNICEF, piloted it in targeted slums as a two-year learning laboratory for a community-based approach. The project design initially involved 750 community change agents (CCAs) who conducted door-to-door information, education, and communication (IEC) activities on safe water, hand-washing, food, sanitation, diarrhoea management, and immunization. CCAs also offered the product for sale. Safewat was also made available at retail outlets. The mid-term evaluation found that retention of message was high among households visited by CCAs. However, householders did not know where to obtain Safewat, they questioned the credibility of CCAs, and use was low. Major mid-course changes were made. CCAs were exclusively dedicated to IEC, and Safewat was made available only through stores, chemist shops, and doctors clinics. Classic social marketing behaviour change techniques were added—highly visible events, including outdoor video shows, street theatre, healthy baby contests, point-of-sale demonstrations, and neighbourhood community meetings on water and sanitation—to create a conducive social environment. **Results:** Retention of message among households visited by CCAs remained high, while CCA standing in the community has risen. Householders were aware of where to obtain Safewat, and the rate of adoption improved steadily. **Conclusion:** CCAs are best deployed in a communication function, as disinterested advocates of diarrhoea-prevention measures, separated from the selling function, which is best managed by sales staff.
Acknowledgements: The Population Services International is grateful to World Health Organization, United Nations Children's Fund, and Centers for Disease Control and Prevention, USA, for providing technical assistance and funding.

2:30 pm-4:30 pm (Venue: Meeting Room 2)
Scientific Session 12: Health Systems 3 and Nutrition 4

Health Systems 3

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Union-level Healthcare: Can the Poor Afford it?

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Background: In 1999, LAMB restructured its community health services from mobile clinics to union-level static clinics as per the government health strategy (HPSP). **Objective:** Present the financial results to date of the LAMB healthcare model at union level. **Methodology:** LAMB has helped communities develop union-level healthcare centres (HCCs) as part of a strategy that provides health and development services from village level to tertiary care (at LAMB Hospital). Village Health Volunteers work in the villages and link with the union HCCs. ESP-plus services are provided at HCCs by 3 local women paramedics/community midwives trained at LAMB and supported by a chowkidar and an ayah. HCCs are managed by a local Health and Development Committee (HDC) which sets fee and subsidy rates. **Results:** There are now clinics in 9 unions, with plans for 2 more. LAMB provides most of the initial set-up costs, including training, and aims to hand over HCC to the community within 3-5 years. In 2002, at the oldest clinic, there were 1,552 general patients, 3,781 antenatal patients, and 67 deliveries. For all the 9 clinics, the figures were 7,468 general patients, 15,647 antenatal patients, and 118 deliveries. In 2002, the oldest clinic was able to cover all of its costs, including all staff, from local income. Projections for 2003 are that two clinics will cover all of their costs. Local income is mostly fee income but includes local donations, profit from the local savings scheme, and other income-generating activities. **Conclusion:** ESP-plus services can be provided at the union level by well-trained local individuals. This service can be paid for by well-organized poor communities. **Acknowledgements:** LAMB wishes to acknowledge its partners in this work: Department for International Development, PLAN, and Tear Fund (UK).

Effectiveness of an NGO Health Programme in Rural Bangladesh: Evidence from the Management Information System

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Background: Many non-governmental organizations (NGOs) have provided basic maternal and child health services in rural areas under the Bangladesh Population and Health Consortium (BPHC) programme. Monitoring performance and access for the poor has been a major priority. **Objective:** Review data on coverage of selected child and maternal health services and death rates among the registered catchment population (2 million) from 1999 to 2002. **Methodology:** Data from the management information system for 27 partner NGOs combined and also data from 12 NGOs working in the same areas from 1999 to 2002 were analyzed for 2001 and 2002. Coverage of selected services and death rates was based on service statistics, household members registered, and births and deaths in each quarter recorded by fieldworkers. Rates were disaggregated for the poorest one-third and others based on surveys of monthly household expenditure. **Results:** Estimates of coverage of maternal and child health services in the 27 NGO areas combined in 2002 were relatively high for the poorest and others: 3 antenatal care visits (69.4% and 75.5%); 2 tetanus toxoid vaccinations for pregnant women (85.7% and 92.0%); and one postnatal care visit within 42 days of delivery (70.2% and 74.4%); measles vaccination (76.1% and 79.4%); treatment of cases of diarrhoeal disease (83.1% and 85.5%); and acute respiratory infection (85.0% and 84.0%). Child death rates in 2002 for the poorest and other children were: neonatal, 21.1 and 20.6 per 1,000 livebirths; postneonatal, 13.5 and 10.8 per 1,000; and child (1-4 year(s)), 11.0 and 8.8 per 1,000. For the 12 NGOs working in the same areas for 1999-2002, the neonatal death rate fell by 50%, from 32.0 to 15.9 per 1,000 livebirths, while the postneonatal and child (1-4 year(s)) death rates were quite stable. **Conclusion:** If genuine, low child (0-4 year(s)) mortality and rapid decline in neonatal death rates among the poorest and others could be linked to the high coverage of child and maternal health services. Other development activities of NGOs could also have contributed. There is no evidence that the decline in death rates was due to changes in reporting of births and deaths, although some under-reporting of deaths is expected (30% in 1997-1998). The death rates and service coverage estimates need to be verified through independent household surveys. **Acknowledgements:** The financial support for the BPHC programme has been provided by the Department for International Development, UK.

Introduction of Urban Depot-holders under the NGO Service Delivery Program

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Background: The NGO Service Delivery Program (NSDP) supports 41 local NGOs to deliver the Essential Services Package (ESP) in Bangladesh through urban and rural static and satellite clinics. It has about 8,700 female depot-holders working as service provider/promoters in rural areas. NSDP is developing strategies to address the needs of the poor in urban areas by introducing depot-holders. **Objective:** Conduct a baseline evaluation of depot-holders as they are being introduced in urban areas, which will allow for monitoring of changes in practice over time. **Methodology:** A baseline survey was conducted among all depot-holders trained in 3 urban areas (n=60). In-depth interviews were conducted on a sub-sample of 30 depot-holders, 10 in each area. Also, 4,800 women of reproductive age (15-49 years) were surveyed to ascertain their patterns of healthcare-seeking behaviour (analysis in progress). **Results:** Preliminary results showed that all depot-holders received 6 days' training. About 20% thought that the training was not adequate. Most of them were married, had 1-2 child(ren), and had at least an eighth grade of education. The mean age of depot-holders was 28 years. The mean length of residence in their localities was about 20 years. Eight depot-holders dropped-out. The depot-holders from one area (A type municipality) reported that rich people from non-slum areas are not interested to seek services from them. Some depot-holders' poor customers preferred to go to the adjacent NGOs or to the government facilities because they offer services at lower cost or no cost. Some of them asked for more commodities to increase their earning (they receive half of the profit from sales). Most of them were satisfied with their supervision by service promoters and could cope with the official record-keeping system. **Conclusion:** Depot-holders can be successfully introduced in all types of urban communities, but drop-outs are a concern. It can be anticipated that more comprehensive/continuous training will be required and some strategy for reaching non-slum dwellers. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged. NSDP and its partner NGOs provided all programme-related support for the study.

Child Injury: An Emerging Child Health Problem in Bangladesh

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Background: In this era of epidemiological transition, injury has emerged as one of the major causes of mortality and morbidity in both developed and developing countries. However, there is a lack of valid and representative information on injury to depict child injury situation in Bangladesh. **Objective:** Explore the magnitude of injury among 0-18-year old children in Bangladesh. **Methodology:** A cross-sectional survey has been conducted in 24 unions of 12 randomly-selected districts of the country. In total, 133,563 households, comprising 648,455 population, were screened to identify morbidity and mortality. Causes of mortality and morbidity were determined by verbal autopsy and verbal diagnosis methods respectively. When injury cases were identified, detailed information on injury, including sociodemographic and environmental factors, was collected. Data were collected by 48 trained data collectors during January-June 2003. **Results:** Proportional mortality and morbidity due to injury among children was 13.4% and 30% respectively. Proportional mortality due to injury sharply increased from the age of one year. The highest proportional mortality due to injury (49%) was observed among children aged 15-17 years. The injury mortality and morbidity rate among children aged 0-17 year(s) was 46.8/100,000 person-years and 1447/100,000 person-years respectively. Drowning was the major cause of injury deaths (88%) among 1-4-year old children. However, among the older children, road traffic accident was the major cause of death. **Conclusion:** It has been observed that morbidity and mortality patterns have been changed especially among children in Bangladesh. Injury has been emerged as one of the major causes of childhood mortality and morbidity, and drowning is the major cause of early childhood mortality. RTA becomes the major cause of injury-related deaths in adolescents. **Acknowledgements:** The technical and financial support of UNICEF, Bangladesh, TASC, USA, and Centers for Disease Control and Prevention, USA is acknowledged.

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Reduction of Protein-energy Malnutrition by Slashing Episodes of Diarrhoea among Children Aged 0-5 Year(s)

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Background: Diarrhoea is a leading cause of illness and death among children in developing countries where an estimated 1.8 billion episodes and 3 million deaths occur each year. Overall, these children experience an average of 3.3 episodes per year, but in some areas the average exceeds 9 episodes; this contributes protein-energy malnutrition to a greater extent. **Objective:** Study the association between diarrhoeal episodes and protein-energy malnutrition and slashing of protein-energy malnutrition by slashing diarrhoeal episodes among children aged 0-5 year(s). **Methodology:** The study was carried out among 160 families having children aged 0-5 year(s). In total, 380 children aged less than 5 years were selected, and data regarding the incidence of diarrhoea and prevalence of protein-energy malnutrition were gathered through their parents. Interventions were applied, and results were correlated. **Results:** The number of diarrhoeal cases was reduced by 62% due to adoption of health methods, such as exclusive breast-feeding (56%), increased use of oral rehydration solutions (71.15%), and adoption of purification methods (82.5%). The study observed improvement in all grades of malnutrition towards normal. **Conclusion:** Reduction in episodes of diarrhoea shows improvement in protein-energy malnutrition cases.

Outcome of Severely-malnourished Children Who Refuse to Undergo Nutritional Rehabilitation: Results of a Follow-up Study

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Background: Nutritional rehabilitation of severely-malnourished children is critically important; however, the outcome of children who refuse nutritional rehabilitation and are discharged from the hospital against medical advice need to be understood for defining strategy for their efficient management. **Objective:** Investigate compliance to outpatient follow-up and outcome of severely-malnourished children discharged from the hospital without nutritional rehabilitation. **Methodology:** Parents of severely-malnourished children (weight-for-length <70%, bipedal oedema, or weight-for-age <50%) attending the Dhaka Hospital of ICDDR,B for treatment of diarrhoea and other acute illnesses during May 2001-June 2003 were counselled on the importance of nutritional rehabilitation. Children of parents refusing hospitalized nutritional rehabilitation were enrolled for outpatient follow-up 15 days and 1 month after discharge. During follow-up, micronutrients were provided, and intercurrent illnesses were treated. **Results:** Of 1,067 children eligible for nutritional rehabilitation, 658 (62%) underwent nutritional rehabilitation. Domestic problems and parental work disruption were the most common causes of refusal to undergo nutritional rehabilitation. One hundred of 409 children refusing nutritional rehabilitation and living within 10 km of the hospital were randomly selected for follow-up. Family income of these children was around US$ 2 per day; 37% were males; the median age was 18 months; weight-for-length was 67.2±6.6%; and 11% had bipedal oedema. Thirty-five (35%) children could not be traced after discharge, and of those reporting, 62% reported voluntarily, while 38% were escorted from residence. Cough, fever, and diarrhoea were the most common illnesses. The median weight gain at first and second follow-up visits was 4.0 and 2.3 g/kg.d respectively. Three children died during the follow-up period. **Conclusion:** Severely-malnourished children not undergoing nutritional rehabilitation have poor follow-up compliance, and poor outcome, e.g. excess morbidity, deaths, and poor weight gain. There is an urgent need to find an effective alternative for management of such children using a health systems approach. **Acknowledgements:** The financial support of the World Bank, Swiss Development Corporation, and United States Agency for International Development (USAID), are acknowledged.

Water and Electrolyte Salvage in Experimental Model of Dehydration and Malnutrition

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Background: Oral rehydration therapy is now well-standardized, except for children with severe malnutrition. Recently, an ORS, called Resomal, was specifically designed for severely-malnourished children with diarrhoea. **Objective:** Assess the consequences of malnutrition on kidney and intestinal response to dehydration, with special reference to water and electrolyte absorption from Resomal and reference WHO-ORS. **Methodology:** Malnutrition was achieved by providing half amount of food that well-nourished animals consumed on the previous day for 30 days. Dehydration was achieved by water removal for 46 hours in healthy and in malnourished rabbits. At 46 hours, dehydration was assessed by change of body weight (g), urine volume (mL) and osmolality (mosm/kg), BUN (mg/dL), active colonic and jejunal electrolyte transport in Ussing chamber. Small intestinal water and electrolyte absorption was measured by *in vivo* and *in vitro* methods. **Results:** Compared to controls, the dehydrated animals reduced 12% of body weight and 87% of urine volume and increased urine osmolality (110%) and BUN (94%). Short-circuit current (Isc) and net sodium transepithelial flux (JNa net) increased in the dehydrated rabbit's colon. Malnourished dehydrated animals exhibited identical results. Net *in-vivo* absorption of water and electrolytes increased during infusion of WHO-ORS in jejunum of both the dehydrated groups. During Resomal infusion, water absorption was not different from that of WHO-ORS, but absorption of sodium was reduced, and potassium increased in both the groups. *In vitro*, compared to control, glucose-stimulated Isc, Jna, and G increased in both the groups. **Conclusion:** During experimental dehydration, the kidney and large intestine salvage water and electrolytes, thus reducing the consequences of dehydration. Jejunal water absorption from Resomal and WHO-ORS increased during dehydration, but Resomal allows absorption of less sodium and more potassium in both the groups. **Acknowledgements:** The financial support of the French Foreign Ministry is acknowledged.

Outpatient Management of Severe Child Malnutrition: Compliance with Feeding Advice and Its Sociodemographic Co-variates

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Background: From 1 March 2001 to 31 December 2002, ICDDR,B and 3 pilot NGO clinics serving the urban poor in the Dhaka metropolitan area enrolled 465 severely underweight weaning-age children for protocolized nutrition rehabilitation on an outpatient basis. Management of these children consisted of counselling on home feeding of nutrient-dense complementary foods with continued breast-feeding, supplementation of micronutrients, infectious disease control, scheduled weight-gain monitoring and, if indicated, referral to hospital. Within this package of interventions, frequent feeding, in adequate quantity, of the recommended nutrient-dense food was considered especially important. **Objective:** Examine the extent of compliance with feeding recommendations during weeks 1, 5, and 9 after children's enrollment and also identify sociodemographic co-variates of compliance with emphasis on potential constraints. **Methodology:** This study was conducted with respect to 184 weaning-age (6-23 months) severely underweight children newly identified by the pilot clinics from 1 October 2001 to 30 June 2002. Trained ICDDR,B interviewers conducted semi-structured household-level interviews with children's mothers or other main caretakers to obtain 24-hour feeding histories and to collect related sociodemographic information with a focus on potential constraints to compliance. Co-variates of compliance were examined by univariate analysis methods. **Results:** Compliance with feeding recommendations was categorized based on the 24-hour feeding histories, taking into account frequency, quantity and types of foods and their ingredients, while also considering breastfeeds. While only 16%, 14%, and 11% of caretakers were considered as fully compliant with feeding advice during weeks 1, 5, and 9, partial compliance was at or above 68% in the course of all 3 visits. Non-compliance increased, however, from 16% in week 1 to 21% in week 9. There were only weak, mostly non-significant associations between compliance and sociodemographic co-variates. Full compliance was lower in slum households, but higher if fathers contributed to household expenses and were not considered as abusive or irresponsible by the children's mothers. Non-compliance was higher in households in extremely crowded dwellings and if mothers perceived no improvement in the child's health. Non-compliance in weeks 5 and 9 also went hand in hand with mothers having sought advice elsewhere. Partial compliance, which was consistently observed for over two-thirds of the enrolled children, was, however, sufficient to achieve recovery from severe underweight for 61% of the enrolled children, with a mean improvement of 0.85 z-scores or 0.29 z-score per month. **Conclusion:** Counselling on improved feeding practices for severely underweight children should be interactive so as to respond to household-level conditions and constraints. Since adequate improvements could be achieved with only partial compliance with recommended feeding regimens, feeding advice can be flexible. **Acknowledgements:** The study was conducted with financial support from the World Bank grant to the ICDDR,B Nutrition Centre of Excellence, BINP Operations Research contract funds, Government of Bangladesh Gates Award Counterpart funds, and ICDDR,B core funds.

2:30 pm-4:15 pm (Venue: Meeting Room 3)
Scientific Session 13: Vaccine

102 (208)

Reduced Doses of Oral Killed Enterotoxigenic *Escherichia coli* Plus Cholera Toxin B Subunit Vaccine is Safe and Immunogenic in Bangladeshi Infants and Children

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Background: The oral inactivated ETEC vaccine, composed of a mixture of formalin-inactivated whole-cell ETEC bacteria expressing 6 colonization factors (CFs) and one 1 mg of recombinant cholera toxin B subunit (Swedish Bacteriological Laboratories, Stockholm, Sweden), has been extensively tested in Bangladeshi adults and children down to 18 months of age in whom it was found to be safe and immunogenic (Qadri *et al*. Vaccine; 21, 2003; 18, 2000). Since ETEC infections are most common in the first two years of life, testing of the vaccine is needed in younger children and infants. **Objective:** Test the suitability of the ETEC vaccine in Bangladeshi children and infants. **Methodology:** Double-blinded placebo controlled trials and dosing studies were designed in age groups from 17 days to 6 months. **Results:** A full dose of either vaccine or placebo (*E. coli* K12) gave rise to adverse events in the form of vomiting and the study had to be terminated prematurely after 42/158 children had been enrolled. Dosing studies were carried out using half and quarter amounts of the full dose of the vaccine. This showed that, in infants aged 17 days-6 months, the quarter dose appeared to be most suitable. The quarter dose was, therefore, tested in a randomized placebo controlled design on 158 children. The lowered dose of vaccine was well-tolerated. IgA-antibody secreting cell responses and IgA-plasma antibodies were seen to vaccine antigens (CFs and CTB) after the first or second dose ($p=0.01-<0.001$). The immune response did not differ significantly from those seen in children who had received a full or half dose (p=NS), but were significantly increased compared to the placebo recipients ($p<0.05$). **Conclusion:** The study demonstrates that reduced doses of the ETEC vaccine can be both safe and immunogenic, and careful considerations must be placed on dosage when new vaccines are being tested or introduced for infants in developing countries. **Acknowledgements:** The research was supported by the United States Agency for International Development (USAID) (grant no. HRN-A-00-96-90005-00) and the Swedish Agency for Research and Economic Cooperation (SIDA-SAREC) (grant no. 1998-05440).

Future Promise of a Live Oral Rotavirus Vaccine: Trial with Human Strain (RIX4414)

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Background: A safe and effective rotavirus vaccine is urgently needed to reduce the enormous disease burden associated with rotavirus illness. Unanticipated adverse events (intussusception) experienced with the US-licensed tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV) have accelerated efforts to develop and evaluate alternative vaccine candidates so that a safe and effective public-health tool would be available. An oral live-attenuated rotavirus vaccine (RIX4414) is under development based on the human rotavirus strain 89-12 (AVANT, Immunotherapeutics). The strain belongs to the serotype G1P1A and genotype P[8]. The 89-12 vaccine is safe, immunogenic, and efficacious (89-100%) (Bernstein DI *et al*. Lancet 1999;354:287). **Objective:** Evaluate the safety and reactogenicity of the new oral live human rotavirus vaccine (RIX4414) in Bangladeshi toddlers. **Methodology:** A double-blind, randomized, placebo-controlled study was conducted in urban Dhaka to assess the safety of the RIX4414 vaccine. Ninety children aged 2-3 years were randomized such that 30 children received study vaccine of $10^{5.8}$ ffu and 30 children of $10^{6.7}$ ffu, and 30 children received the placebo. Reactogenicity and adverse events were assessed after a single dose of vaccine or placebo through daily home visits for the first two weeks and then twice weekly for another 4 weeks. **Results:** The first data generated in Bangladesh of the RIX4414 vaccine showed that this vaccine appeared to be safe and well-tolerated in toddlers. The symptoms elicited (diarrhoea, fever, vomiting, and irritability) were generally mild and of short duration. No treatment group was associated with significantly higher numbers of adverse events. There were no cases of intussusception. **Conclusion:** The newly-developed human rotavirus vaccine (RIX4414) shows promise, and larger trials are needed to confirm its safety, immunogenicity, and efficacy. **Acknowledgements:** The research was funded by by the United States Agency for International Development, NVPO, and World Health Organization.

Seroconversion to Rotavirus Non-structural Protein4 Following Rhesus-Human Reassortant Rotavirus Vaccine Immunization in Indian Infants

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Background: Rotavirus non-structural glycoprotein NSP4, a multifunctional protein, has been recently shown to be a viral enterotoxin, inducing diarrhoea in neonatal mice. More importantly, antibodies to NSP4 have been shown to protect infant mouse pups from diarrhoea induced by homotypic and heterotypic viruses. NSP4 may be a key determinant of rotavirus pathogenesis and a target for vaccine development. Immune responses to NSP4 and its role in protection against disease in humans have not been thoroughly investigated. **Objective:** Evaluate the rate of IgG seroconversion to NSP4 protein in infants following rhesus-human reassortant rotavirus (RRV-TV) vaccine immunization and compare them with IgA and neutralizing antibody responses in these infants. **Methodology:** The NSP4 gene of rotavirus strain RRV corresponding to amino acid residues 78-175 was expressed as 38 kDa GST-NSP4 fusion protein, which strongly reacted to both rotavirus (RV)-positive human sera and anti-GST rabbit sera in western blot. NSP4-specific serum antibody response was measured in pre- and post-vaccinated sera samples by ELISA, using affinity-purified GST-NSP4 (derived from RRV) and GST protein as antigens. Rotavirus-specific IgA antibodies were determined using rotavirus culture lysate and mock culture lysate by a similar method as described for NSP4. Neutralizing antibody titre to RRV was determined using microneutralization antigen-reduction assay. Seroconversion was defined as ³4-fold rise in antibody titre between pre- and post-vaccinated samples. **Results:** Pre- and post-vaccinated sera samples from 19 children aged 6-14 weeks immunized with RRV-TV or placebo (14 received the vaccine and 5 received the placebo) were all assessed for NSP4-specific IgG antibodies, anti-rotavirus IgA antibodies, and neutralizing antibodies to RRV. RRV-TV-immunized children showed a high seroconversion rate: 8/14 for anti-NSP4 IgG, 13/14 for anti-RV IgA, and 9/14 for neutralizing antibodies to RRV respectively. Further, all the children who were seroconverted to NSP4 also seroconverted to rotavirus IgA. Most children who did not seroconvert to NSP4 were shown to have anti-NSP4 antibodies in their baseline sera. None in the placebo group showed positive seroconversion to NSP4. A correlation between rotavirus shedding and immune response to NSP4 was also implied. **Conclusion:** The present study shows rotavirus non-structural protein NSP4 evokes serum IgG response in infants immunized with rhesus-human reassortant rotavirus vaccine (RRV-TV).

Profile of Use of Optional Vaccines in the Immunization Clinic of a Tertiary-care Hospital in India

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Background: In developing countries like India, children receive only 6-7 antigens as part of the national immunization programme in contrast to their counterparts in developed nations, who receive about 12-13 antigens. This vaccine gap is another example of inequitable distribution of health services between the rich and the poor contributing to the marked differences in morbidity and mortality due to vaccine-preventable diseases. Generating awareness for judicious and timely administration of these optional vaccines (hepatitis B, M.M.R., Hib, chickenpox, typhoid, hepatitis A) can greatly reduce mortality among children aged less than 5 years. **Objective:** Evaluate the level of awareness about optional vaccines among parents, the extent of use, and the factors associated with their use. **Methodology:** In a cross-sectional study, a pre-designed and pre-tested structured questionnaire was used for interviewing either of parents (n=1,392) accompanying children for routine immunization after obtaining an informed verbal consent. The study was undertaken at the immunization and health guidance clinic of S.C.B. Medical College, Cuttack, during July 2002-December 2002. The study variables included socioeconomic status, educational status, gender, place of residence, and source of awareness. Statistical analysis was done using chi-square test and percentage. **Results:** 60.2% of parents were aware of one or more type(s) of optional vaccines. Awareness regarding individual vaccines, except for hepatitis B, was very poor (<10%). Awareness was higher among parents of high-socioeconomic status and better educational background, which was statistically significant. In the case of 54.2% of respondents, health providers were the source of information. Surprisingly, rural population was better informed (marginal difference). Reasons for not vaccinating were mostly lack of awareness but not the cost factor. **Conclusion:** Awareness regarding newer vaccines is inadequate. Necessary information, education, and communication activities should be intensified. Participation of healthcare providers of related field is recommended.

Programmatic and Non-programmatic Determinants of Low Immunization Coverage in Bangladesh

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Background: Despite remarkable progress and continuing concerns and interests of all stakeholders on EPI, around half of children are not fully immunized. **Objective:** Document in-depth understanding of programmatic and non-programmatic determinants of low immunization coverage. **Methodology:** A cross-sectional survey of 2,702 randomly-selected 12-23-month old children from 6 upazilas was conducted, and categories of fully immunized, drop-outs, left-outs, and invalid dose cases were identified. In-depth interviews of mothers from each category and interviews with other adult family members, service providers, and programme managers were conducted. Prospectively, infants aged 0-4 week(s) were followed up till the DPT3 dose was administered. Sub-sample of children completed DPT3 and eligible for measles were also followed. Service statistics were reviewed, and inventory of vacancies and logistics was prepared. **Results:** The immunization coverage until first birthday showed a wide variation by upazila. The coverage of fully-immunized cases ranged from 51% to 89%. Drop-outs varied from 35% to 8%. Invalid doses were 2-3% in high-performing upazilas to 1% in low-performing ones. Multiple factors were documented for drop-outs, notably irregular EPI sessions, lack of effective supervision, and insufficient communication regarding subsequent doses. Reasons behind complete immunization included parents convinced about benefits of EPI, vaccination spot in close proximity, and community influence. Provider's inadequate knowledge of invalid dose resulted in providing subsequent doses ahead of schedule and also injecting measles vaccine with the third dose of DPT as in Sylhet, where the vaccination schedule started late at 3 months after birth. Left-outs resulted from dependence on fate and living in hard-to-reach areas. **Conclusion:** The majority of the determinants attributable to low immunization coverage appears programmatic. Programme needs to ensure regular holding of vaccine sessions. Refresher training for providers and effective managerial supervision would help clear the concept of valid/invalid doses and facilitate better communication with clients regarding benefits of completing all doses timely. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Screening for Unmet Need for Immunization: Challenge for Improving Immunization Coverage in Bangladesh

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Background: Despite high coverage under the Bangladesh Expanded Programme on Immunization, only half of children are fully immunized. Studies in Latin America have shown that unmet needs can be identified at clinics using simple screening tools, leading to the increased coverage of services. **Objective:** Assess unmet needs for immunization and evaluate the impact of using a screening tool on identifying these needs and addressing them in clinics in Bangladesh. **Methodology:** The study design was quasi-experimental with a non-equivalent control group. It was conducted in clinics in two government and two NGO areas. Use of a screening tool was evaluated comparing selected indicators in intervention and comparison clinics at the beginning and at the end of the intervention period (April 2002-March 2003). Exit interviews were conducted with 400 consecutive clients in each of the 4 study areas to ascertain the unmet needs for services. **Results:** In the NGO intervention clinics, overall 4,792 additional needs were identified—201 for child immunization and 848 for tetanus toxoid (TT) vaccination. For every 100 clients requesting child immunization, 13 other clients needed this service. For every 100 clients requesting TT vaccination, 49 other clients needed this service. There was a large increase in the proportion of additional needs that were met from 1.9% to 13.7% for child immunization and from none to 68.4% for TT vaccination. The increase in meeting additional TT needs was statistically significant in both NGO and government clinics and significantly greater than that in the comparison area. The increase in meeting additional child immunization need in NGO clinics was also statistically significant. **Conclusion:** Identification of additional need for immunization increased following the introduction of the screening tool, and more unmet needs were addressed, contributing to improved immunization coverage. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Spatial Analysis in Vaccine Trials

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Background: The utility of conventional vaccine trial methods has recently come into question. In particular, it has been suggested that public-health practitioners cannot use traditional protective efficacy measures to make decisions about whether or not to vaccinate diverse populations. Many trials, including those measuring the efficacy of the Ty21a typhoid fever vaccine, have produced conflicting results in different settings because of varying exposure levels to disease. The Ty21a vaccine, for example, had an efficacy of 96% in Egypt, 77% in Chile, and 53% in Indonesia. Vaccines may not work as well when exposure to a disease is higher. **Objective:** Develop methods that can be used for calculating different efficacy values for different spatial subsets of vaccine trial areas, develop and describe methods that control spatial bias in outcomes due to spatial confounders, such as differential access, to passive surveillance facilities, describe how spatial information can be used for measuring whether herd immunity is important for a vaccine, and also describe how spatial information can be used for supporting the logistics of a vaccine trial. **Methodology:** The study uses health, demographic and GIS data from Bangladesh to develop and test the methods that incorporate a spatial component into vaccine trails. The spatial subsets are defined using spatial distributions of known risk factors for the disease. In turn, this facilitates the stratification of efficacy calculations by exposure to the disease (i.e. high-risk areas represent areas with higher exposure). **Results:** Traditional vaccine trial methods have an underlying assumption that the effect of the vaccine is the same throughout the trial area. Since the force of infection varies in space, the vaccine efficacy is not the same throughout most trial areas. Also, accessibility to hospitals is a determinant of hospitalization rate; thus, it could be a confounder for measuring vaccine efficacy. **Conclusion:** Incorporating a spatial component into vaccine trials provides specific information that is necessary to determine whether and how to vaccinate different populations. The study will provide methods for addressing spatial heterogeneity of the effectiveness of a vaccine that is not represented in traditional non-spatial phase III vaccine trial results.

4:30 pm-5:15 pm (Venue: Meeting Room 1)
Plenary 5: Pathogenesis

109 (367)

The Colonic Bacteria Ecosystem. The Awaited Partner of Infectious Diarrhoea?

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From the origin of humanity, diarrhoea was associated with daily life and recognized as a common symptom related to the health status of individuals. In the twentieth century, the infectious origin of diarrhoea was searched but hard to establish. One major scientific starting point was the discovery in the sixties of an experimental model of diarrhoea based on the use the interaction between *Vibrio cholerae* toxin in small intestine epithelium. The observed intestinal secretion of water and electrolytes explained the watery diarrhoea and dehydration. As a consequence, the first two partners causing diarrhoea were infectious agents interacting with the intestinal epithelium. *Infectious diarrhoea was then regarded as a disease of the intestinal epithelium.* With the discovery of glucose-stimulating sodium and water absorption, food and its constitutive nutrients became the second group of partners. Rice, which was already used in so many countries for so many years, became the most popular food to prevent and treat dehydration. In addition, the peak of diarrhoea observed at weaning indicated that *infectious diarrhoea is a disease linked with the environment*. It was then recognized that the intestinal epithelium is part of a more complex entity called the intestinal mucosa that also participates in the mechanism of response to infectious agent. It is composed of all the different systems that are also present in the body, including nerves, immune cells, endocrine cells, fibroblasts, and blood cells. *Infectious diarrhoea was then regarded as a disease of the whole body.* The last identified partner is the colon. With the discovery that short-chain fatty acids stimulate sodium absorption in the colon, amylase-resistant starch, which is metabolized in the colon in short-chain fatty acids, was found to reduce stool output in cholera. Interestingly, the bacteria that are physiologically present in the colon in great quantity were not easily integrated in the understanding of infectious diarrhoea. It may be in part because, after Pasteur and Koch, bacteria are essentially regarded as infectious agents. It may also be that the biodiversity of the colonic ecosystem was not an easy system to study. Nevertheless, several attempts of treatment based on bacteriae properties were successful in the prevention and treatment of diarrhoea. Fermented food, essentially milk, and then bacteria added to food without fermentation, were often found to be effective. The terms probiotic given to those bacteria and prebiotic given to carbohydrates metabolized in the colon point to an interaction between ‘good’ and ‘infectious’ bacteria. But not much is known about the mechanism of action of these external agents. Not until recently the biodiversity of the ecosystem could be studied. It is now at hand with the simple recognition that a molecular signature present in the DNA coding for the ribosomal RNA, and the possibility to look at a glance at all the species present in a sample of stool (or colonic content) could identify bacteria. Thus, rather than examining one specific microorganism after the other, as it has been done for searching infectious agents, the colonic flora is regarded as one system together. Its properties have to be searched in the community of the different bacteria. Among them, the control of the colonic function to handle water and electrolytes is essential in the prevention and treatment of watery diarrhoea. In addition, the colonic flora has a barrier function against external bacteria that may also be critical in dysenteric diseases. Finally, if, as it is presently proposed, the bacteriae ecosystem is determined by the genetic background and way of life of a population, *infectious diarrhoea will be regarded as a common symptom related to the health status of a community*.

4:30 pm-5:30 pm (Venue: Meeting Room 2)
Scientific Session 14: Environmental Health 2

110 (219)

Reliability of Arsenic Field-test Kit

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Background: Contamination of ground water with arsenic is a widespread concern in Bangladesh. Field kits are currently used for classifying tubewells as delivering arsenic below 50 µg/L as safe, painted green or above 50 µg/L unsafe painted red. **Objective:** Ascertain the reliability of field-test kit by standard laboratory method and evaluate the efficiency of field kits. **Methodology:** Trained field teams collected water samples from 1,933 tubewells from the ICDDR,B Matlab field area in 20 mL scintillation vials and transported to laboratory for arsenic assay using atomic absorption spectrophotometry (AAS) attached with hydride generation. They tested water from the same sample on the spot using Merk field kit. Results were used for immediate feedback and for classification of tubewells into unsafe ($\geq$50 µg/L) as 2 and marked in red: safe (<50 µg/L) as 1 and marked green. Arsenic test was calibrated using standard reference materials bought from the National Institute of Standard and Technology, USA. **Results:** According to the kit method, 53% of the water samples had arsenic more than permissible limit of 50 µg/L. The AAS results revealed that 51% of tubewell water had more than 50 µg/L arsenic concentration. Of water samples those marked as 1, 98% had <50 µg/L arsenic concentration, and 96% marked as 2 had >50 µg/L arsenic concentration according to the AAS method. The validation result showed high correlation (r^2=0.94) between AAS and Merk field-test kit. **Conclusion:** These kits can be used for initial screening in a large-scale national survey under careful monitoring. **Acknowledgements:** The study was supported by SIDA, World Health Organization, and United States Agency for International Development (USAID).

Investigation of Effects of Arsenic on Pregnancy Outcomes and Child Health and Development in West Bengal, India

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Background: Health effects of ingested inorganic arsenic in drinking-water been shown in different regions of the world almost exclusively in adults. The impact of pre- and postnatal arsenic exposures on pregnancy outcomes and child development is largely unknown. **Objective:** Study reproductive effects, including fertility, spontaneous abortions, stillbirths, and intellectual and behavioural child development, and also study possibly modifying effects of nutrition and metabolic susceptibility on arsenic toxicity. **Methodology:** In West Bengal, India, a retrospective cohort study is being conducted in families in which one member had skin lesions. From these families, pregnancy histories of 100 women known to be drinking more than 400 µg/L of arsenic in 1995 and 100 controls with exposure levels less than 50 µg/L of arsenic, and their living children, are being compared. Detailed arsenic lifetime exposure histories are being assessed, and arsenic concentration is being measured in water of tubewells subjects used as sources of drinking-water. Arsenic metabolites are being measured in urinary samples. Sociodemographic factors are being assessed for all subjects. Women are undergoing detailed interviews about their pregnancy histories and other potentially relevant factors. Every child (age range: 5-15 years) is being tested for cognitive abilities and learning achievements with structured culturally-adapted testing methods and examined medically. Detailed nutritional assessment of each family is being carried out, and blood micronutrient levels are being analyzed, to search for a potential modifying role of nutrition on arsenic effects. Methylation patterns in urine samples are being examined, which may help identify a potentially susceptible subgroup. **Results:** Preliminary exposure data, health and demographic characteristics of mothers and children are being presented. **Conclusion:** The very high exposure to inorganic arsenic in drinking-water, mainly above 400 µg/L, experienced by the study participants make this study a unique opportunity to assess the impact of inorganic arsenic on pregnancy outcomes and child development.

Anti-oxidants in Treatment of Arsenicosis: A Randomized Clinical Trial in Bangladesh

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Background: Published reports and the animal study conducted by the authors demonstrated that arsenic toxicity is mediated by impairment of oxidant/anti-oxidant balance in the body. **Objective:** Evaluate the therapeutic effects of anti-oxidant vitamins and minerals in reducing chronic arsenic toxicity in patients. **Methodology:** A randomized clinical trial was carried out in 319 men and women aged 12-65 years, with chronic arsenic poisoning through drinking contaminated water. The patients were given either a recipe of ß-carotene, ascorbic acid, alpha-tocopherol, selenium, zinc, and folic acid (anti-oxidant group) (n=158) or placebo (n=161) for 12 months. All subjects were given arsenic-free water. Treatment effects were assessed clinically and by laboratory determination of arsenic concentrations in biological samples. **Results:** Treatment with anti-oxidants increased body weight, haemoglobin concentration, and improved skin lesions in arsenicosis patients. The improvements were greater than those observed in the placebo-treated group. After 3 months of treatment, concentration of arsenic in spot urine sample reduced by 50% in both anti-oxidant and placebo groups. However, subsequent urine samples showed steady increase in arsenic excretion in the anti-oxidant group, which was greater than the initial values at the end of 12 months. The placebo group did not show any significant change during this period. Anti-oxidant treatment gradually reduced arsenic concentrations in scalp hair and fingernail. There was no change in the placebo group. Arsenic speciation study showed greater excretion of dimethylarsinic acid in urine due to anti-oxidant treatment compared to the placebo. **Conclusion:** Vitamins and minerals given along with arsenic-free water are useful in reducing arsenic toxicity. **Acknowledgements:** The financial support of the Ministry of Science and Technology, Government of Bangladesh, and UNICEF-Dhaka is acknowledged.

Flocculent-Disinfectant Point-of-use Water Treatment for Reducing Arsenic Exposure in Bangladesh

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Background: An estimated two-thirds of all tubewells placed in Bangladesh to provide safer sources of drinking-water may be contaminated with harmful levels of arsenic. **Objective:** Evaluate the effectiveness of treating arsenic-contaminated tubewell water with a point-of-use flocculent-disinfectant for reducing exposure to arsenic. **Methodology:** The study was conducted in Matlab, Bangladesh. Flocculent-disinfectant was provided to 105 households drawing their drinking-water from tubewells contaminated with ≥50 ppb arsenic (determined by a rapid field-test) for 12 weeks. One woman in each household was taught how to treat tubewell water and was instructed to drink treated water only. Drinking-water and spot urine samples were collected at baseline and at 2, 5, 9, and 12 weeks after intervention. Drinking-water was analyzed for total arsenic and spot urines for the sum of arsenite, arsenate, monomethylarsonic acid, and dimethylarsinic acid (total urinary arsenic) by atomic absorption spectrophotometry. **Results:** The mean baseline arsenic concentration in tubewell water was 162 ppb. The mean total urinary arsenic concentration at baseline was 439 µg/g creatinine. Following initiation of the intervention, arsenic concentrations in drinking-water decreased by a mean of 138 ppb (85%); 88% met the Bangladesh standard of <50 ppb. Urinary arsenic concentrations decreased by a mean of 163 µg/g creatinine (37%). **Conclusion:** The flocculent-disinfectant markedly reduced arsenic levels in tubewell water and, to a lesser extent, urinary arsenic in women who consumed treated water. The lesser reduction in urine arsenic may be a result of continued exposure (through untreated drinking-water, other dietary or environmental sources), or metabolic factors. **Acknowledgements:** The study was funded by the Procter and Gamble Health Sciences Institute, USA.

4:30 pm-5:30 pm (Venue: Meeting Room 3)
Scientific Session 15: Nutrition 5: Child Development and Childcare

114 (066)

Do Severely-malnourished Hospitalized Children Differ in Their Development and Behaviour from Severely-malnourished Children Attending Community Nutrition Centres?

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Background: Severe malnutrition of childhood not only results in impaired physical growth but also poor mental development, which can be permanent if not treated at an appropriate time by means of psychosocial stimulation. **Objective:** Compare mental and psychomotor development and behaviour of severely-malnourished children admitted to an urban, hospital-based nutrition rehabilitation unit (NRU) and those attending rural community nutrition centres (CNCs) under the Bangladesh Integrated Nutrition Programme (BINP). **Methodology:** Thirty severely-malnourished children (wt/age <-3SD) aged 6-24 months, admitted to NRU of Dhaka Hospital of ICDDR,B, were compared with 30 severely-malnourished, age- and sex-matched children attending CNCs of BINP in Monohordi upazila. Children's development was assessed using the revised version Bayley Scales of Infants Development and behaviour rated on a five, 9-pointed scale by Wolke, and mothers were asked about temperament of thir children. Differences between the groups were compared by bivariate and multivariate analyses. **Results:** The study groups did not differ in parental years of schooling, fathers' occupation, and presence of sanitary latrine. The NRU children lived in significantly less-crowded houses (p=0.03), were significalntly more wasted, had smaller heads, and thinner arms, and all had recovered from diarrhoea along with pneumonia, septicaemia, and dyselectrolytaemia, while the community children had minor illnesses. The mean±SD mental development index (MDI) and psychomotor development index (PDI) of the NRU children were 68.67±16.44 and 56.57±11.09 respectively and of the CNCs children were 83.40±14.37 and 78.97±19.23 respectively (p<0.001 for both the comparisons). The NRU children were significantly less active, less cooperative, more fussy, and less vocal, and their mothers also assessed them to be more fussy (p=0.03), less active (p=0.005), and less oriented (p=0.05), although they were more soothable (p=0.002) compared to the community children. **Conclusion:** Severe malnutrition associated with serious illnesses adversely affects development and behaviour of malnourished children. In addition to treatment of underlying diseases and nutritional supplementation, management of severely-malnourished children should include psychosocial stimulation to improve their development.

Mental Development and Behaviour of Low-birth-weight Bangladeshi Infants Living in a Poor Urban Community

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Background: In developed countries, low-birth-weight (LBW) infants, who are mostly preterm, are reported to have lower developmental consequences than normal-birth-weight (NBW) infants. However, very few reports are available from developing countries, where LBW is mainly due to intrauterine growth retardation. The authors also failed to find any such report from Bangladesh, where the prevalence of LBW is as high as 30%. **Objective:** Compare mental and psychomotor development and behaviour of LBW infants with that of NBW infants. **Methodology:** This report is based on post hoc analyses of a randomized controlled trial of the effects of supplementing pregnant women with fish-oil on development of their infants at 10 months of age. Pooled data from treated and placebo groups of LBW infants (weight <2,500 g, n=66) and NBW infants (weight >2,500 g, n=183) were compared on later development using Bayley Scales of Infants Development (BSID-II) that has two sub-scales—Mental Development Index (MDI) and Psychomotor Development Index (PDI). Infants' activity, emotional tone, vocalization, approach to the tester, and cooperation during the test were rated on 9-point scales of Wolke. Bivariate and multivariate analyses were performed to examine group differences. **Results:** The LBW children performed significantly worse than the NBW infants on MDI (99.5±7.0 vs 102.9±8.0, $p<0.002$), and PDI (96.8±10 vs 102.7±10, $p<0.001$), and they were also less active (5.3±1.4 vs 6.0±1.4, $p<0.001$) and less happy (5.2±1.5 vs 5.8±1.5, $p<0.014$). The family of NBW infants had significantly higher income, better housing, and more assets, and these babies had longer gestational age. Mothers' age, body mass index, and mid-upper arm circumference were also significantly higher in the NBW group. There was no difference in the number of NBW and LBW children receiving either of the treatments. After controlling for all possible confounders, the LBW group had significantly lower scores on MDI (B=2.7, se=1.1, 95% CI 0. 6-4.8) and PDI (B=3.5, se=1.3, 95% CI 1.0-6.0) and was also significantly less active (B=0.5, se=0.2, 95% CI 0.1-0.8). **Conclusion:** In a poor urban Bangladeshi community, mental and psychomotor development of LBW infants is significantly lower, and they are less active than NBW infants at 10 months of age.

At Which Level of Malnutrition do Children Tend to Develop Mental and Psychomotor Deficits and Behavioural Problems?

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Background: Malnutrition causing developmental delay has been reported in many studies, and it is also reported that malnourished children have more behavioural problems than those adequately nourished. Only few studies have looked at different levels of malnutrition, but no reports could be found from Bangladesh, where 56% of children are undernourished. **Objective:** Compare development and behaviour of rural Bangladeshi children of varying nutritional status and determine the level of malnutrition at which developmental deficits occur. **Methodology:** This is a post hoc analysis of a case-control study where malnourished children (W/A <-2SD), aged 6-24 months, attending 20 Community Nutrition Centres of Bangladesh Integrated Nutrition Programme in Monohordi upazila, were compared with better-nourished children (W/A ≥-2SD) matched for age, sex, and village. Bayley Scales of Infant Development comprising Mental (MDI) and Psychomotor (PDI) Development Indices were used in assessing children's development, and their behaviour was rated on Wolke's five, 9-point scales. Children were grouped as W/A £-3SD (n=65), -2 to -2.99SD (n=48), -1 to -1.99SD (n=78), and >-1SD (n=48) and compared using ANOVAs controlling for age and extensive socioeconomic variables. Individual group differences were examined with post hoc *t*-tests. **Results:** There were significant group differences in development and behaviour. Compared to children of W/A >-1SD, MDI significantly declined in children with W/A £-3SD, whereas a significant decline in PDI was noted in children with W/A <-2SD. Children with W/A £-3SD had very low scores (adjusted mean±se MDI: 84.8±1.6; PDI: 77.2±1.9) compared to children with W/A >-1SD (MDI: 91.4±1.9; PDI: 93.5±2.3). Children with W/A of £-3SD were significantly less friendly, cooperative, and vocal; those with W/A of £-2SD were less active; and those with W/A of £-1SD were less happy. **Conclusion:** Children's development began declining around -2SD W/A. Considering the high prevalence of malnutrition in Bangladeshi children (56% <-2SD), these results have serious implications both for individuals and national development. Urgent attention is required for prevention of and reduction in childhood malnutrition. Furthermore, psychosocial stimulation should be integrated into community management of undernourished children to improve their development.

DAY 3: TUESDAY, 9 DECEMBER 2003

8:30 am-9:15 am (Venue: Meeting Room 1)
Plenary 6: Nutrition and Agriculture

117 (368)

Agriculture in Asia—Irrevocable Links to Nutrition and Health

Barbara Burlingame

Food and Agricultural Organization, Rome, Italy

Much of the world's 'double burden of disease', that is communicable and non-communicable diseases, and even more of the world's 'double burden of malnutrition', that is over- and undernutrition, are directly or indirectly attributable to agriculture. No region has been more profoundly affected than Asia. Although many other factors contribute to the disease burden in Asia, good agriculture is able to provide solutions, while bad agriculture exacerbates or even causes the problems. The nutrition and health statistics for Asia are not entirely encouraging. Data from the Food and Agriculture Organization (FAO) and World Health Organization (WHO) show a grim picture of high infant and maternal mortality; very high prevalences of wasted, stunted, and underweight children; low dietary energy, protein and fat supplies; and high prevalences of micronutrient deficiency diseases. Superimposed upon these problems is the emergence of obesity and chronic diseases. Agriculture and food supply policies at the international, regional and national levels have achieved some remarkable results in recent decades. FAO reports that the world has enough food now to more than adequately feed its population, so increased production is not the solution. This paper will address other aspects of agriculture that impinge upon the disease burden: use of agricultural statistics in the formulation of health policy, the effects of industrial vs small-scale agriculture, agricultural biotechnology and biodiversity, global vs local markets, agricultural mechanization and human energy requirements/expenditure, food-based micronutrients vs micronutrient fortification and supplementation, 'feeding the cities', and agro-industrial waste and communicable diseases.

118 (100)

Prevalence of Tuberculosis among HIV-seropositive Individuals in Kolkata

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Background: HIV and TB co-infection is a major challenge nowadays. HIV-infected persons carry 5-fold increased risk of developing tuberculosis than the general population, and every third HIV-infected person dies of tuberculosis worldwide. However, no information is available about the prevalence of tuberculosis among HIV-seropositive persons in Kolkata, India. **Objective:** Find out the prevalence of pulmonary tuberculosis among HIV-seropositive individuals in Kolkata. **Methodology:** Persons who reported having high risk factors for HIV infection were screened for their HIV status at the NICED Health Counselling Centre at I.D. and B.G. Hospital. Of them, 120 HIV-positive cases, included in this study, were screened for pulmonary tuberculosis by sputum examination for AFB and chest X-ray. Those who were sputum-positive and/or chest X-ray-positive were defined as suffering from pulmonary tuberculosis. **Results:** Of the 120 HIV-seropositive individuals, 79 were males and 41 were females. Sputum examination revealed that 29 (24.17%) individuals were positive for AFB, of which 23 (29.11%) were males and 6 (14.63%) were females. However, findings of chest X-ray were suggestive of 19 (15.83%) cases as positive for TB. Finally, of the 120 HIV-seropositive cases, 36 (30%) were positive for active TB, and 84 (70%) were negative for TB. **Conclusion:** TB is a major opportunistic infection among HIV-seropositive persons in Kolkata.

Digital Cameras versus Film Digitizers for Chest Radiograph Archival

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Background: Chest radiographs can be used for categorizing pneumonias, refining estimates of the proportion due to certain pathogens, and quantifying the preventable fraction using new vaccines. Digital imaging allows for rapid electronic transfer of data; recent improvements in digital camera technology may present high-quality options that are cheaper than film digitizers. **Objective:** Compare two digital image modalities for capturing chest radiographs, digital camera, and film digitizer, to a hard-copy gold standard. **Methodology:** Each radiograph was scanned on a Vidar film digitizer and photographed using a Sony Mavica camera according to standard written guidelines. A panel of radiologists read chest radiographs from 192 patients with suspected pneumonia. Using the hard-copy readings as the gold standard, sensitivity and specificity of other modalities were calculated. A receiver operator characteristics (ROC) analysis was conducted to evaluate how well the camera and film digitizer performed at diagnosing pneumonia compared to the hard-copy images at different levels of sensitivity. **Results:** Of the 192 radiographs, 185 (96%) were classified as possible pneumonia on the hard copies. The camera was 92% sensitive and 100% specific, and the film digitizer was 86% sensitive and 71% specific. In the ROC analysis, the value for the area under the curve was 0.94 for the camera and 0.91 for the film digitizer. The camera set cost $ 1,008 and the film digitizer $ 3,000. **Conclusion:** The ability of the radiology panel to detect evidence of pneumonia on the digital camera images was as good as the film digitizer images. The 3-fold lower cost of the digital camera makes this technology an affordable and widely accessible alternative for large vaccine trials, surveillance systems, and perhaps clinical use.

Who is to Blame? Sociocultural Influences on Caring Practices for Acute Respiratory Illnesses

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Background: Acute respiratory infections (ARIs) remain a major cause of child mortality in Bangladesh, accounting for 25% of all childhood deaths. However, little is known about the influence of sociocultural factors on the management of illness episodes. **Objective:** Examine how illness beliefs guide treatment strategies in a pluralistic health system. **Methodology:** In-depth qualitative research was conducted over a 10-month period from October 2002 to June 2003 as part of the Matlab IMCI Evaluation Study. Methods included key-informant interviews, open-ended interviews with primary caretakers of young children, free-listing and rating exercises, hypothetical case scenarios and detailed accounts of self-treatment, and sequences of healthcare-seeking behaviours from confirmed pneumonia cases. **Results:** Causal interpretations for acute respiratory illnesses are frequently linked to humoral beliefs and relate to over-consumption of foods that possess cooling properties or exposure to cold substances, causing the child to contract what is locally perceived as a 'cold' condition. In fear of being blamed for poor caring practices, mothers are reluctant to share the illness with other family members until the child's condition becomes dire, prolonging the administration of potentially harmful home remedies, such as the application of kerosene, and delaying care-seeking. The findings illuminate distinct trends in care-seeking. While initial treatment is with local health providers, the type of practitioner selected varies according to such factors as the child's age, aetiologic interpretations of the condition, and perceptions of appropriate therapeutic measures. **Conclusion:** Intervention strategies designed to reduce mortality associated with ARI need to recognize and address the constraints mothers face in informing other family members about the child's condition and seeking prompt care. The modern health sector must acknowledge the role of local health providers in the treatment of ARI and strive to identify ways to involve these practitioners in the overall approach to reducing infectious childhood illnesses. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged. The Matlab IMCI Evaluation Study is supported by the World Health Organization and the United States Agency for International Development (USAID).

Incidence of Pneumonia and Risk Factors Relating to Severity of Illness in a Cohort of Newborns from Birth to 24 Months of Age in Rural Mirzapur, Bangladesh

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Background: Pneumonia is the major cause of mortality among young children. Most studies do not separately report the incidence of pneumonia and risk factors relating to development of severe pneumonia. **Objective:** Examine the incidence and risk factors for pneumonia among young children from a rural community of Bangladesh. **Methodology:** During 1994-1997, a cohort of 252 newborns was prospectively followed twice a week from birth to 24 months of age. The community health workers identified a case of acute lower respiratory tract infection (ALRI) at the household level during routine household visits, and the cases were referred to the hospital for investigation and treatment. In the hospital, the physician diagnosed a case of pneumonia using the WHO criteria. Socioeconomic information was collected at the household level at the beginning of the study. Multiple regression analysis identified the factors associated with severe pneumonia. **Results:** The incidence of pneumonia was 15.5 per 100 child-years. Among the pneumonia cases, 55% and 30% occurred during the first six months and second six months of life respectively. The lowest incidence was observed during the summer season. About 73% of the newborns had severe pneumonia. Children living in a bigger family and who had a father with no steady income were about 4 times more likely to develop severe pneumonia [odds ratio (OR)=3.85, CI 1.03-14.29, and OR=4.1, CI 1.17-14.6 respectively] than children living in a smaller family or with a father who had a steady income. **Conclusion:** Reduction in ALRI, particularly pneumonia mortality, could be achieved by early recognition of a pneumonia case. Efforts should be made to educate parents to recognize the signs and symptoms of pneumonia for seeking early treatment. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Impact of Community-based Training on Bangladeshi Rural Mothers' Perception on Home Care Management of Childhood Pneumonia

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Background: Since physicians advise mothers for home care management (HCM) for <5 years old children with pneumonia, it is important that mothers adequately perceive doctors' advices on HCM of children with pneumonia. **Objective:** Assess if periodic training on WHO-recommended HCM of children with pneumonia increases the level of rural mothers' perception in managing a child with pneumonia, properly, right at home. **Methodology:** Following a baseline survey, 132 randomly-selected rural mothers (each for intervention and control group) were imparted a series of community-based training (3 tri-monthly sessions, each consisted of 5 days) in Pirgacha, Rangpur. Pre- and post-intervention means of mother's perception (based on 25 key questions) were compared. **Results:** Around 92% of the mothers at the baseline never heard about HCM of children with pneumonia. More pre-trained mothers (94.7%) had wrong conception of massaging oil, which contrasted by 92.4% in post-intervened ones indicating warm cloth to keep child warm. Only 21.9% of pre-trained mothers knew about HCM of pneumonia in contrast to 92.4% of post-trained ones. Further, 9.2% of pre- and 92.4% of post-intervened mothers could identify basic/obvious differences between pneumonia and severe pneumonia ($p<0.001$). These included age-specific respiration rates (7.5% vs 92.4%) and difficult breathing (9.9% vs 90%) respectively ($p<0.001$). Mothers' perception was graded based on a weighted score of 25 key questions on HCM of children with pneumonia. None of pre-trained mothers obtained grade A+, A, or B (>90%, 80-89%, and 60-79% marks respectively) compared to 48.3% of intervened mothers having A+ through B. However, 2.6%, 12.8%, and 84.5% of pre-trained mothers obtained grade C, D, and F (indicating 40-59%, 20-39%, and <20%) respectively, which came down after training (3.3%, 0.3%, and 0%) significantly ($p<0.001$). **Conclusion:** The findings re-emphasize the need of further training for rural mothers on HCM of children with pneumonia which will help the country's child-survival programme. **Acknowledgements:** The study was supported by the Bangladesh Medical Research Council, through the Government of Bangladesh's HPSP, funded through WHO and World Bank. The authors are grateful to all physicians at the Pirgacha Upazila Health Complex and Medical Technologist at the Institute of Public Health, Dhaka, for their individual and collective approach and expertise. The authors sincerely thank the rural mothers for their painstaking and active cooperation in conducting the training sessions.

Serological Evidence of *Chlamydia pneumoniae* among Bangladeshi Children Suffering from Pneumonia

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Background: Despite major improvements in the diagnosis of pathogenic organisms causing childhood pneumonia (Pn), sufficient details of *Chlamydia pneumoniae* are not yet studied and so not well-understood. **Objective:** Study the role of *C. pneumoniae* in causing pneumonia in Bangladeshi children. **Methodology:** As part of one of ongoing longitudinal research on clinico-epidemiological study on acute respiratory infection (ARI), 64 children suffering from pneumonia or severe pneumonia, diagnosed clinically employing WHO's standard case management protocol for ARI, were studied at the paediatric out-patient department of Dhaka Medical College Hospital during September-December 2000. In addition to complete blood count, plain X-ray chest and bacteriological culture of naso-pharyngeal swabs (performed at Institute of Public Health Laboratory), antibody titres from paired sera (performed on preserved serum transported to Toho University, Japan) were measured against *C. pneumoniae*, first during acute (at day 1 of diagnosis) and at convalescent phase (on day 14) employing (i) micro-IF using Tar slides (BION) as antigen and (ii) ELISA with Hitazyme *C. pneumoniae* kit (Hitachi Co. Ltd., Japan), findings of which were read as positive if antibody titre showed a 4-fold rise for micro-IF or if developed characteristic colour to specific chromogenic substrate added in ELISA. **Results:** While 56 (87.5%) of the samples yielded various bacteria (45.3% *H. influenzae*, 45.3% *M. catarrhalis,* and 7.8% *S. pneumoniae* and mixed infections), 8 (12.5%) were culture-negative. Antibody titres from paired sera (based on 4-fold rise) revealed that 20.3% (n=13) of children were infected with *C. pneumoniae* (based on positive finding either on micro-IF or ELISA, or both). **Conclusion:** *C. pneumoniae* may be an important emerging pathogen for childhood pneumonia, which may have been overlooked or underestimated, particularly in developing countries like Bangladesh. **Acknowledgements:** The study was supported by a grant for the International Health Cooperation Research, Ministry of Health and Welfare, Japan. Technical assistance was rendered by Norishige Hashiguchi (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.) and Chikako Mashida (Hitachi Chemical Co. Ltd.), and, partly by ACIH and Kumamoto National Hospital, Japan. The authors are grateful to all physicians/medical technologists at the Institute of Public Health, Dhaka, and Dhaka Medical College Hospital, and laboratory staff at the Kumamoto National Hospital, Japan, for their individual/integrated expertise.

8:30 am-9:15 am (Venue: Meeting Room 3)
Special Session 3: *Helicobacter pylori*

124 (000)

Helicobacter pylori Infection: Immune Response in Children

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9:15 am-10:00 am (Venue: Meeting Room 1)
Plenary 7: Genetics

125 (369)

Quorum Sensing, Biofilm Formation, and *Vibrio cholerae* Epidemiology

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The ability of *Vibrio cholerae* strains to cause epidemic cholera in humans requires the expression of cholera toxin (CT) and a pilus colonization factor TCP. These *V. cholerae* virulence genes are regulated by quorum sensing, a regulatory process that occurs at high cell density. Quorum sensing specifically plays a role in the development and dissolution of biofilms, and this process may significantly affect the pathogenic cycle and transmissibility of *V. cholerae*. A biofilm is defined as a community of microorganisms attached to each other and to a surface. Several studies have suggested that biofilm-mediated attachment to abiotic surfaces may be important for survival of *V. cholerae* in the environment. Compared to the wild-type strain, *luxO* mutants (which have a quorum-sensing constitutive phenotype) fail to produce significant biofilms after 24 hours, while *hapR* mutants (which have a quorum-sensing repressed phenotype) form much thicker biofilms. Mutants defective in both *luxO* and *hapR* form biofilms as thick as those of *hapR* mutants, indicating that quorum-sensing control of biofilm formation acts via HapR. To identify HapR-regulated genes involved in biofilm formation, whole-genome microarray analysis was used for comparing the transcriptional profiling. As expected, expression changes were observed for *hapA* (encoding HA protease), which is directly activated by HapR but also *vps* genes (encoding VPS, an extracellular polysaccharide). Deletion of *vps* genes completely abolished biofilm formation in both wild-type and *hapR* mutants, confirming the essential roles of *vps* genes in biofilm formation in *V. cholerae*. Animal infection experiments on various mutants suggest that quorum sensing in vivo probably occurs late in the infection cycle and then down-regulates expression of TCP, CT, and VPS while up-regulating HA protease expression. Because *V. cholerae* must survive passage through the acidic environment of the stomach before reaching its site of replication in the host upper intestine, it was hypothesized that biofilm formation might increase the ability of *V. cholerae* to withstand acid shock and thus enhance infectivity. Planktonic cells were readily killed after exposure to pH 4.5 for 30 minutes. In contrast, biofilm-associated cells were more than 1000-fold more resistant to acid shock than were planktonic cells. In animal experiments, mutants defective in quorum sensing were defective in colonization only when the innoculum consisted of cells in a biofilm structure. Taken together, data suggest a working model for how quorum sensing may influence critical aspects of the *V. cholerae* infection cycle. The biofilm structure may be critical during entry into the host to protect vibrios against acid shock in the stomach. After reaching the intestine, dispersal of individual cells from the biofilm enables these cells to shut off quorum sensing-mediated repression of the CT and TCP genes, thus permitting maximal colonization of intestinal sites. Later in the infection, the number of *V. cholerae* in the intestine increases, and quorum sensing again represses CT and TCP production and activates HA protease production promoting detachment from the epithelium. Bacteria shed as detached biofilms might be more infectious. Biofilm formation in the environment on abiotic or biotic surfaces may also promote infectivity by increasing resistance to stomach acid.

9:15 am-10:00 am (Venue: Meeting Room 3)
Special Session 4: Zinc Scale Up

126 (069)

Scaling Up Zinc as a Treatment for Childhood Diarrhoea in Bangladesh: Project Planning and Implementation

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Background: The treatment of diarrhoea with zinc (20 mg per day for 10 days) in children aged less than 5 years reduces disease severity and duration. It also lessens the likelihood of subsequent diarrhoeal illness and mortality over the next 6 months. Given zinc's proven potential to reduce disease burden in young children, the challenge now is to reach all children with a diarrhoeal illness, in particular those undernourished, and treat them with zinc. **Objective:** Develop and implement a comprehensive scale-up plan that includes the production, distribution, marketing, and monitoring of zinc as a treatment for childhood diarrhoea in Bangladesh. **Methodology:** The following studies have been implemented: (1) formative research upon which to model caretaker practices and prepare provider counselling and mass-media messages relating to scaling up of zinc, (2) a baseline observational study of incident cases of childhood diarrhoea in rural and urban sub-districts, (3) national cluster coverage and caretaker practice surveys, and (4) safety and compliance monitoring. A 3-phase business plan has been developed by the Social Marketing Company (SMC) which covers production, distribution, marketing, training, and monitoring of sales. The transfer of the technology required to produce a blended, non-metallic tasting, premix from Nutriset (French Laboratory) to a local, Bangladesh laboratory/pharmaceutical manufacturer will begin with an open competition. **Results:** Zinc will be provided in a dispersible, 20-mg tablet formulation. These will be packaged in a 10-tablet blister pack (tablets will be made available for individuals to sample). The initial 1 million tablets for research purposes will be compressed and packaged by the Square Pharmaceuticals. A subsequent 200,000 million tablets will be blended, compressed, and packaged by Nutriset. Through SMC, a multimedia campaign has been prepared. Sales officers have been trained to work with private-sector providers. Distribution and sale of zinc blister packs is scheduled to begin in April 2004. Integration of zinc treatment into Ministry of Health and Family Welfare, NGO, and private sector delivery systems is planned. Implementation teams for each sector have been established and training of trainers initiated. **Conclusion:** Taking a proven, effective intervention, such as zinc, and scaling it up to the point it will have a significant impact on the health of Bangladeshi children but it is a complex task. It requires multiple partnerships that bring together a wide range of skills and expectations. Particularly challenging is the integration of research findings into the business plan and marketing of zinc. **Acknowledgements:** The Project is funded through support from the Bill and Melinda Gates Foundation and the United States Agency for International Development (USAID).

10:15 am-10:45 am (Venue: Meeting Room 1)
Plenary 8: Neonatal Health

127 (370)

Improving Newborn Health in Developing Countries: Improving Child Survival by Providing Essential Newborn Care

Stephen N. Wall

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Despite substantial gains in child survival over the past 25 years, the death rate of newborn infants—babies aged less than one month—has been virtually unchanged in developing countries. The results are sobering: a child's risk of dying is 15 times greater in the first month of life than at any other month in the first years of life. Of more than 5 million deaths of newborn infants each year, 98% occur in developing countries. The United Nations Millennium Summit in September 2000 called for a reduction by two-thirds in deaths of children aged less than 5 years before 2015. Child health experts predict that this goal is not attainable without a reduction in the number of deaths of newborn infants by at least half, as 40% of all child deaths occur in the newborn period. Improving newborn survival can be achieved by incorporating effective, evidence-based interventions into the existing health sector programmes, especially safe motherhood and child-survival programmes. Research shows that many neonatal deaths (and stillbirths) could be prevented by addressing health needs of pregnant women and by improving the quality of healthcare for mothers and newborns during delivery and the immediate postpartum period. Similarly, research shows the effectiveness of targeted interventions to prevent, identify, and treat serious neonatal illness during the postnatal period. Essential care to mothers during pregnancy should include the following: (1) tetanus toxoid immunization, (2) supplemental iron and folic acid (to all women of childbearing age), (3) adequate and balanced protein and energy intake, (4) counselling about the importance of immediate and exclusive breast-feeding, (5) screening and treatment of syphilis in endemic areas, and (6) treatment with antimalarials and promotion of insecticide-treated bed nets in malaria endemic areas. During labour and delivery, specific interventions to improve newborn survival should focus on ensuring clean delivery (including cord-cutting) practices and providing appropriate resuscitation to newborns who fail to breathe normally after birth. Postpartum and postnatal care is essential to the health of both mother and newborn. For the newborn, this care should include (1) the prevention and management of hypothermia, (2) exclusive breast-feeding, (3) identification and management of low birth-weight (appropriate referral, use of kangaroo mother care), and (3) recognition and treatment of serious infections, such as sepsis and pneumonia. Two-thirds of neonatal deaths occur in the first week of life. Therefore, the greatest opportunity to reduce child mortality through improving newborn health is by implementing interventions to improve care to women during pregnancy, to mothers and newborns during labour and delivery, and to newborns during the postnatal period.

10:45 am-12:30 pm (Venue: Meeting Room 1)
Special Session 5: Neonatal Health

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Achieving Programme Scale: Experiences of Replication from Integrated Nutrition and Health Project

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Background: The Integrated Nutrition and Health Project (INHP) is a 10-year project (1996-2006) implemented by CARE with the goal of achieving "sustainable improvement in the nutrition and health status of women and children". Building on the lessons learned from INHP I in 8 Indian states (1996-2001), CARE-India has designed INHP II (2001-2006), which focuses on 6 technical interventions. This programme is being implemented in 100,000 villages across 8 states of India. The technical interventions include (1) targeted supplemental feeding of pregnant and lactating women and children aged less than 6 years; (2) maternal and childhood immunization; (3) antenatal care, including enhanced focus on nutrition, tetanus toxoid immunization, iron-folate supplementation, and birth planning; (4) vitamin A supplementation for children aged less than 3 years, and (5) newborn care at the community level (a focused package of newborn care to be implemented at the community level to improve health and survival of newborns, including improvement in care-seeking for newborns). **Objective:** Describe the experiences of replication from the Integrated Nutrition and Health Project. **Methodology:** An internal assessment has been conducted to establish the links between the processes being adopted and the expected outcomes. The sites with complete project intervention were compared with randomly-selected sites with no/minimal project implementation. **Results:** Some of the project interventions that showed significant differences between the intervention and the control sites are receipt of 90+ IFA tablets (65% vs 49%), childhood immunization (82% vs 39%), early breast-feeding (78% vs 44%), and appropriate complementary feeding (65% vs 41%). **Conclusion:** The experiences of replication from this project offer valuable lessons for nutrition and health projects at scale in Asian countries. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Selected Findings from the Baseline Survey of CARE/India's Newborn-care Evaluation Research: Implications for CARE Programme

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Background: Community-based newborn-care interventions need to be systematically evaluated both in terms of their effectiveness in reducing neonatal mortality and their feasibility of large-scale implementation. **Objective:** Establish the baseline for evaluation research being carried out to assess the effectiveness of a community-based newborn care-intervention package on large-scale on neonatal mortality. **Methodology:** This is an evaluation research study aimed at assessing the implementation processes of the CARE-India's Newborn Care Package and its effectiveness on reducing newborn mortality. A pre-test post-test control group quasi-experimental design was used. A household survey has been carried out to establish baseline levels for process and outcome indicators, including neonatal mortality. In all, 56,155 households were surveyed, and a KPC survey of 17,706 women, who delivered in past 2 years, was done. **Results:** The neonatal mortality rates were estimated to be 47.8 and 45.1 per 1,000 livebirths in the intervention and the control district respectively. In the intervention district, only 28% of the newborns were wrapped before their placenta was cut, and about 85% were bathed within 12 hours of birth. In more than half of the babies, even drying was delayed till the placenta was delivered. Breast-feeding was initiated at least after 2 days in more than 90% of the babies. In about three-fourths of the children, something (mainly mustard oil) was applied to the cord. Only 6% received any kind of medical check-up during their first month of life. **Conclusion:** Coverage with essential newborn-care practices in this community is low. A strong behaviour change communication strategy, particularly one-to-one communication, is required to promote these healthy practices. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and the technical support from the John Hopkins Bloomberg School of Public Health are acknowledged.

Saving Newborn Lives Initiative in Bangladesh—Save the Children-USA, Bangladesh Field Office

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Background: Bangladesh has made substantial health gains over the past decade, particularly in reducing infant and child mortality rates. These newborn deaths account for almost half of all deaths among children aged less than 5 years. Currently, 9 of 10 deliveries take place at home in Bangladesh with no skilled attendance, and many more mothers and newborns go without any care during the most vulnerable first days after birth. Low birth-weight is also very high in the country. For sustaining gains in child survival in the country and achieving MDGs, reducing the newborn mortality rate must become a national priority. Findings from different parts of the world suggest that the majority of newborn deaths are preventable and that solutions do not lie in expensive technology. Proven, low-technology health interventions can make the difference between life and death for the majority of newborns. Promotion and support of healthy home care for delivery and newborn practices is just as important as ensuring essential care at the health facilities. **Objective:** Improve the health and survival of newborns in Bangladesh. **Methodology:** Existing materials on newborn care were reviewed, and key stakeholders were interviewed followed by a national strategic planning workshop. **Results:** Save the Children-USA, Bangladesh Field Office is implementing a programme on saving newborn lives in Bangladesh that aims at strengthening and expanding the maternal and essential newborn-care practices in the country. Bangladesh is one of the focus countries of SAVE's global initiative, Saving Newborn Lives (SNL). The SNL Bangladesh Programme has 3 central goals: (a) Provision of essential care for all newborns in the country; (b) Identification and appropriate treatment of sick newborns; and (c) Specific more intensive care for low-birth-weight newborns. **Conclusion:** The present priorities of the SNL Bangladesh Programme include advocacy, newborn services, training, research, and behaviour change communication. The technical solutions to saving newborn lives are simple, inexpensive, and effective. What is important now is to act together and decisively by all who have an interest in the newborn health in Bangladesh. **Acknowledgements:** The financial support of the Bill and Melinda Gates Foundation is acknowledged.

Newborn-care Practices in Selected Upazilas of Bangladesh: What Evidence is Drawn from Community-based Research?

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Background: Little is known about newborn-care practices at the household level in Bangladesh, although neonatal mortality remains consistently high. **Objective:** Understand prevailing newborn care practices in the community and the socioeconomic, cultural and behavioural factors associated with such practices. **Methodology:** A baseline survey and qualitative research were carried in Saving New Born Lives project areas in 12 upazilas of 6 divisions. 3,325 mothers with infants aged less than one year were selected for interview using a systematic two-stage cluster sampling procedure. For the qualitative component, 64 focus-group discussions and 163 in-depth interviews were conducted with mothers of newborns, family members, and service providers. **Results:** Lack of proper information was the most commonly-reported reason for not attending any antenatal check-up (32%). Only 10% of deliveries was assisted by skilled birth attendants. Poverty, easy accessibility of unskilled providers, and family decisions were key reasons. Postnatal check-ups were very low (2% within 3 days of delivery) and were attributed to religious customs and non-responsive family members. Early bathing of newborns was common and was influenced by traditional practice and perceptions. Only 14% of the newborns were dried, and 26% were wrapped immediately after birth. About one-third of the mothers reported putting the baby to the breast within one hour of birth, and 88% of the mothers reported giving colostrum. **Conclusion:** The study has shown a baseline picture of the care that a mother and newborn receives at home in Bangladesh. It is evident that saving newborn lives will depend largely on how well these practices could be improved at the community level. An adequate policy response to this effect would, thus**,** be critical. **Acknowledgements:** The financial support of the Bill and Melinda Gates Foundation is acknowledged.

Delivery and Newborn-care Practices in Two Rural Communities in Bangladesh

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Background: Neonatal mortality remains unacceptably high (over 40/1,000 livebirths) in Bangladesh. Paucity of knowledge on what contributes to neonatal deaths and lack of an effective service strategy are critical challenges. **Objective:** Understand delivery and newborn-care practices and associated beliefs and barriers in selected rural communities of Bangladesh, as a prelude to developing and evaluating an intervention package. **Methodology:** Qualitative data were collected through in-depth interviews and focus-group discussions involving recently-delivered women, members of families, and the community. A cross-sectional survey was then conducted among 25,592 recently-delivered women in Sylhet and Tangail districts. **Results:** About half of the women received antenatal care during pregnancy, and about 40% had at least two doses of tetanus toxoid injection. Untrained traditional birth attendants and family members were the predominant birth attendants in Sylhet (81%). Among those who delivered at home, 48% in Sylhet and 62% in Tangail used a boiled cord-cutting instrument. None in the family specifically was responsible for taking care of the newborn during the first days of life. Widespread common beliefs were that birthing process is a 'polluting' event, and the newborn needs to be bathed before qualifying for any care or ritual. More than 80% of the newborns in both the areas were bathed immediately after birth, and less than 5% were dried and wrapped before the delivery of placenta. Less than 1% of the newborns in Sylhet were put to breast before the delivery of placenta. **Conclusion:** The findings show several practices that need to be modified if substantial improvement in neonatal health is to be expected. A package of maternal and neonatal healthcare was then developed based on these findings and is being implemented for evaluation. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and the Bill and Melinda Gates Foundation, through a grant to the Saving Newborn Life Initiative, Save the Children–USA, is acknowledged.

Results of Behavioural Trials for Alternative and Family-endorsed Newborn-care Practices

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Background: To help design the intervention package for improved obstetrical and neonatal care in a community-based intervention research project in rural Bangladesh, formative qualitative research was conducted to understand key practices and beliefs. The formative research identified specific issues needing further investigation regarding practices relating to cutting of umbilical cord, treatment of asphyxia, wrapping methods, bathing, and feeding. **Objective:** Understand and evaluate key messages and interventions in terms of cultural acceptability and obtain information that can be used for empowering workers with alternative options for negotiating with the families on these key practices so as to increase the likelihood of compliance by the family members. **Methodology:** The behavioural trials consisted of unstructured in-depth interviews and demonstration of alternative caring practices to recently-delivered women (RDW), mothers-in-law, and mothers of RDWs. **Results:** Acceptance of options by the caregivers varied widely. Immediate cutting of the cord before placental expulsion, cutting of the cord by someone other than the mother giving birth, and avoiding pre-lacteals after birth were deep-rooted and potentially difficult to modify. Administering mouth-to-mouth breathing and massaging the back of the baby in case of asphyxia had intermediate level of acceptance. Delaying bathing for 3 days or wiping with wet cloth or sponge bath were better accepted. For thermal care of the newborn, immediate wrapping and covering from head to toe was also acceptable. Many respondents referred to the need of approval of these modified practices from the family. **Conclusion:** With carefully-designed behavioural trials, it is possible to elicit and find solutions or alternatives to apparently 'difficult to change and harmful' practices in traditional communities. It was also evident from these findings that there is a critical need to involve other household decision-makers. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and the Saving Newborn Lives Initiative (SNL) of Save the Children–USA is acknowledged.

Topical Skin Barrier Therapy and Infection-control Measures Improve Survival of Hospitalized Very Low-birth-weight Neonates

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Background: Low-birth-weight (LBW) infants are at an increasingly greater risk of mortality as gestational age decreases. Premature infants aged less than 33 weeks gestation are particularly susceptible to infections and transcutaneous loss of fluid and heat due to developmental immaturity of their skin barrier. **Objective:** Determine the impact of (1) an infection-control programme and (2) topical therapy with skin barrier-enhancing emollients, sunflower seed oil, or aquaphor, on morbidity and mortality of preterm infants aged less than 33 weeks gestation. **Methodology:** An infection-control programme was introduced to the Special Care Nursery (n=300), Dhaka Shishu Hospital and emphasized education of staff and caregivers in measures to decrease risk of nosocomial infections, particularly hand-washing and strict asepsis during procedures, and prudent use of antibiotics. In total, 385 neonates were randomized to topical application of sunflower seed oil or aquaphor 3 times daily, or to the control group (i.e. usual nursery practices). **Results:** Infection-control efforts resulted in the decline in episodes of suspected neonatal sepsis (47%), cases of culture-proven (61%) and clinical diagnoses (53%) of neonatal sepsis, and neonatal mortality (20%). Emollient therapy significantly reduced the odds of neonatal mortality by 25% (OR 0.75, 95% CI 0.57-0.98). Emollient therapy reduced the odds of mortality by 35% (OR 0.63, 95% CI 0.46-0.87) among infants weighing 1,250 g or less. **Conclusion:** Infection-control practices and topical therapy with skin barrier-enhancing emollients are simple, inexpensive interventions that have significant potential for improving survival of very LBW neonates. **Acknowledgements:** The financial support of the Thrasher Research Fund, the Johns Hopkins Family Health and Child Survival Cooperative Agreement with the United States Agency for International Development (USAID), Save the Children Federation–USA through a grant from the Bill and Melinda Gates Foundation, and a William Weston Research grant from the Society for Pediatric Dermatology is acknowledged.

10:15 am-12:30 pm (Venue: Meeting Room 2)
Scientific Session 17: Infectious Diseases 4

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Molecular Analysis of *Vibrio cholerae* ElTor O1 Bacteriophage

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Background: The phage typing scheme for *Vibrio cholerae* O1 biotype ElTor has been developed at the National Institute of Cholera and Enteric Diseases. Most of the cholera phages were successfully used for typing purposes. So far, the genome of none of the phages used for phage typing has been mapped or sequenced without which its structure-function cannot be understood at the molecular level. One of the phages, N-4, is an ElTor O1 phage of the new phage typing scheme is selected for molecular characterization. **Objective:** Characterize *V. cholerae* ElTor O1 phages at the molecular level. **Methodology:** Phage DNA was purified and isolated according to the procedure of Watarai *et al.* (1998). High titre phage stock 3-4x10^{10} pfu/mL was prepared by broth lysis procedure. The phage stock was purified using polyethylene glycol (10% W/V). Phage DNA was isolated using phenol: chloroform treatment. In total, 28 enzymes were used for digesting the phage DNA. **Results:** DNA could be digested with only 4 enzymes, Hind III, AccI, Hae III, and EcoRV. Additionally, EcoT14I, EcoT22I, TthHB 81, and MspI have been also found which could digest the phage DNA. Hind III was also used for digesting the DNA partially. Since the phage DNA was resistant to a large number of enzymes, it might be assumed that the DNA was highly methylated. **Conclusion:** It has been calculated that the length of DNA seems to be approximately 30 kb and has at least 15 Hind III sites. Presently, Hind III and other enzymes are employed to clone the phage DNA.

Molecular Characterization of Multidrug-resistant *Shigella dysenteriae* type 1 Strains from Recent Outbreaks in West Bengal, India

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Background: Dysentery caused by multidrug-resistant strains of *Shigella dysenteriae* type 1 is a major public-health problem in developing countries. During the past one year, many dysentery outbreaks due to *S. dysenteriae* type 1 were reported from different parts of West Bengal, India. In all these outbreaks, treatment failure with antimicrobial agents commonly used for treating shigellosis was a matter of concern. Most *Shigella* species have developed resistance to commonly-used drugs. **Objective:** Determine the clonal relationship of multidrug-resistant strains of *S. dysenteriae* type 1 isolated from dysentery outbreaks and characterized using molecular methods. **Methodology:** In total, 15 *S. dysenteriae* type 1 strains, isolated during dysentery outbreaks in the past one year, were analyzed in this study. Standard biochemical methods were employed for isolation and identification of the strains. Antimicrobial susceptibility testing was performed by an agar disk-diffusion method, as recommended by the National Committee for Clinical Laboratory Standards. MIC was determined using E-test (AB Bio Disk, Solna, Sweden) following the manufacture's instructions. Plasmid DNA was extracted from the isolates by modification of the alkaline lysis method of Kado and Liu. For PFGE, total genomic DNA was embedded in agarose plugs and digested with *Xba*I. **Results:** Antimicrobial susceptibility test showed that *S. dysenteriae* type 1 strains were resistant to most antimicrobial agents used for treating shigellosis, including ciprofloxacin and norfloxacin. The strains were, however, sensitive to ofloxacin. The MIC range of ciprofloxacin and norfloxacin was 2 to 6 and 6 to 24 microgram per millilitre respectively. PFGE and plasmid profile of all strains showed almost identical pattern but was different when compared to strains collected from early outbreak strains. **Conclusion:** It is concluded that the strains isolated from different outbreaks belonged to a single new clone.

Emergence of Mutidrug-resistant *Shigella dysenteriae* Type 1 in Eastern and North-eastern India: Is It the Forerunner of a Major Pandemic?

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Outbreaks of dysentery caused by multidrug-resistant *Shigella dysenteriae* type 1 have been a recurrent challenge in many parts of the developing world. Beginning in February 1984, an extensive outbreak of bacillary dysentery predominantly due to *S. dysenteriae* type 1 (Shiga bacillus) swept through West Bengal and subsequently spread to other parts of India. *S. dysenteriae* type 1 isolated were highly sensitive to nalidixic acid. Subsequently, due to its indiscriminate use, *Shigella* strains became resistant to nalidixic acid. After a lapse of about 18 years, an outbreak of bacillary dysentery due to *S. dysenteriae* type 1 was reported in April 2002 among the labourers of tea gardens in eastern India. *S. dysenteriae* type 1 strains were resistant to ampicillin, co-trimoxazole, nalidixic acid, norfloxacin, and ciprofloxacin. However, they were sensitive to ofloxacin. Similar outbreaks were reported from Diamond Harbor (near Kolkata) and Aizawl. It is feared that these small outbreaks may be the forerunner of a major pandemic.

Iron-deficiency Anaemia is Associated with Past but not Current *Helicobacter pylori* Infection

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Background: Iron-deficiency anaemia and *Helicobacter pylori* are both common in India. *H. pylori* impairs iron absorption and may contribute to iron-deficiency anaemia. **Objective:** Determine if there is an association between *H. pylori* infection and iron-deficiency anaemia. **Methodology:** The study sample comprised 217 children, aged 5-15 years, with recurrent abdominal pain of unknown cause. Children with haemoglobin <12 g% and serum ferritin of <15 mg/dL were considered as cases and others served as controls. Active *H. pylori* infection acquired recently or in the past was defined by a positive C14 UBT and a positive IgG serology to *H. pylori*. Positive IgG antibodies and a negative C14 UBT indicated past *H. pylori* infection. Negative C14 UBT and IgG serology indicated absence of *H. pylori* infection. **Results:** Fifty-one subjects were considered as cases and 166 as controls. The relationship between active and past *H. pylori* infection with iron-deficiency anaemia was examined separately. The odds of having iron-deficiency anaemia were 3 times higher in children with past infection compared to those without any infection (OR 2.9 95% CI 1.3-7.1, p=0.006). This remained significant after adjusting for age, sex, and type of toilet. There was a significant interaction of overcrowding with the association between past *H. pylori* infection and iron-deficiency anaemia (test of homogeneity p=0.04). Active infection was not associated with iron-deficiency anaemia (OR 1.15 95% CI 0.18-5.1, p=0.84). **Conclusion:** Iron-deficiency anaemia may be a residual effect of past *H. pylori* infection. The effect modification by overcrowding may be by virtue of frequent reinfection and selection of better colonizing strains in the host.

Quantitative Biofilm Assay for Enteroaggregative *Escherichia coli* and Characterization of Enteroaggregative *E. coli* strain in Japan

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Background: Enteroaggregative *Escherichia coli* (EAEC) is defined by an aggregative adherence to HEp-2 cells. The gold standard for identification remains the HEp-2 adherence test. However, this test requires specialized facilities. Although a DNA probe and an *aggR*-PCR method have been used for identification, some EAEC are probe- or *aggR*-negative. **Objective:** Evaluate the usefulness of the quantitative biofilm assay to screen for EAEC in clinical *E. coli* isolates and characterize virulence factors among EAEC strains in Japan. **Methodology:** In total, 1,042 *E. coli* strains from diarrhoeal children were examined for biofilm and *aggR* gene. For quantitative biofilm assay, bacteria were incubated overnight in high glucose DMEM in polystyrene microtitre plate. The plate was stained with crystal violet after washing. Biofilm was quantified using ELISA plate reader at 570 nm. EAEC was identified by HEp-2 adherence test. The *aggR* and other virulence genes were evaluated by PCR. **Results:** The strains were classified into 3 groups according to the absorbance: group I (<0.2)-62 strains, group II (0.1-0.2)-36 strains, and group III (<0.1)-944 strains. The incidence of EAEC in groups I and II was 77.4% (48/62) and 0% (0/35) respectively. All 50 strains examined for HEp-2 assay in the group III were not EAEC. The incidences of *aggR* were as follows: group I-33.9% (21/62), group II-0% (0/36), and group III-0.2% (2/944). Twenty-one *aggR*+ and 27 *aggR*-EAEC strains were screened by this assay. The AggR+ strains showed various virulence factors of EAEC. **Conclusion:** Although the confirmation by HEp-2 assay is needed to identify EAEC, the quantitative biofilm assay using microtitre plate is useful to screen for EAEC. The heterogeneity was observed among the EAEC strains in this study.

Zinc Supplementation in Malnourished Bangladeshi Children with Cholera

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Background: Cholera is characterized by massive rice watery stool, vomiting, rapid dehydration, acidosis, and sometimes death in absence of treatment. Antibiotic use and rehydration have been the mainstay of treatment, yet there are deaths due to diarrhoea. In the meantime, zinc has been proven to deduce volume of diarrhoeal stool and duration in acute diarrhoea and reduction of water and electrolyte secretion in response to cholera toxin-induced secretion in animal models. Therefore, it is hypothesized that zinc supplementation may reduce diarrhoea due to *Vibrio cholerae*-associated infection. **Objective:** Evaluate whether zinc supplementation, in addition to antibiotic therapy, in cholera patients has any effect on the duration and severity of cholera. **Methodology:** A double-blind, randomized placebo-controlled clinical trial was conducted among children aged 3-14 years. In total, 164 children (82 in each group) of either sex having watery stool for not more than 24 hours and dark-field positive for cholera were enrolled in the study after initial correction of dehydration by intravenous (IV) fluid (cholera saline) or oral rehydration solution (ORS) based on the WHO guidelines. After recruitment, all children were randomly allocated to receive either 30 mg of elemental zinc per day or placebo till recovery or maximum for 7 days. Each patient, irrespective of the study group, received syrup erythromycin 50 mg/kg/day divided into 4 doses for 3 days. **Results:** On recruitment, baseline characteristics, such as age, sex, mean duration of diarrhoea before enrollment, dehydration status, and time needed for rehydration were comparable among the groups ($p>0.05$). Children without zinc supplementation had 14% (64.0±24.2 vs 73.0±27.5, $p=0.03$) longer duration of diarrhoea than children of the zinc-supplemented group. The effect was more pronounced in malnourished children (weight-for-age median <75%) where it showed 22% longer duration of recovery (61±23 vs 75±30, $p=0.02$) in the non-supplemented group. There was no significant difference in total (5 days) stool output between the supplemented and the non-supplemented children, but when the children were stratified by more malnourished sub-groups (WHZ <-2SD), 36% more diarrhoeal stool was passed by children who did not receive zinc ($p<0.03$), and they had 22% longer duration of illness than zinc-supplemented counterpart. Survival analysis showed that 55% of children of the zinc-supplemented group recovered by 2.5 days of treatment, whereas in the placebo group, only 35% of children recovered during the same period ($p<0.05$). **Conclusion:** The study confirmed the beneficial effects of zinc supplementation in malnourished children with cholera. Zinc supplementation should, then, be routinely provided in conjunction with antibiotic therapy in children with cholera, especially in malnourished children, to achieve greater benefits.

Intra-familial Transmission of *Salmonellae* in Slum Dwellers of Kolkata, India

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Background: *Salmonella* endemicity has been shown to be maintained through the cyclic order from man to environment and vice-versa. Person-to-person transmission has also been implicated. However, the exact mode of its transmission has not been elucidated so far. **Objective:** Determine the mode of transmission of *Salmonella* in the community in Kolkata. **Methodology:** Faecal samples from diarrhoea patients were tested for isolation of *Salmonellae*, as per standard protocol, which were later confirmed by biochemical and serological tests. Different water samples and other sources of the locality were examined for *Salmonella* organism. **Results:** Three of 610 faecal samples were positive for *Salmonella enterica* serovar Typhimurium. Of the 3 positive samples, one was index-positive case, and two were isolated from the household contacts. Of 26 water samples, 3 were positive for *S. enterica* serovar Typhimurium, of which 2 were isolated from pond water and one from stored water source from the same household as the index case. **Conclusion:** *S. enterica* serovar Typhimurium could be detected from stool culture and water samples. The study indicated that there was evidence of intrafamilial transmission in the family of the index case. Stored water and pond water are also two potential vehicles which may be responsible for the spread of *Salmonella* infections in the endemic zone like Kolkata.

Increasing Spectrum in Antimicrobial Resistance of *Shigella* Isolates in Bangladesh: Resistance to Azithromycin and Ceftriaxone and Decreased Susceptibility to Ciprofloxacin

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Background: Antimicrobial therapy has been recommended for patients with shigellosis because it can limit the clinical course of illness and reduce the risk of complications and duration of faecal excretion of organism, reducing spread of infection. A major problem, however, is the increasing resistance of *Shigella* spp. to useful antimicrobials. **Objective:** Investigate the current trends in antimicrobial resistance of *Shigella* isolates and compare these with previous data to suggest timely recommendations for empirical antimicrobial therapy. **Methodology:** Antimicrobial resistance of *Shigella* isolates in Bangladesh during 2001-2002 was studied and was compared with those isolated in 1991-1992. **Results:** By studying *Shigella* isolates in Bangladesh during 2001-2002 and comparing them with those isolated in 1991-1992, significant increase in resistance to trimethoprim–sulphamethoxazole (TMP-SMZ, from 52% to 72%, $p<0.01$) and nalidixic acid (19% to 51%, $p<0.01$), high resistance to tetracycline (79%), ampicillin (56%), and chloramphenicol (42%), and emergence of resistance to azithromycin (complete resistance 16%, intermediate 62%), mecillinam (complete resistance 3%, intermediate 3%), and ceftriaxone/cefixime (2%) were identified. Overall, 63% of 266 isolates (2001-2002) were resistant to $\geq$3 (multidrug-resistant, MDR) of the following antimicrobials: ampicillin, TMP-SMZ, tetracycline, nalidixic acid, mecillinam, ciprofloxacin, azithromycin, and ceftriaxone, which was 52% of 369 strains ($p<0.01$) in 1991-1992. Seventy-one percent of 154 isolates (2001-2002), tested by E-test, had decreased ciprofloxacin–susceptibility (MIC $\geq$0.064-0.38 µg/mL), which were detected as ciprofloxacin-susceptible and nalidixic acid-resistant by the disk method and associated with MDR traits ($p<0.01$). High modal MICs were observed to azithromycin (MIC 6 µg/mL) and nalidixic acid (MIC 128 µg/mL) and low to ceftriaxone (MIC 0.023 µg/mL). Ceftriaxone resistance was due to extended spectrum ß-lactamase production, encoded by a 50-MDa R-plasmid, transferable to *E. coli* K12 and wild *S. sonnei* by conjugation. **Conclusion:** The findings are worrying and mandate monitoring of resistance and new guidelines for antimicrobial therapy of shigellosis. When indicated, mecillinam or ciprofloxacin might be considered for treating shigellosis. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Epidemiology and Surveillance of Multidrug-resistant Tuberculosis in Urban and Rural Areas of Bangladesh

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Background: Bangladesh is ranked as the fourth highest tuberculosis disease burden country among 210 countries in the world in 2003. However, there is a scarcity of epidemiological data on the incidence, prevalence, age distribution, seasonality, transmission, and microbial resistance patterns. **Objective:** Study the epidemiology of tuberculosis and monitor drug-resistance patterns of *M. tuberculosis* to commonly-used anti-tuberculosis drugs. **Methodology:** The study has been conducted in a rural area at Matlab and in urban Dhaka. A community-based tuberculosis surveillance system has been set up in the Matlab Health and Demographic Surveillance System (HDSS) area in a defined population of 107,000. Community health research workers detect suspected TB cases having cough for less than 3 weeks among persons aged 15 years and above during their monthly visit. A detailed history of illnesses and sociodemographic data are also collected. These cases are referred to the Matlab government hospital for diagnosis based on sputum test and clinical examination. Antimicrobial resistance has been monitored at the Shyamoli TB clinic in Dhaka. All sputum-positive samples from Matlab and 3 positive samples from Shyamoli were cultured daily and tested for antimicrobial resistance by standard technique. **Results:** In total, 57,726 (85%) persons aged ³15 were interviewed in Matlab. The prevalence of cough for more than 21 days was 7%, and it was significantly higher among males than among females (9.1% vs 5.6%). Among 3,826 persons from whom sputum samples were examined for acid-fast bacilli (AFB), 52 (1.4%) were smear-positive, more common among males (2.1%) than among females (0.6%). The overall population-based prevalence of smear-positive cases was 96/100,000, significantly higher among males (191/100,000) than among females (32/100,000). Of 641 *M. tuberculosis* strains tested, resistance to streptomycin, isoniazid, rifampicin, and ethambutal was observed in 45.1%, 14.2%, 6.5%, and 8.6% respectively. Multidrug resistance, defined as resistance to both isoniazid and rifampicin, was observed in 5.5%. **Conclusion:** The high burden of tuberculosis among rural population warrants appropriate measures to control tuberculosis in Bangladesh. The higher prevalence of persistent cough and AFB-positive sputum among males and the emerging drug resistance need further exploration. **Acknowledgements:** The study was funded by the United States Agency for International Development (USAID).

10:15 am-12:00 noon (Venue: Meeting Room 3)
Special Session 6: Probiotics

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Food Sanitation Today in Japan

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Background: In Japan, food sanitation has historically been controlled on different levels. During the last century, food sanitation has improved dramatically by introducing many regulations on different levels of authority and reached the present situation. Food sanitation has been dramatically improved after the World War II. This was conducted by improving education, implementation of many regulations and general environment conditions in food industries, transportation and marketing systems, and in the individual families as well. **Objective:** Overview the past and present food sanitation systems with pictures representing them on different levels in Japan. **Methodology:** Important phrases have been drawn from the previous and present food sanitation laws. They have been taken from the Internet sources of the government and private sectors and compared between the previous and present. Pictures of food presentation at shops and restaurants and of eating habits on different levels were taken with a digital camera, Model no. C 2020 Zoom, and processed by a computer. **Results:** Food sanitation was controlled by local police until 1877 for the safety of drinks, diary products, and meats. In 1900, the first prototype of food sanitation law was introduced, and in effect until in 1947 when a new and modern law 'Food Sanitation Law' was introduced. Since then, it was amended many times without much improvement, catching up with the changes in the society with rapidly continuous changes in eating habits and food industries that fed them. A big amendment was introduced recently, and another one will be implemented next year to meet the requirements for the safety of foods and their resources in future, effective 2006. **Conclusion:** In Japan, education of food sanitation at different levels, such as regulations, schooling and individual families, seems most important for reducing further the present level of food poisoning and infections of the gastrointestinal tract. **Acknowledgements:** This presentation was financially supported in part by the University of Tokushima (no. 0364) and Association of JICA Experts in Tokushima.

A Trend of Food Poisoning and Preventive Measures in Japan

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Background: Food poisoning in Japan has observed only a small decrease during the past 5 decades with about 40,000 cases per year in recent years. Recent changes in eating habits and modern food processing in Japan may fear an accidental big outbreak in future. Food poisoning in Japan has reached the lowest level and did not decrease in the number of cases during the past 5 decades. **Objective:** Analyze chronologically the factors responsible for food poisoning in view of historical eating habits and processing of foods, making suggestions for further prevention of food poisoning in Japan by comparing foods during the period, the way of cooking, processing and transportation, and others, and also give some preliminary data showing herbal plants with antibacterial activities for possible prevention of bacterial food poisoning. **Methodology:** Statistical data were collected from the Web sources of the Ministry of Health, Welfare and Labour of Japan. A practical and possible way of preventive measures was tested by the growth of bacterial colonies after imprinting the fingerprints treated in various ways. Water extracts of several herbal plants were tested for antibacterial activity by reading an inhibitory zone around the paper disc immersed with the extract and placed on to a thin film of bacterial suspension overlaying the enriched agar medium plates. **Results:** The main causative agents are microorganisms, such as *Salmonella, Vibrio, Clostridia*, and small round-structured viruses. Death is usually due to ingesting toxic poison of dogfish and certain mushrooms resulting in several cases every year. Big outbreaks usually occur with commercial foods due to some sabotage of the handlers involved in food processing. Prevention of food poisoning in Japan may be more effective by eating foods cooked in the old way, washing hands, and keeping daily basic rules during process of foods in industries than applying antibacterial agents to tables, cookeries, and facilities involved. **Conclusion:** Knowledge of risk factors for food poisoning at individual levels is most important. Prevention of food poisoning in Japan may be more effective by eating foods fresh or heated as in the old way. It may also be possible by washing hands regularly and keeping daily basic rules during process of foods in industries and using many antibacterial natural plants. **Acknowledgements:** The presentation was financially supported in part by the University of Tokushima and the Association of JICA Experts in Tokushima.

Sound in Human Life

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Background: From the early development of mankind, sound has occupied an important position in human life as the nutrition for mind. On the other hand, the sound has not always been regarded as important, because the sound has been natural phenomenon and considered not to have fatal effect on human life. But, with the development of the human society, the sound in human life becomes to attract keen attention as a medium necessary for healthy life. **Objective:** Give proper knowledge on the relationship between sound and human-being, with the expectation that the proper knowledge will assist pure development in human mental life. **Methodology:** Materials used in this paper are taken from the past achievements of the author and researchers in this field. The history of development in research is first overviewed, and the characteristics of sound are explained, as the knowledge on the characteristics of sound is necessary for future progress. Many illustrations are being presented to make the subject easy to understand. **Results:** The sound was the object of the philosophy before 17^{th} century. With the discovery of physical property of sound, the study on sound was transferred from the philosophers' hands to the natural scientists. The engineers began to work on sound with the inventions of the telephone and the gramophone in 19^{th} century. After these inventions, the psychological and biological studies on human auditory sense have been progressed to improve the quality of sound. It is shown that audible sound carries little energy. Why the sound can give a big effect on the human being is discussed. The reason is that the sound carries a lot of information necessary human life.

Speech and Hearing

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Background: The scientific study on speech began in the early 20th century. Despite its importance in human life, it does not have long history. The speech has been used by the human-being since the human-being appeared in the world and has been the most important medium for communication in the society. The development in communication engineering demanded the study on speech. The study on speech made clear that hearing was indispensable for utterance of speech. The speech processing in the brain is now a focus of research in information science. **Objective:** Give proper knowledge on speech and hearing which is necessary for realizing the healthy human life. **Methodology:** The materials used in this paper are taken from the past achievements of the author and researchers in this field. The mechanism of speech utterance and speech recognition carried out in brain is first made clear, and the role of hearing in speech recognition and in speech acquisition is described with the characteristics of speech and hearing. **Results:** The speech is the most important medium for human activity. The speech is uttered as the consequence of activity of brain. Words and linguistic rules stored in the brain memory are necessary to form the language to speak. The brain commands articulatory muscles to generate speech. The uttered speech is heard by ears and fed back to the brain. The feedback of speech is necessary not only for good pronunciation but also for acquisition of speech in infant ages. The brain works well in speaking language, and the brain can be trained and refreshed by reading aloud.

Probiotics against Pandemic Strains of *Vibrio parahaemolyticus*

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Background: Lactic acid bacteria are normal residents of the gastrointestinal tract. Commercial lactic acid bacteria in yogurt play an important role as probiotics which prevent the growth of harmful bacteria. **Objective:** Evaluate the probiotic properties of lactic acid bacteria against pandemic strains of *Vibrio parahaemolyticus*. **Methodology:** In total, 328 strains of lactic acid bacteria were isolated from 24 samples of Thai-fermented foods and were screened for probiotic properties based on their ability to survive in high acidity and bile salt. Their oxygen and vitamin B12 requirement and antibiotic susceptibility were also investigated. They were tested against 13 indicator pathogenic bacteria, and strains which exhibited strong probiotic properties were tested against pathogenic strains of *Vibrio*, especially the pandemic strains of *V. parahaemolyticus*. **Results:** All strains of lactic acid bacteria were screened, and 5 strains were selected. All of them were tested against pathogenic strains of *Vibrio,* and 1 strain designed as PSU-LA71 showed better inhibition against *V. cholerae* and *V. parahaemolyticus* compared to lactic acid bacteria in commercial yogurt. Using the agar spot technique, PSU-LA71 was investigated on 10 pandemic strains of *V. parahaemolyticus* O3:K6, and the inhibition zone was between 11.1 and 15.0 mm. Finally, the co-culture technique showed that the percentage of inhibition of PSU-LA71 on pandemic strains of *V. parahaemolyticus* were between 52.1 and 92.0. PSU-LA71 was isolated from fermented pork and was identified as *Lactobacillus plantarum*. The mechanism of inhibition on pandemic strains of *V. parahaemlolyticus* was discussed. **Conclusion:** Isolated *L. plantarum* showed antagonistic effect on pandemic strains of *V. parahaemolyticus* O3:K6.

Controlled Trial to Study Efficacy of Tyndalized *Lactobacillus acidophilus* in Acute Onset Childhood Diarrhoea

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Background: Most western literature proving beneficial effect of *Lactobacillus* sp. in acute diarrhoea have been either done in live strains of species, such as *Lactobacillus* GG or in combinations. *Lactobacillus* GG and live strains of any *Lactobacillus* sp. are not available in India. But *Lactobacillus* is widely used in India increasing the cost of treatment of acute diarrhoea. **Objective:** Evaluate the efficacy of tyndalized *Lactobacillus acidophilus* in acute diarrhoea. **Methodology:** This double-blind, randomized controlled trial was conducted in children aged 6 months-12 years reporting to DTTU with acute diarrhoea. *L. acidophilus*/placebo was given to children for 3 days, along with WHO-ORS and feeding. Intake-output records were done 4 hourly. **Results:** In total, 98 children—48 in *Lactobacillus* group and 50 in placebo group—were included in the study. On comparison, ORS consumed, frequency of stools, time taken for appearance of first semi-formed stool, time taken for first rehydration, and duration of hospital stay were all showing decreasing trends in the *Lactobacillus* group but the difference was not significant. Weight gain was more in the *Lactobacillus* group, but the total intravenous fluid required was also more in the same group with the differences between the 2 study groups being statistically insignificant. There were 4 treatment failures in the *Lactobacillus* group, but none in the placebo group. Of 13 rotavirus-positive cases, 5 were in the *Lactobacillus* group and 8 in the placebo group. Of the rotavirus-positive cases, the decreasing trends in all the outcome variables in the *Lactobacillus* group were further enhanced, but without reaching the statistically significant difference from the placebo group. **Conclusion:** There is no significant benefit of tyndalized *L. acidophilus* in the onset of acute childhood diarrhoea. Studies with larger samples of rotavirus-positive cases need to be undertaken to further evaluate the benefits.

Probiotics in Gastrointestinal Disorders: Future Prospect

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Background: Probiotics are non-pathogenic bacterial and fungal organisms that benefit the host by improving the microbial balance of the gut. The beneficial roles of those organisms in the intestinal lumen were described in the last century, and possible mechanisms include modification of toxins or toxin receptors leading to the prevention of adhesion and overgrowth of pathogenic bacteria, immunomodulation, immune tolerance against dietary antigens, stimulation of intestinal enzymatic activities, and increasing synthesis and secretion of mucins. **Objective:** Review the existing publications and present summaries of findings of studies conducted in different gastrointestinal disorders and address future prospects. **Methodology:** Identification of publications was performed by a computerized literature search in Medline database from 1960 to December 2002 using the index terms probiotics, *Lactobacillus*, *Saccharomyces, Bifidobacterium*, and diarrhoea. **Results:** Results of preliminary studies done in the past decade suggest a promising role of probiotics in the management of lactose intolerance, antibiotic-associated diarrhoea, traveller's diarrhoea, irritable bowel syndrome, intestinal allergy, and necrotizing enterocolitis, and also in other gut conditions, such as inflammatory bowel disease. Lactobacilli are perhaps among the most attractive probiotic agents because of long-standing use in fermentation of foods and historical record of safety. This agent has been investigated with regard to its antidiarrhoeal properties since the 1960s. A number of recent controlled trials have shown that certain strains of *Lactobacillus*, such as *L. casei* strain GG, *L. reuteri*, and *L. acidophilus* have therapeutic and prophylactic benefits in viral gastroenteritis. In Bangladesh, a recently-concluded study has also demonstrated a beneficial effect of a new probiotic agent *L. paracasei* in children with acute watery diarrhoea caused by organisms other than *Vibrio cholerae* and rotavirus. In addition to *Lactobacillus*, several other organisms, including a few species of *Saccharomyces*, a yeast, and bacteria, such as *Bifididobacterium bifidum* and *Streptococcus thermophilus,* have shown therapeutic and prophylactic potentials in the management of acute viral gastroenteritis. **Conclusion:** The benefits documented thus far are of varying degrees and are most likely dependent on the specific type of probiotic agent, the dose, the dosing pattern, and the characteristics of the host and its underlying luminal microbial environment. In this regard, specific agents with proven efficacy in developed countries need to be tested in tropical developing countries because of different microbial ecosystems and immunocompetence in such population. The safety and specificity of a particular probiotic agent and method of delivery to such population for a particular purpose should also be carefully documented before making broad recommendations. Research is also probably essential for the positioning of probiotic preparations either as food, a food supplement, or as a pharmaceutical preparation.

2:00 pm-2:45 pm (Venue: Meeting Room 1)
Plenary 9: Nutrition

150 (371)

How Training Programme Can be Delivered to Make a Difference: Example of WHO Treatment of Severe Malnutrition

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Malnutrition contributes to an estimated 60% of deaths in children aged less than 5 years. Typically, the median case-fatality rate for severely-malnourished (<-3Z weight-for length/height and/or oedema) children ranges from 30% to 50%, and this has not changed over the past 5 decades. However, it is possible to reduce mortality rates substantially by modifying treatment to take account of the physiological and metabolic changes that occur in these children. The WHO case management guidelines for severe malnutrition with appropriate training aim to improve the quality of hospital care and reduce mortality. The case-fatality rates have decreased in centres where the WHO guidelines were implemented and/or training was provided with relatively low levels of investment. For example, the case-fatality rate dropped from 17% in 1999 to 9% in 2001 in Myanmar, and in Africa it fell to 21% from a pre-training 46%. But in one hospital, after an initial drop to 19%, mortality increased to 38% when new staff was appointed, and in Nepal mortality fell to a modest 15% from an earlier 17%. Staff turnover was identified as one of the reasons for unsustained achievement and increased mortality as the untrained staff did not acquire the special skills needed to manage severely-malnourished children. Quality of care improved with implementation of the WHO guidelines, and the mortality rates fell. The main constraint to be addressed is: inadequate medical teaching and weaknesses in the healthcare system. The WHO guidelines for in-patient treatment of severe malnutrition should be part of the medical and nursing curricula in developing countries where malnutrition is a major public-health problem.

151 (029)

Study on *Cyclospora* Associated with Diarrhoeal Diseases in Nepal

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Background: Diarrhoeal diseases are the most common illness of children and endemic in developing countries. Enteric parasites, particularly diarrhoeagenic protozoa, are common among poor population. **Objective:** Find out the prevalence of *Cyclospora* associated with diarrhoea in Nepal. **Methodology:** The study was conducted during October 1999-August 2002 (Year 1: October 1999-September 2000, Year 2: October 2000-September 2001, and Year 3: October 2001-August 2002). In total, 1,397 diarrhoeal faecal samples collected from all age groups at different healthcare centres in Kathmandu city and its suburb (n=1,326) and public schools, both inside and outside of Kathmandu Valley (n=71), were studied. *Cyclospora* was detected by the direct microscopic method by ultraviolet and differential interference contrast microscopes in Japan. **Results:** The overall positive rate was 9.2% (128/1,397) with an equal positive rate in males (9.1%) and females (9.3%). A higher positive rate was observed in children aged 10 years and less than 10 years (11.1%) and lowest in the age-group of 51-60 years (3.1%), however, without any significant difference ($p>0.05$). The highest positive rate was found in Year 1 (17.9%), followed by a marked decrease in two successive years (Year 2: 8.8%; Year 3: 2.0%) ($p<0.01$). A significantly higher positive rate was observed in summer (rainy season) (12.6%), followed by autumn (6.8%) and winter (2.4%), with the lowest prevalence in spring (dry season) (1.8%) ($p<0.05$). Only males were positive in winter, whereas in summer, females showed a higher positive rate ($p<0.01$). Month-wise, the peak incidence was observed in July, followed by August and zero in December, January, and February. The positive rate was closely associated with rainfall in the first two years but not in Year 3. A significantly higher prevalence was observed in females in summer ($p<0.05$). **Conclusion:** The findings indicate that the parasite is endemic in Nepal and widely distributed irrespective of sex, location, and age-groups.

Analysis of Collagen-induced Pathogenic Gene Expression in *Entamoeba histolytica* by DNA Microarray

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Background: *Entamoeba histolytica*, the aetiological agent of invasive amoebiasis, is the fourth leading parasitic cause of mortality in the world. One of the major issues in *E. histolytica* is how does it destroy the human tissue during invasive amoebiasis. Since collagen is a major component of the extracellular matrix and basal lamina of the intestine where *E. histolytica* first comes in contact with host and performs all vital activities, including tissue destruction, it is thought that the studies on events taking place during *E. histolytica* and collagen interaction will throw some light on the molecular mechanism of pathogenesis in amoebiasis. **Objective:** Develop an *E. histolytica* genomic DNA microarray and identify global variation in gene expression during *E. histolytica* and human collagen interaction. **Methodology:** Approximately, 1 kb inserts from an *E. histolytica* random fragment genomic library were isolated to generate 'shotgun' microarrays. The arrays were probed with differentially-labelled cDNAs prepared from total RNA of collagen-activated and non-activated *E. histolytica* trophozoites. PCR products on the arrays that showed differential hybridization were sequenced. **Results:** Analyzing 9,600 random inserts of *E. histolytica* shotgun DNA microarray by labelled cDNA of collagen-activated *E. histolytica*, 14 differentially-expressed clones that showed reproducible upregulation were identified and selected for sequencing. Sequence homology showed resemblances with components of a signaling cascade previously hypothesized to transmit responses to cell attachment, adapter proteins for vesicle formation, and proteins implicated in cytoskeletal reorganization and locomotion. Two known virulence factor genes—cysteine proteinases and amoebapore—were also upregulated in response to collagen stimulation. **Conclusion:** The cluster of genes identified by microarray analysis provides an insight into the collagen-induced signaling in *E. histolytica* and supports the hypothesis that a genetic programme is stimulated by interaction with extracellular matrix, resulting in the release of virulence factors that play a role in the invasiveness of the host. **Acknowledgements:** The study was supported by a grant from the Sandler Center for Basic Research in Parasitic Diseases and National Institutes of Health (NIH) (grant no. U19-AI056517-01). A. Debnath was the recipient of UNESCO-ASM Travel Award (2002). The financial support of the Indian Council of Medical Research is also acknowledged.

Cryptosporidiosis in Bangladeshi Children with Diarrhoea: Molecular Epidemiology and Immune Response

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Background: *Cryptosporidium* spp. cause persistent diarrhoea and growth impairment in children. Molecular epidemiology of, and host antibody responses to, this infection have not been reported from Bangladesh. *Cryptosporidium parvum* (Cp) is the most common species that infects humans, and two major genotypes are identified. Most human infections are caused by genotype-I isolates. **Objective:** Investigate molecular epidemiology of, and immune response to, *C. parvum* and correlate genetic and immunological information with epidemiological and clinical features. **Methodology:** Forty-six of 1,672 children, aged 15 days-60 months, attending the Dhaka Hospital of ICDDR,B, with diarrhoea and Cp identified in stool specimens either by direct modified acid-fast stain or ELISA and confirmed by microscopy were enrolled with appropriate age-matched controls and followed up 21 days later. Genotypic diversity was analyzed by PCR–RFLP of SSU-rRNA locus for species determination and TRAP-C1 and COWP-loci for genotyping. Serum levels of Cp-specific antibodies were determined on enrollment and at follow-up by ELISA using an oocyst lysate as capture antigen. **Results:** Most (96%) infections occurred in children aged less than 24 months. Watery diarrhoea and vomiting were the common clinical presentations. Cp-infected cases had a significantly longer duration of diarrhoea and were more likely to develop persistent diarrhoea. DNA from 45 stool samples could be PCR-amplified. All 45 were Cp 89% genotype-I. There was no significant difference in serum antibody levels between the Cp-infected and the uninfected children on enrollment. There was a significant increase in serum Cp-specific antibody levels over a 3-week period in children with acute diarrhoea, but not in those with persistent diarrhoea. **Conclusion:** No correlation was observed between the genotypes and the clinical and epidemiological characteristics. Studies with a larger sample size might be useful in understanding the molecular epidemiology of cryptosporidiosis. **Acknowledgements:** The study was funded by Opportunity Pool Funds of the International Collaborations in Infectious Disease Research Program of the U.S. National Institute of Allergy and Infectious Diseases.

2:00 pm-3:00 pm (Venue: Meeting Room 2)
Scientific Session 19: Genetics 2

154 (096)

Virulence Genes of *Vibrio cholerae* Thailand Isolates

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Background: Cholera, a severe diarrhoeal disease of man, is caused by a gram-negative bacterium named *Vibrio cholerae*. The disease is endemic in southern Asian countries, including Thailand, parts of Africa, and Latin America. Previous molecular analysis of epidemic isolates of *V. cholerae* revealed clonal diversity among strains during different epidemics. It was proposed that there had been a continual emergence of new clones of toxigenic *V. cholerae* which replaced the existing clones, possibly through natural selection involving unidentified environmental factors and immunity of the host population. **Objective:** Determine the presence of virulence genes of *V. cholerae* O1 strains. **Methodology:** *V. cholerae* were isolated from patients with severe diarrhoea from various endemic areas in Thailand during 2001-2002 and were compared with those isolated during 1999-2000 using specific primers in polymerase chain reaction (PCR) or multiplex PCR. These genes included *ctxAB, zot, ace, toxR, hlyA*, and *tcpA*. Eighty-five *V. cholerae* serogroup O1 strains were isolated during March 1999-April 2000 and December 2001-February 2002. Seventeen standard *V. cholerae* strains, i.e. a Thai O1 strain isolated in 1990 and a Thai O139 strain isolated in 1993 and fifteen O1 and O139 strains were also included. Additionally, the genetic heterogeneity between the two groups of *V. cholerae* O1 isolates was studied and compared by pulse-field gel electrophoresis (PFGE). **Results:** The study revealed that most organisms possessed the virulent determinants. Additionally, the differences in the PFGE banding patterns could be used for the epidemiological tracing of the organisms. It was interesting to note that certain provinces had a limited number of pattern variation, whereas others had a wider pattern variation. **Conclusion:** The numbers of variation patterns found in different provinces may reflect the transmission of different strains/clones of the organism at certain period of time. **Acknowledgements:** This work was financially supported by the Japan Health Science Foundation and the Thailand Research Fund.

Role of Fibrillarin and snRNA in Post-transcriptional Modification of rRNA in *Giardia lamblia*

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Background: *Giardia lamblia*, a binucleate-flagellated intestinal protozoan parasite of humans and other mammals, has been demonstrated as one of the most primitive extinct eukaryotic organisms. In eukaryotes, rRNA processing has shown involvement of small ribonucleoprotein particles (RNPP) containing fibrillarin protein and small nucleolar RNAs (snoRNAs). Although *G. lamblia* contains no nucleolus, fibrillarin and small nuclear RNAs (snRNAs) have been identified in the parasite. However, no data are available on the conformational studies of snRNA fibrillarin interaction. Studies on these aspects will be useful in future for rational drug designing in giardiasis. **Objective:** Localize fibrillarin within *Giardia* and its role in the formation of RNPP. **Methodology:** Three snRNAs of *G. lamblia,* namely J, H, and D and fibrillarin were cloned by RT-PCR. Recombinant fibrillarin was over-expressed, purified by Ni chelating column, and antibody was raised in rabbit. Northwestern blotting, gel shift assay, fluorescence resonance energy transfer (FRET), and flow cytometric energy transfer (FCET) by molecular beacon were performed to show RNA protein interactions in vitro and in vivo. N terminal glycine arginine rich (GAR)-conserved domain-truncated fibrillarin was cloned, over-expressed, and purified. Gel mobility shift assay was performed to show the role of this GAR domain in snRNA binding and RNPP formation. **Results:** Purified fibrillarin has clearly shown its role in the formation of RNPP complex with all the 3 snRNAs by gel shift, northwestern blot, FRET, and FCET. N terminal GAR domain has been identified in *Giardia* fibrillarin which showed its active participation in snRNAs fibrillarin complex formation. **Conclusion:** In *G. lamblia,* RNPP complex formation involves fibrillarin and snRNAs. N terminal GAR domain of fibrillarin is responsible for binding with snRNAs. FRET and FCET techniques, using antisense molecular beacon, have been used for the first time and found to be useful for determination of in vivo RNA-protein complex formation. **Acknowledgements:** The financial support of the Indian Council of Medical Research is acknowledged.

Prevalence and Virulence Gene Profile of Shiga Toxin-producing *Escherichia coli* from Cattle, Sheep, and Goats in India

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Background: Domestic animals, especially cattle and sheep, are recognized as main reservoirs of Shiga toxin-producing *Escherichia coli* (STEC). These organisms cause certain life-threatening infections (bloody diarrhoea, haemolytic-uraemic syndrome (HUS), thrombocytic purpura, etc.) in children and adults. However, detailed information on the prevalence of STEC in animals from India, vis-à-vis as source of infection to humans, is lacking. **Objective:** Study the prevalence and virulence gene profile of STEC in various animal species in Kashmir valley. **Methodology:** 291, 154, 229, 59, and 413 strains of *E. coli* were isolated from cattle, sheep, goats, rabbits, and poultry respectively. DNA from *E. coli* strains was extracted from their overnight cultures by boiling. Bacterial lysate was centrifuged and the supernatant used as template for polymerase chain reaction (PCR). Detection of virulence genes *stx1, stx2* (coding for Shiga toxins)*, eaeA* (coding for intimin), and *hlyA* (coding for enterohaemolysin) of STEC strains was carried out by multiplex-PCR using specific primers. Some samples were also screened for presence of *Saa* (STEC autoagglutinating adhesin) gene. *E. coli* isolates were serogrouped on the basis of their O antigen from National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, H.P., India. **Results:** STEC strains belonging to different serogroups (O2, O4, O6, O8, O22, O75, O76, O81, O115, O131, O132, O157, untypable) were isolated from cattle, sheep, and goats with and without diarrhoea. Faecal samples from rabbits and avian species (poultry, free-flying pigeons) did not possess STEC strains. However, a few *E. coli* strains isolated from poultry and rabbits carried *eaeA* and *hlyA* genes. 23.6%, 17.5%, and 13.9% of *E. coli* strains from ovine, caprine, and bovine species respectively were Shiga toxigenic. s*tx1*, *stx2*, *eaeA,* and *hlyA* genes alone or in combination were carried by STEC strains isolated from bovine, ovine, and caprine species. None of the *E. coli* strains studied carried *saa* gene. **Conclusion:** High percentages of STEC belonging to different serogroups are prevalent in cattle, sheep, and goats in Kashmir valley and could be source of infection to humans, including children. Some STEC belonged to serogroups known for certain life-threatening diseases in humans. These findings strongly suggest conductance of such studies in humans to precisely work out significance of these strains in diarrhoeal diseases in this part of globe. Faecal samples from poultry and rabbits did not carry STEC strains and may not be regarded as vehicles for spread of STEC in Kashmir valley. **Acknowledgements:** The authors are indebted to the Director, National Salmonella and Escherichia Centre, Kasauli, India, for serogrouping of *E. coli* isolates.

Shigella sonnei Undergo High-frequency, Adaptive Mutations to Stable Expression of Lactose Utilization: A Potential Mechanism to Escape Diagnostic Detection

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Background: *Shigella* spp. cause endemic disease worldwide and are responsible for approximately 650,000 deaths annually. Recent CDC estimates indicate that >440,000 cases of shigellosis occur annually in the USA, and >80% of these are due to *Shigella sonnei*. *S. sonnei* are characterized as slow lactose-fermenters, but the current studies have revealed that this species mutates to complete lactose-positivity under adaptive conditions. **Objective:** Examine how *S. sonnei* of higher primates remain undetected, particularly in countries with high sanitary standards. **Methodology:** The lactose-utilization phenotype of *S. sonnei* strains was studied on lactose minimal, LB, and MacConkey media under various growth conditions. Phenotypic lactose-utilizing mutants were isolated and studied for genetic stability, and the PCR-amplified *lac* gene region of wild-type Lac^- and several independent Lac^+ isolates were sequenced to determine the location and type of responsible mutation. **Results:** When plated onto MacConkey lactose agar, resulting *S. sonnei* colonies present as pink (i.e. slight Lac+) during the first 12-24 hours and then turn white (i.e. Lac-) after 36 hours. Continued incubation at 25-37 °C results in the formation of Lac+ papillae appearing in all colonies (i.e. high-frequency mutations). When restreaked onto MacConkey lactose, these Lac+ papillae remain strongly Lac+. This mutation occurs only in the presence of lactose and only during growth in stationary phase (i.e. an adaptive mutation). These Lac+ mutants do not occur during log phase growth even in the presence of lactose. DNA sequence analyses of 4 independent Lac+ mutants have revealed that all mutations occur in the *laci* repressor gene, but involve at least 2 different molecular mechanisms: slip-strand repair mutagenesis and IS element insertion. **Conclusion:** The observation that *S. sonnei* undergo high-frequency adaptive mutations to Lac+, together with the isolation of mixed Lac+ and Lac- *S. sonnei* from infected humans, suggests that Lac+ *S. sonnei* occur in nature and likely escape standard clinical microbiological detection.

3:00 pm-5:00 pm (Venue: Meeting Room 1)
Scientific Session 20: Neonatal Health and Diarrhoeal Disease 3

Neonatal Health

158 (019)

Women-focused Development Intervention Reduces Neonatal Mortality in Rural Bangladesh: A Study of Pathways of Influence

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Background: There has been a substantial reduction in hazard of childhood deaths over the period. The reduction is statistically similar for children aged 1-4 year(s), irrespective of their mothers' participation in the development programme. The reduction is much greater among infants whose mothers participated in the development programme. However, how the development intervention does influence neonatal survival is not known. **Objective:** Study the impact of BRAC's Rural Development Programme on neonatal mortality in Matlab. **Methodology:** A case-control study, including 117 cases (died within 28 days) and 351 controls (live children and taken from the nearest door of cases) who born during 1999-2000, was the main method employed. Twelve case studies of both cases and controls were done to complement the quantitative data. The ICDDR,B surveillance database provided the sampling frame. The risk ratio was estimated to see how BRAC programme participation reduces the risk of mortality, the stratified analysis was done to see the effects of third variables over the association, and the log regression was done to see the net effect of variables on neonatal death. **Results:** Neonates of BRAC non-members were at 1.9 times increased risk (CI 1.09-3.25) of dying compared to neonates of BRAC members. This association works through two intermediate variables, including antenatal care and family planning. Age of mothers and occupation of fathers acted as confounders over this association. Mothers' physical violence and psychological stress, pre-maturity, and low birth-weight had respectively 2.2, 1.7, 13, and 2 times increased risk of neonatal death. However, BRAC membership did not have any influence over these factors. When simultaneously accounting for all variables in a multivariate log regression, a dose-response association was maintained for antenatal care, family planning, physical violence, and pre-maturity. **Conclusion:** There has been a substantial reduction in neonatal morality among mothers who participated in BRAC intervention. For an effective reduction in the number of these deaths, programme planners should think about ways to address all risk factors together rather than only antenatal care and family planning. **Acknowledgements:** The financial support of the Ford Foundation and the Aga Khan Foundation is acknowledged.

Perinatal Death Audit: A Useful Strategy to Improve Patient Management

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Background: Perinatal and maternal mortality rates are important indicators of health in a community and standards of healthcare provided. Perinatal death audit is a well-established practice with evidence that suggests real benefit from the process. A workable perinatal death audit process is presented in the rural Bangladesh setting. With quality assurance being demanded by donors and consumers, audit provides a robust framework for identifying and addressing problems in perinatal care. **Objective:** Present the method and outcomes of running a local perinatal death audit. **Methodology:** LAMB Integrated Rural Health and Development Project, Dinajpur, is a referral centre performing many complicated obstetric deliveries. A monthly retrospective chart audit reviewed perinatal and maternal deaths from January 2000 to December 2002. Data were analyzed by the computerized Perinatal Problem Identification Programme. Overall findings and individual cases were discussed at monthly multidisciplinary meetings involving nurses, doctors, and chaplains in an anonymous and non-blame environment. At the end of 3 years, significant clinical management practices that had changed as a result were listed. **Results:** In this 3-year period, 6,211 babies were delivered. The hospital perinatal death rate was 85 per 1,000 births (twice as high for <2.5 kg babies at 169/1,000). Obstetric causes of perinatal death were antenatal/intrapartum hypoxia and hypertensive disorders. The main causes of hospital early neonatal deaths were hypoxia and infection. 76% of perinatal deaths were linked to patient-associated avoidable factors (not seeking antenatal care or delay in seeking care in labour). The hospital maternal mortality rate was 354 per 100,000, chiefly from hypertensive disorders and primary pulmonary hypertension. Management choices reviewed, improved guidelines developed, and training needs of community and staff being addressed were considered significant changes. **Conclusion:** Regular locally-run perinatal death audit is useful to identify the magnitude of risk of perinatal mortality. As a Christian project, LAMB seeks to promote the value of each individual's life and premature death as a tragedy to be averted. The maternal and perinatal death audit seeks areas where specific interventions could improve chances of a healthy productive life for mothers and children, as God intends.

Participatory Community Health Enquiry and Planning for Birth and Newborn Care in Urban Slums

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Background: The neonatal mortality rate for urban low-socioeconomic group in Madhya Pradesh is 69.7 (70% of infant mortality). The urban health programme targets sustained improvement in neonatal health through an NGO-CBO partnership approach. **Objective:** Undertake participatory health enquiry and planning to identify facilitators, barriers, and realizable options in use of neonatal health services for guiding the programme in Indore. **Methodology:** Women with children aged less than 2 years from 6 slums, covering 2,500 households, were engaged in participatory discussions over a 10-day workshop. Outcomes were represented visually by the community through maps and diagrams. NGO-CBO teams continued this process over the next 3 months in 35 slums. **Results:** All slums posed similar challenges of limited availability and access to basic health services. Pregnant women received TT immunization from public and private facilities. Timely TT immunization ranged from 57.7% to 82.6% in intervention areas. Service provision by public-sector workers was restricted to influential members, excluding 'others' in the lower socioeconomic strata. Preparedness for birth was limited. Not all contacted a trained birth attendant (60-63%) and made monetary and transport arrangements primarily due to proximity, accessibility, and faith on the private providers. Practice of five cleans, provision of warmth to the newborn, and timely initiation of breast-feeding were not common. However, traditional beliefs against these behaviours were not deep-rooted, but might be overcome through sustained discussions and counselling. **Conclusion:** The programme aims to enhance demand, service coverage, and adoption of optimal behaviours at households by strengthening linkage between the public sector and the private providers, identification of champions from the community to promote these behaviours, and capacity-building of community volunteers.

Effect of Smokeless Tobacco Consumption on Birth-weight among Pregnant Slum Dwellers in Dhaka City

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Background: The adverse effect of tobacco smoking on birth-weight has been extensively studied. However, this relationship has rarely been evaluated for smokeless tobacco consumption. **Objective:** Assess the effect of smokeless tobacco consumption on birth-weight. **Methodology:** In a community-based cohort study among pregnant women (>20 weeks gestation), 267 consumers of smokeless tobacco and 488 non-consumers were followed up to compare birth-weight of their newborns. The subjects were randomly selected from among 307 consumers of smokeless tobacco (chewers or users of tobacco preparations as dentifrice) and 1,107 non-consumers of tobacco, identified in a door-to-door survey, in 92 slums in Dhaka city. A team of 8 female interviewers and 32 traditional birth attendants followed-up the subjects at home during August-December 2002. Until the end of the study, 211 consumers and 473 non-consumers with pregnancy outcomes of 197 and 472 livebirths respectively were followed-up. For home-deliveries, the interviewers weighed the babies within 48 hours of birth using UNISCALE, whereas for facility-deliveries, birth-weights in discharge certificates were used. **Results:** Babies (n=194) born to tobacco consumers were, on average, 200 g lighter than those (n=469) of non-consumers (2.57±0.45 vs 2.77±0.47 kg, $p<0.05$). There was a significantly higher proportion of low birth-weight (<2.5 kg) among consumers than among non-consumers (39.18±3.50 vs 24.52±1.99%, $p<0.05$). After adjusting for possible confounding factors (maternal education, gravidity, place of delivery, and preterm birth), tobacco consumption during pregnancy was about twice as likely (OR=1.82, 95% CI=1.20-2.77) to result in low birth-weight than non-consumption. **Conclusion:** Smokeless tobacco consumption by mothers during pregnancy is associated with decreased birth-weight. There is a need to build up community awareness on its negative health consequences. **Acknowledgements:** During the study implementation period, the first author (M.E. Chowdhury) was affiliated with the Bangladesh Institute of Research for Promotion of Essential & Reproductive Health and Technologies. The financial support of the World Health Organization and field support of Radda MCH-FP Centre are acknowledged.

Perinatal Care: A Special Area of Concern for Bangladesh

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Background: Perinatal mortality and morbidity is very high in Bangladesh due to suboptimal perinatal care. Perinatal health situation can reflect some aspect of this suboptimal care. **Objective:** Assess the perinatal health situation in Bangladesh and focus on the needs of actions based on the baseline information. **Methodology:** Review literature on perinatal healthcare and personal communication made with activists in this field. **Results:** The main causes of maternal mortality are: haemorrhage, abortion, toxaemia of pregnancy, and obstructed labour. The main causes of neonatal death are perinatal asphyxia, neonatal infection, preterm/low birth-weight, congenital anomalies, and birth injuries. Perinatal care includes: care before birth, care at birth, immediate newborn care, and newborn care within first 7 days. Scenario of care before birth reveals: poor prenatal check-up (only 18%), poor prenatal counselling by health workers in regards to delivery preparation (59%), breast-feeding (74%), danger signs (53%), poor delivery preparation only (10%), and delivery conducted by skilled birth attendants. Scenario of care at birth reveals: 93% are home deliveries; there is inadequate newborn counselling by health workers; 52% of newborns are not dried soon after birth; and 15% of newborns are not wrapped soon after birth. Scenario of postnatal care reveals: 38% of newborns are bathed within one hour after birth, and skin-to-skin contact is not practised. **Conclusion:** To reduce perinatal morbidity and mortality, it is necessary to establish neonatal healthcare service based on low-cost appropriate technology in all healthcare facilities and continue research on perinatal health problems in institutions and in the community. Survey search and intervention research should be conducted. Information collected through research should be disseminated to professionals, policy-makers, and implementing agency.

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Evaluation of Rapid Diagnostic Assays for *Vibrio cholerae* O1: Does Sensitivity Vary by Skill Level?

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Background: Cholera outbreaks often occur in areas with limited laboratory facilities and financial resources. Timely confirmation of outbreaks could be achieved by low-skilled personnel using sensitive diagnostic tests. **Objective:** Evaluate diagnostic assays for *Vibrio cholerae* O1 and report preliminary findings. **Methodology:** Every 50th symptomatic patient at a diarrhoea treatment centre in Dhaka, Bangladesh, were enrolled. The SMART™, Medicos™ Cholera Dip Stick, and an immunochromatographic dipstick from the Institut Pasteur (IP) were performed on stool by high- and low-skilled staff and compared to stool culture. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative (NPV) predictive value were calculated. **Results**: Of 70 patients enrolled so far, 47% were females. The median age was 8 years (range 4 months-80 years). Most patients reported watery diarrhoea (99%) and vomiting (94%). The median number of stools per day was 15.5 (range 4-40). *V. cholerae* O1 was isolated from 28 (40%) stools. The Se, Sp, PPV, and NPV for clinical diagnosis of cholera was 89%, 62%, 61%, and 90% respectively. The performance measures of SMART™ were 31%, 94%, 71%, and 73% for low-skilled staff and 75%, 91%, 94%, and 82% for high-skilled staff. The performance measures of Medicos™ dipstick were 82%, 69%, 64%, and 85% for low-skilled staff and 93%, 75%, 72%, and 94% for high-skilled staff. The performance measures of IP dipstick were 93%, 67%, 65%, and 93% for low-skilled staff and 93%, 64%, 63%, and 93% for high-skilled staff. None of the culture-negative, assay-positive specimens had the genes that encode the O1 antigen or cholera toxin by multiplex PCR. **Conclusion:** This evaluation demonstrates that the IP dipstick had the highest sensitivity, irrespective of skill level. A sensitive and affordable diagnostic tool could aid in rapid assessment of suspected cholera outbreaks.

Phenotypic and Genotypic Characterization of *Vibrio cholerae* Non-O1 Non-O139 from Clinical and Environmental Sources in Bangladesh

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Background: The non-O1 non-O139 serogroups of *Vibrio cholerae* are ubiquitous members of the heterotrophic microflora of aquatic environs and are recognized as a cause of sporadic diarrhoea and localized outbreaks of diarrhoeal illness, but the potential of disease-producing factors and the difference between environmental and clinical strains are not yet clearly known. **Objective:** Understand the mechanism by which *V. cholerae* non-O1 non-O139 cause acute secretory diarrhoea in Bangladesh and understand the association of any new subset of *V. cholerae* with disease. **Methodology:** In total, 205 *V. cholerae* non-O1 non-O139 strains isolated from clinical (n=145) and environmental (n=60) sources during 2000-2001 were selected for phenotypic and genotypic characterization. One hundred twenty-four clinical and 31 environmental isolates were serogrouped according to the international serotyping scheme. All these strains were included for adherence assay in HeLa cells and invasion assay in Hep-2 cells. The strains were screened for *ompW, ctxA, toxR and tcpA* genes by PCR. **Results:** Of the clinical strains, serogroups O49 (n=21), O5 (n=14), O176 (n=11), O24 (n=6), and O145 (n=5) were predominant. Of the environmental strains, serogroups O179 (n=8), O4 (n=7), O121 (n=5), and O70 (n=5) were predominant. None of the environmental serogroups were isolated from clinical samples. Most clinical strains showed either localized or diffused or aggregating type of adherence but only a few environmental strains produced aggregative type of adherence in HeLa cell. Both clinical and environmental strains ranged from 0.01% to 0.5% invasive for Hep-2 cells (calculated as percent of inoculum which survived gentamicin treatment) which can be explained as new type of strong adherence that can contribute to the production of diarrhoea in humans. Most clinical strains and two-thirds of the environmental strains were positive for *toxR*. None of the strains were positive for *tcpA* or *ctx,* except for one clinical strain. Results derived from *ompW* gene corroborated the results of conventional biochemical identification of these strains. **Conclusion:** Clinical strains and an environmental strain do not belong to identical O-serogroups and vary in their virulence and adherence properties. **Acknowledgements:** The financial support of Bill and Melinda Gates–Government of Bangladesh fund of ICDDR,B is acknowledged.

Association of Cytolethal Distending Toxin Locus *cdtB* with Enteropathogenic *Escherichia coli* Isolated from Acute Diarrhoea Patients in Kolkata, India

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Background: Cytolethal distending toxin (*cdt*) constitutes a novel class of genotoxin that induces characteristic cellular distention and death. *Cdt* is considered an important factor in intestinal pathogenesis. Although the expression of *cdt* is well-studied, epidemiology and molecular characterizations of *Escherichia coli*-harbouring *cdt* are few. **Objective:** Investigate the incidence and molecular characteristics of *E. coli*-harbouring *cdt* isolated from hospitalized patients with acute diarrhoea. **Methodology:** In total, 284 stool specimens collected from hospitalized patients with acute diarrhoea were screened by *cdt*-PCR using stool enrichment cultures. From PCR-positive cultures, 30-300 colonies were screened by *cdt* probe hybridization assay. The probe-positive isolates were identified by conventional tests. In addition, 138 enteropathogenic *E. coli* (EPEC) were included in the hybridization assay. *Cdt* expression was detected on HeLa cells line. The *cdtB* DNA sequences of representative strains were compared with published sequences. To check the clonality, pulsed-filed gel electrophoresis (PFGE) was performed after digestion of DNA with *Xba*I. **Results:** The incidence of *cdtB*-positive *E. coli* among diarrhoea cases was 1.4%, and 89% of the cases were infants. The *cdtB*-harbouring *E. coli* strains were identified as EPEC, and 67% of the isolates belonged to the classical serogroup. High titre *cdt* effect was observed with strains isolated from bloody diarrhoea cases. Sequence analysis of the *cdtB* from 3 representative strains showed close homology with other EPEC strains (U03293 and AF373206). In PFGE, most strains exhibited distinct pattern, and in dendrogram analysis, all the *cdt*-positive strains formed unique cluster. **Conclusion:** Although the incidence level of *E. coli*-harbouring *cdtB* was low, the most affected age group was infants. Most strains expressed *cdt* in the tissue culture assay and belonged to distinct clones. **Acknowledgements:** The United States-Japan Cooperative Medical Science Program and the Japan International Cooperation Agency supported the work.

3:00 pm-5:00 pm (Venue: Meeting Room 3)
Special Session 7: Vaccine (IVI)

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Research Needs for Introducing Vi Vaccine into Public-health Programmes

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Background: Typhoid fever is a major cause of morbidity with an estimated global prevalence of 16-33 million cases per year. In the absence of an affordable programme to assure safe water and better sanitation conditions in less-developed countries, efforts need to be directed towards primary prevention through vaccines. **Objective:** Describe key elements of the DOMI Typhoid Programme and some of the preliminary results. **Methodology:** The DOMI Typhoid Programme is designed to provide the evidence base to accelerate the introduction of Vi vaccine in a rational way and to assist in technology transfer and licensure of Vi to qualified local producers. The Programme includes coordinated activities across the spectrum of vaccine development, introduction, and evaluation. These include epidemiological studies, disability burden assessment, cost-effectiveness analyses, technical assistance in vaccine production and regulation, policy and economic studies of vaccine introduction, and behavioural studies of vaccine acceptability. **Results:** The heat-phenol-inactivated, parenteral whole-cell typhoid vaccine, which provides approximately 65% protection, has limited usefulness because of the adverse reactions it evokes. In contrast, the live, attenuated oral vaccine, Ty21a, and the injectable subunit vaccine, Vi polysaccharide, have been shown to be safe. Unlike Ty21a, the protective efficacy of Vi has been consistent in all field trials. Vi vaccine is a one-dose, heat-stable vaccine that is not patent-protected and can be produced by both international and local vaccine manufacturers. For these reasons, Vi vaccine is the licensed, modern typhoid vaccine that has been targeted by the Diseases of the Most Impoverished (DOMI) programme for accelerated introduction into public-health programmes in developing countries. **Conclusion:** The study focuses on the crucial steps required to generate the evidence needed for policy-makers to make rationale vaccine introduction decisions. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

Why Is It Important for Development of a Vaccine Against Shigellosis that We Have Accurate Data on Disease Burden and Distribution of Species and Serotypes?

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Background: Shigellosis causes considerable morbidity and mortality worldwide. Outside of China no vaccine to protect against shigellosis is licensed. To accelerate the development of a safe and effective vaccine against shigellosis, accurate data on disease burden and distribution of species and serotypes are needed. **Objective:** Measure age-specific incidence of shigellosis and distribution of species and serotypes. **Methodology:** Standardized epidemiological and laboratory methods were used for measuring prospectively the shigellosis disease burden in 6 Asian countries. **Results:** 568,000 individuals in 6 Asian study sites have been under surveillance. So far, 62,867 episodes of diarrhoea have been recorded. Of them, 2,944 (5%) episodes of shigellosis have been detected. Very high incidence rates of shigellosis were reported in China (52/1,000 children/year) and Bangladesh (48/1,000 children/year). Considerable heterogeneity of *Shigella* species and serotypes was observed between sites. In 5 sites, *Shigella flexneri* was the most frequently-isolated *Shigella* species. In Thailand, the large majority (80%) of shigellosis cases were caused by *S. sonnei*. Significant differences in *S. flexneri* serotypes and subtype distribution between sites were detected. **Conclusion:** In the absence of cross-protection, vaccine to protect against shigellosis has to contain several *Shigella* species and serotypes. There may be a need for individual vaccines for the Asian region. **Acknowledgements:** This work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

Taking Oral Cholera Vaccine from a Traveller's Vaccine to a Public-health Tool for the Poor

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Background: It has been more than a decade since the pivotal studies on the oral killed whole-cell cholera vaccines were conducted at ICDDR,B. Despite its proven efficacy and its licensure in many countries, currently the vaccine is mainly used by western tourists. There is very limited use of the vaccine in countries where the disease is endemic or where there is a high-risk of outbreaks. In the late 1980s, the technology for vaccine production was transferred from Sweden to Vietnam. Initially, a monovalent (O1) vaccine with a slightly different composition than the Swedish vaccine was produced in Vietnam and was shown to be safe and protective in an open trial. In the mid-1990s, due to the emergence of *Vibrio cholerae* O139, a bivalent (O1 and O139) vaccine was developed and shown to be safe and immunogenic. **Objective:** Increase the use of cholera immunization as a potential public-health tool. **Methodology:** The International Vaccine Institute–Diseases of the Most Impoverished (DOMI) programme is or will be conducting several projects. These include: technology transfer of the Vietnamese oral killed whole-cell cholera vaccine to local producers in India and Indonesia; assisting in quality control of oral killed whole-cell cholera vaccine production by developing country manufacturers; collaborating on a large Phase III trial of the oral killed whole-cell bivalent vaccine in India; a demonstration project of the Swedish vaccine in an endemic non-refugee population in Mozambique; and Phase I/II trials of a new generation live vaccine in Bangladesh; and providing cost-effectiveness analysis for policy-makers and marketing data for producers. **Results:** The projects are still ongoing, and an update of activities is being presented. **Conclusion:** Through these efforts, it is hoped that cholera immunization can be brought to those who need it most. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

Bringing DOMI Vaccines to the Poor: What Is the Role of Local Producers

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Sustainable production of new generation, affordable ophan vaccines requires that qualified local producers enter the market. Local producers must acquire the technology to produce high-quality vaccines at a large scale. The DOMI programme has coordinated technology transfer and production of oral killed whole-cell cholera vaccine between VABIOTECH (Vietnam) and Shantha (India). Unfortunately, VABIOTECH is not yet pre-qualified by the World Health Organization and, therefore, the vaccine cannot be considered for evaluation or use outside of Vietnam. By transferring the technology to Shantha Biotechnics, a WHO pre-qualified producer, this hurdle will be overcome. To gain international acceptance, the DOMI programme will conduct a placebo-controlled field trial of the vaccine in Calcutta. Similarly, the National Institutes of Health, USA, transferred Vi PS technology to Lanzhou Institute in China, which is not yet pre-qualified by WHO. The DOMI programme is coordinating the transfer of the Vi PS technology from Lanzhou Institute to other pre-qualified producers, such as Shantha in India. Glycoconjugation of vaccines can overcome the limitations of existing typhoid and cholera vaccines and perhaps offer the opportunity to develop an effective *Shigella* vaccine. Production of conjugates at affordable prices by local producers is feasible. However, there is a dearth of institutions with the capacity to transfer this technology to local producers. The opening of the IVI's new laboratories facilities has created an opportunity for IVI to become the first international vaccine research centre focused in producing clinical lots of conjugate vaccines against enteric diseases for the developing world. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

DOMI's Sociobehavioural Research Programme

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Background: Shigellosis, typhoid fever, and cholera continue to contribute significantly to morbidity in developing countries, particularly among infants and children. While shigellosis vaccines are still under development, there are viable typhoid fever and cholera vaccine candidates, which could be implemented into public-health programmes in the near future. The International Vaccine Institute's DOMI (Disease of the Most Impoverished) programme is currently conducting shigellosis disease-burden studies, and typhoid fever and cholera vaccine-demonstration trials in Bangladesh, China, India, Indonesia, Pakistan, Thailand, and Vietnam. In conjunction with these studies, socio-behavioural research is being conducted with local leaders, healthcare providers, and residents. These studies are a joint effort between in-country social scientists and members of the DOMI Social Science Task Force. **Objective:** Understand local populations' perceptions, attitudes, knowledge, and experience regarding these diseases and vaccination. Data can be used for improving the design and implementation of vaccine effectiveness trials, increase participation in these trials, and provide information for policy-makers in relation to future campaigns. **Methodology:** The methods employed for these studies are both qualitative and quantitative and include semi-structured interviews, case studies, key informants, and household surveys. **Results:** Similarities and differences across sites are being explored in relation to how such factors as severity, vulnerability, disease causality, healthcare use, and previous vaccine experience influence respondents' acceptance of a particular vaccine. **Conclusion:** To maximize participation in vaccination trials and programmes, it is necessary to know local social and cultural specific factors, which influence individuals' decision-making about vaccination. **Acknowledgements:** The Diseases of the Most Impoverished (DOMI) programme at the International Vaccine Institute, South Korea, is funded by the Bill and Melinda Gates Foundation.

Economics and the DOMI Agenda: Beyond Cost-effectiveness Calculations

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The DOMI (Diseases of the Most Impoverished) programme aims to help rational decision-making about the introduction and use of vaccines against typhoid fever, cholera, and shigellosis in DOMI countries by providing evidence from economics and policy analyses since real life data on costs and demand from the field are almost totally absent for these diseases. Along with vaccination-cost study, the DOMI Economic Team attempts to measure private economic benefits of cholera, typhoid, and shigellosis vaccines, using economic methods, such as cost of illness (COI) study and willingness to pay (WTP) study. The COI study for typhoid fever vaccine in Delhi suggests: (1) the most logical age groups to be targeted are 2-5-year olds and 5-19-year olds based on economic considerations alone, and (2) there is a potential for private vaccine provision in this area. The WTP study, which provides a more comprehensive benefit measure, for typhoid fever and cholera from Hue, Vietnam, implies that: (1) currently-available vaccines are fairly acceptable for household decision-makers and (2) benefits of cholera and typhoid fever vaccines exceed costs for all age groups in Hue. Once all data from economics studies and other DOMI studies, such as disease-burden study and sociobehavioural study, are collected, they will be synthesized to provide cost-effectiveness and cost-benefit measures in a National Policy Dialogue Tool so that the policy-makers can make a reasonable decision on allocating resources to alternative vaccination programmes. In conclusion, the DOMI economics study provides more comprehensive evidence for vaccination programmes against these enteric diseases beyond cost-effectiveness calculations.

3:00 pm-5:00 pm (Venue: Meeting Room 3)
Scientific Session 21: Pathogenesis

172 (031)

Novel Inhibitors of Cholera Toxin-induced Intestinal Fluid Secretion

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Background: L-histidine has been shown to reduce cholera toxin (CT-)-induced fluid loss in mice, and when added to oral rehydration solutions, the amino acid improves the clinical course of adult cholera patients. The molecular basis of this protection was not understood. **Objective:** Determine the mechanism by which L-histidine reduces fluid loss in cholera by evaluating the protection afforded by a molecular adduct, PGE_2-L-histidine, formed in mixtures of PGE_2, and L-histidine. **Methodology:** PGE_2-L-histidine was purified by C18 reverse phase chromatography, and the structure was established by mass spectrometry and nuclear magnetic resonance analysis. The effect of the adduct on cAMP and intestinal fluid accumulation were determined using the mouse intestinal loop assay, and cAMP levels in cultured cells were determined by cAMP-specific ELISAs. The effect of the adduct on adenylyl cyclase was measured by *in vitro* enzyme assays. The capacity to inhibit chloride transport was determined with forskolin- and CT-stimulated HT-29 cell monolayers mounted in Ussing chambers. Possible modes of interaction of PGE_2-L-histidine with the active site of adenylyl cyclase were suggested, using structural analysis programmes. **Results:** The PGE_2-L-histidine adduct was a new molecular species formed by chemical reaction between C11 of PGE_2 and *tau* nitrogen of the imidazole ring of L-histidine. The adduct reduced CT-induced fluid loss and cAMP levels in murine intestinal segments and decreased cAMP levels in cultured cells (e.g. Chinese hamster ovary cells and HT-29 human colonic epithelial cells) exposed to CT. Further CT-induced ion transport in HT-29 cells was similarly reduced. The PGE_2-L-histidine adduct was shown to be a competitive inhibitor of adenylyl cyclase in a cell-free enzyme assay. Structural analysis of the enzyme's active site identified potential docking sites that could explain the inhibitory activity of the compound. **Conclusion:** PGE_2-L-histidine appeared to inhibit experimental cholera by inhibiting adenylyl cyclase and reducing cAMP levels in the intestinal cells, which blocked intestinal secretion that would have culminated in diarrhoea. **Acknowledgements:** The research was supported by research grant no. 1 R01 AI50086 and the James W. McLaughlin Fellowship Fund.

Diffusely Adhering *Escherichia coli* Strains Lacking Afa Gene Cause IL-8 Release from HEp-2 Cells

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Background: Diffusely adhering *Escherichia coli* (DAEC) have been considered as putative diarrhoeagenic *E. coli* (DEC). Although the pathogenic mechanisms of DAEC remain unknown, DAEC possessing Afa (originally identified as a colonization factor in the urinary tract) was recently reported to cause IL-8 secretion. **Objective:** Determine whether non-Afa DAEC was able to induce IL-8 release from epithelial cells. **Methodology:** Forty DAEC strains were isolated between 1997 and 1999, and 21 of them did not possess Afa-gene. Adherence of the 21 strains was examined using HEp-2 cells. IL-8 secretion from the cells was assayed by ELISA. Gentamicin protection assay (GPA) was used for quantifying the number of intracellular bacteria as an index of their invasiveness. **Results:** Although the non-Afa strains possessed an equal ability to adhere to HEp-2 cells, only half of them induced IL-8 secretion. A direct correlation between IL-8 induction and bacterial invasion was observed. GPA revealed that high IL-8 inducers were able to efficiently invade HEp-2 cells, and the invasion indices (percentage of inoculum surviving gentamicin treatment) were more than 0.15%, whereas those of low inducers were less than 0.0005%. It was suggested that the invasiveness of high inducers was dependent upon actin microfilament and microtubule rearrangement because cytochalasin D and colchicine inhibited invasion by about 90% and 70% respectively. Other well-recognized virulence genes or properties for invasion (*invE, eae*, *tia*), adherence (*papC, sfa, clpG*, MRHA), and cytotoxicity (*astA*, *hly*, CNF) were not related to IL-8 induction. **Conclusion:** Some non-Afa DAEC seem to stimulate IL-8 secretion by HEp-2 cells, and invasion may play a pivotal role in cytokine induction.

An Association of 27- and 40-kDa Molecules that Bind A-B Bacterial Enterotoxins to Cultured Cell

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Background: It is well-recognized that Shiga-like toxins (Stxs) preferentially bind to Gb3 glycolipids and cholera toxin (CT) and heat-labile enterotoxin (LTp) bind to GM1 gangliosides. After binding to the cell surface, A-B bacterial enterotoxins have to be internalized by endocytosis. Transport of the toxin–glycolipid complex has been documented in several manners but the actual mechanisms are yet to be clarified. **Objective:** Detect the membrane proteins involved in the binding and transport of A-B bacterial enterotoxins in cultured cells. **Methodology:** A heterobifunctional cross-linker, sulfosuccinimidyl-2-(p-azidosalicylamido)-1,3V-dithiopro-pionate (SASD) was applied. **Results:** Both Stx1 and Stx2 were bound to the detergent-insoluble microdomain (DIM) of Vero cells and Caco-2 cells, which were susceptible to the toxin, but neither was bound to insusceptible CHO-K1 cells. Both CT and LTp were bound to DIM of Vero cells, Caco-2 cells, and CHO-K1 cells. In a cross-linking experiment, Stx1 cross-linked only with a 27-kDa molecule, while Stx2, which was more potently toxic than Stx1, cross-linked with 27- and 20 40-kDa molecules of Vero cells and of Caco-2 cells; moreover, no molecules were cross-linked with insusceptible CHO-K1 cells. LTp was cross-linked only to 27-kDa molecule of these 3 cell types but CT, which was more toxic than LTp, also cross-linked with 27- and 40-kDa molecules of Vero cells, Caco-2 cells, and CHO-K1 cells. **Conclusion:** 27- and 40-kDa molecules might play a role in 23 endocytosis and retrograde transport of A-B bacterial enterotoxins.

Inflamatory Response of a 34-kDa Metalloprotease Showing Haemagglutinating Activity from a Non-toxigenic *Vibrio cholerae* Non-O1 Non-O139 Strain

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Background: The enterotoxigenicity of non-toxigenic *Vibrio cholerae* non-O1 non-O139 strain PL-21 was studied in an *in-vitro* rat intestinal model. Partially-purified proteins from culture supernatants of PL-21 showed significant increase in intestinal short-circuit current and potential difference when added to the luminal surface of rat intestine mounted in Ussing chamber. **Objective:** Purify and characterize this enterotoxic factor. **Methodology:** The enterotoxic factor was purified by gel filtration chromatography (G-200) and ion exchange chromatography (DEAE-52). The 15-N terminal amino acid sequence of this purified protein was analyzed. The enterotoxic response of purified protein was studied in rabbit ileal loop assay. Histological examination was done on the protein-treated rabbit ileum. **Results:** Purification of the enterotoxic factor showed a 34-kDa single band in SDS gel electrophoresis. The 15-N terminal amino acid sequence of this protein showed complete homology to haemagglutinin protease previously purified and characterized from *V. cholerae* O1. The purified protein showed haemagglutinating activity (HA) against rabbit blood, protease activity, and enterotoxic response in rabbit ileal loop assay, inducing accumulation of haemorrhagic fluid response. Histological examination at 20 µg of purified protease-treated rabbit ileal showed presence of erythrocytes and neutrophils in the upper part of the villous lamina propria. At 100 µg of protease, there was gross damage of villous epithelium with necrosis, haemorrhage, and inflammation. The metal chelator EDTA inhibited the effect in Ussing chamber and rabbit ileal loop assay. **Conclusion:** The 34-kDa HA protease is a potent virulence factor of non-toxigenic *V. cholerae* non-O1 non-O139 strains.

Characterization of an *Aeromonas veronii* Strain Isolated from Systemic Infection

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Background: *Aeromonas* is the causative agent of gastrointestinal infection. Although rare, *Aeromonas* septicaemia in immunocompetent persons has also been documented. However, the characteristics of the strains, inducing septicaemia, have not been fully investigated. **Objective:** Characterize an *Aeromonas* strain isolated from an immunocompetent patient who died from septic shock to clarify the virulence factors involved in the development of septicaemia. **Methodology:** The strain was identified to the genomospecies level by restriction fragment length polymorphism of PCR-amplified 16S rRNA gene and biochemical tests. The major secreted extracellular protein was purified and characterized. Biological activity of culture supernatant was assayed using human erythrocytes and various cell lines (Vero, CHO, and HEp-2). The gene-encoding aerolysin was amplified by PCR, cloned, sequenced, and analyzed. An aerolysin-deficient mutant was constructed to investigate whether aerolysin is the only cytotoxin produced by this strain. **Results:** The strain was identified as *A. veronii* biovar *sobria*, hybridization group 8. The major secreted extracellular protein was identified as a 34-kDa metalloprotease of the thermolysin family. Culture supernatant showed haemolytic activity and cytotoxic activity with the 3 cell lines tested. The cloned aerolysin gene showed high homology to other reported aerolysin genes. Aerolysin-deficient mutant did not show any cytotoxic or haemolytic activity. **Conclusion:** The strain was haemolytic and cytotoxic. Aerolysin was the only factor responsible for these activities. Metalloprotease was the major secreted protein in this strain. It is concluded that the strain was a hyper-metalloprotease producer since this was the only protein observed in sodium dodecyl sulphate-polyacrylamide gel electrophoresis of culture supernatant. Since metalloproteases involve in tissue degradation, the hypersecretion of metalloprotease in this strain may have accelerated the dissemination of *Aeromonas*, leading to severe septicaemia.

Helicobacter pylori Infection Is a Cause of Depressed Gastric Acid Secretion in Children and Women of Reproductive Age in Bangladesh

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Background: *Helicobacter pylori* (Hp) is a common gastrointestinal pathogen worldwide with a very high prevalence in developing countries. The effect of Hp infection on acid secretion is poorly defined in such population. **Objective:** Define if Hp infection is associated with abnormal gastric acid secretion in children and women of reproductive age in Bangladesh. **Methodology:** In total, 56 children aged 2-5 years and 122 women aged 20-40 years, living in a peri-urban community near Dhaka city, were studied. Urea breath test (UBT) was used for detecting Hp infection. Gastric acid output (GAO in mmol) was measured during 1-hour basal period (BAO) and 1-hour stimulated period (SAO) with s.c pentagastrin (6 µg/kg). The children and sub-sample of women (n=25) with a positive UBT were re-evaluated for GAO 2 months after a 14-day course of anti-Hp therapy (omeprazole, amoxicillin, and clarithromycin). **Results:** Thirty children (54%) and 96 (79%) women were positive for Hp infection. BAO and SAO (mmol/h) were significantly lower in Hp-infected children compared to non-infected children. In the Hp-infected group, anti-Hp therapy was resulted a significant rise of both BAO and SAO (paired *t* test, before vs after therapy; 0.23±0.30 vs 0.62±0.9, p=0.06 for BAO and 2.04±1.4 vs 3.4±2.5, p=0.001 for SAO, n=25). Acid secretion after anti-Hp therapy reached values equivalent to the Hp-negative children (0.65±0.56 for BAO, O 3.3±2.1 for SAO). Both BAO and SAO were comparable between the infected and the non-infected women. However, anti-Hp therapy resulted a significant rise of both BAO (before vs after therapy; 1.2±1.1 vs 2.2±2.2, p=0.09) and SAO (10.0±3.5 vs14.1±4.1, p=0.02) in infected women. **Conclusion:** The results suggest a compromised gastric acid barrier in population with Hp infection in Bangladesh. Anti-Hp therapy resulted in significant improvement of GAO which suggests a causal link between Hp infection and depressed GAO in this population. **Acknowledgements:** The financial support of the National Institutes of Health (NIH), Maryland, USA and the Swiss Agency for Development and Cooperation (SDC) is acknowledged.

Development of an Animal Model of Arsenic Toxicity in Adult Rabbits Induced by Arsenic Tri-oxide

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Background: Although animal models are important to study human diseases, a useful animal model of arsenic poisoning is not available. This model was developed to study pathogenesis of arsenicosis in animal. **Objective:** Assess the effects of arsenic-induced toxicity by developing a rabbit model in vivo. **Methodology:** Forty New Zealand white adult rabbits (1.4-2.5 kg) were studied in this experiment. After collecting base samples of blood, urine, and hair, As2O3 was orally administered for 7 consecutive days. Doses were 3 mg/kg/day (n=16), 4 mg/kg/day (n=16), and 5 mg/kg/day (n=8). After 7 days, same samples were re-collected and analyzed for arsenic concentrations by hydride generation atomic absorption spectrophotometry. Reduced glutathione (GSH), thiobarbituric acid-reacting substances (TBARS), and NO3/NO2 concentrations were determined by the spectrophotometric method. **Results:** There was a marked increase of dose-dependent arsenic concentrations in urine and hair after arsenic intoxication. Arsenic concentrations significantly increased in urine (3079.78±563.96 vs 27.53±5.62 ppb, $p<0.001$) at dose 3 mg/kg/day and in hair (3.23±0.78 vs 0.61±0.52 µg/g, $p<0.05$) at dose 5 mg/kg/day. Arsenic intoxication reduced GSH concentrations (11.98±0.95 vs 30.68±2.27 mg/dL at dose 5 mg/kg/day, $p<0.001$) and increased NOx and TBARS levels (NOx: 546.17±96.08 vs 339.09±63.42 mmol/L, $p<0.05$ at dose 4 mg/kg/day; TBARS: 9.69±0.89 vs 5.75±0.89 µmol/L, $p<0.05$ at 5 mg/kg/day). There was a negative correlation between TBARS and GSH concentrations ($r=-0.464$, $p<0.01$) and between NOx and GSH concentration ($r=-0.381$, $p<0.05$). **Conclusion:** This animal model could be useful to study the kinetics of arsenic toxicity and evaluate different drug therapies, including antioxidants and NO inhibitors. **Acknowledgements:** Ministry of Science and Information and Communication Technology, Government of Bangladesh and University of Dhaka, Bangladesh.

Intestinal Permeability in Bronchial Asthma

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Background: Bronchial asthma is a T lymphocyte-mediated inflammatory disease of the airways. Activated T lymphocytes are able to migrate from one mucosal site to another. A possible relation between gastrointestinal tract and bronchial asthma has been of increasing interest. **Objective:** Study intestinal permeability in bronchial asthma by studying differential absorption of two non-absorbable sugars, such as lactulose and mannitol, in children with bronchial asthma and compare it with matched control children. **Methodology:** Thirty-two children with bronchial asthma were recruited from the asthma clinic of Mobarak Al Kabir Hospital, Kuwait. The same number of children matched for age, Bsex, and ethnicity was taken as control. The severity of asthma was graded into 4 grades. After an overnight fast, 1 g of lactulose and 1 g of mannitol dissolved in 10 millilitres of water was given to drink. Skin prick test was done, and allergen-specific IGE was determined by rast test. All urine samples were collected during the next 5 hours. Lactulose and mannitol were determined from urine samples using auto-analyzer, CobasMyra. Ratio of lactulose/mannitol was calculated. **Results:** The mean age, body weight, and consanguinity were similar between the patients and the controls. History of asthma among the family of patients was more frequent compared to the controls (56.3% and 50.0%, $p<0.04$). Volume of urine collected from both the groups was almost same. The mean (±SE) of percentage of lactulose in urine of patients and controls was 2.96±0.57 and 0.90±0.14 ($p<0.001$) respectively. The mean (±SE) of lactulose/mannitol ratio in patients and controls was 0.31±0.055 and 0.074±0.008 respectively. Lactulose-mannitol percentage in urine and lactulose/mannitol ratio were not different in different grades of severity of asthma. **Conclusion:** A significant increase in intestinal permeability was observed in children with asthma. No correlation to severity was found. It is suggested that the damaged intestinal mucosa may predispose to entry of macromolecular antigens.

DAY 1: SUNDAY, 7 DECEMBER 2003

2:00 pm-3:00 pm: **Poster Session 1** (Venue: Lobby)

DIARRHOEAL DISEASE 1

P1* (164+)

Molecular Epidemiological Study of *Vibrio cholerae* in Bengal Region

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Background: Toxigenic *Vibrio cholerae* is the aetiological agent of cholera, an acute dehydrating diarrhoea. Among the 206 recognized O serogroups of *V. cholerae*, serogroups O1 and O139 cause epidemics of cholera and are known to carry a set of virulence genes necessary for pathogenesis in humans. This includes genes for cholera toxin (CT) and those encoding a colonization factor known as toxin co-regulated pilus (TCP) and regulatory protein ToxR. Recent studies have indicated that virulence genes or their homologues are also dispersed among diverse serogroups. **Objective:** Undertake molecular epidemiological study of *V. cholerae* strains isolated from environmental waters and clinical samples in Bengal region. **Methodology:** Presence of the virulence genes (*ctxA*, *tcpA* and *toxR*) in the isolates was analyzed by PCR (polymerase chain reaction) method. PFGE (pulsed-field gel electrophoresis) was performed to compare their genetic fingerprint to determine the clonal relationships between clinical and environmental strains. Antibiogram and O serogroup of the isolates were also examined. Isolates from other geographic regions were subjected to similar analysis to compare with the isolates from Bengal region. **Results:** O1 and O139 strains from both clinical and environmental sources were all positive for the 3 virulence genes. On the other hand, non-O1/non-O139 strains from both the sources were all negative for *ctxA* and *tcpA* but positive for *toxR*. PFGE patterns of recent isolates of O1 and O139 were similar in each serogroup regardless of the origins, although some differences were observed by comparing with the past isolates. Non-O1/O139 isolates showed various PFGE patterns. **Conclusion:** The results indicate that there is a clonal relationship between clinical and environmental strains, and the aquatic environments serve as reservoirs of toxigenic *V. cholerae*. **Acknowledgements:** This paper was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Development and Validation of an RT-PCR Method for Detecting Group A Rotaviruses from Stools of Children with Acute Diarrhoea

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Background: Rotavirus is the most important aetiological agent of severe diarrhoea in infants and young children in both developed and developing countries, causing a loss of an estimated 600,000 lives annually. Since the cultivation of rotaviruses from clinical samples is difficult to achieve, the most widely-used diagnostic techniques for detection of rotavirus in faecal samples include enzyme-linked immunosorbent assay (ELISA), electron microscopy (EM), northern hybridization and polyacrylamide gel electrophoresis (PAGE) of rotavirus dsRNA. The reverse transcription/chain termination method, RT-PCR, offers a quick, specific and sensitive alternative for detection of rotavirus from clinical samples. It enables detection of rotaviruses at concentrations substantially lower (1,000 times less) than by other means, which are laborious and time-intensive. **Objective:** Develop and evaluate an RT-PCR technique for the detection of rotavirus shedding in infected children as a rapid and convenient diagnostic procedure. **Methodology:** Twenty rotavirus-positive stool samples were collected from infants and young children with diarrhoea. dsRNA was extracted from 10% faecal suspensions by the phenol chloroform and glassmilk method. RT-PCR was performed using oligonucleotides specific for two overlapping N terminal regions of rotavirus gene encoding the VP3 protein. Sequencing was done using the dideoxy chain termination method followed by analysis using Clustal W programme and MEGA2 software. **Results:** Analysis of the rotavirus VP3 gene from representative genotypes (G1, G2, G3, G4, and G9) of the prevalent rotavirus strains revealed a high nucleotide and amino acid identity (>83%). Region-wise analysis of the gene showed 73-99% sequence identity. All clinical and standard rotavirus strains showed single sharp gene amplicons of the expected length (526 bp) with the VP3 primers even with samples having a low absorbance in ELISA. Sequence analysis and phylogenetic studies showed the VP3 fragments clustering into a single group (bootstrap value 98%) confirming the high conservation of the gene fragment between different genotypes. Analysis using animal strains (representing unusual genotypes) has also been incorporated in the study. **Conclusion:** Results obtained by rotavirus-detection methods, such as ELISA and PAGE, currently employed in the laboratory, is presently being compared to the VP3 RT-PCR method developed. **Acknowledgements:** The financial support of the Indian Council of Medical Research (ICMR) is acknowledged.

How to Study Biodiversity of Bacteria in Stool Samples of Children with Cholera as a Function of Time?

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Background: The understanding of the function of colonic flora in cholera requires knowledge on the biodiversity of the ecosystem. **Objective:** Assess methods to evaluate the number and biodiversity of bacteria in stool samples in cholera. **Methodology:** Counting of bacteria was validated in *Escherichia coli* culture, using 16S rRNA labelling with universal bacterial probe EUB tagged with fluorescent dye indodicarbocyanin (fluorescent *in situ* hybridization [FISH] method). The biodiversity was validated by temporal temperature gradient gel electrophoresis (TTGE). After total bacterial DNA was extracted from faecal sample, fragments of 16S rRNA gene that were amplified by PCR using the universal primer for bacteria were separated according to their GC content using TTGE on a DCode universal mutation detection system. Gel was stained with SYBR green nucleic acid gel stain I and was visualized using an UV transilluminator. A specific marker for *Vibrio cholerae* in stools was cloned and identified by TTGE. **Results:** Bacterial cells were counted easily using their red fluorescent signal against a black background in a microscopic field. On 14 consecutive stool samples of one weaning baby, the number of TTGE bands was always between 8 and 10, and the pattern changed with time. DNA from *V. cholerae* 01 and 0139 presented two major bands in TTGE. Each band was cloned in plasmid pGEM T-Easy vector from Promega and produced one band when transformed into *E. coli*. The cholera bands were found in stool samples of 3 children with cholera when compared with *V. cholerae* 01 reference. **Conclusion:** Methods are now available to study the quantity and biodiversity of colonic microflora as a function of time in children with cholera. **Acknowledgements:** The financial support of the French Ministry of Foreign Affairs is acknowledged.

Comparison of Serotypes of *Campylobacter jejuni* Isolated from Diarrhoeic Patients in Dhaka and Cape Town

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Background: *Campylobacter* is the most frequently-reported cause of bacterial gastrointestinal disease of human and other animals. Infection due to *Campylobacter jejuni* and *C. coli* commonly causes diarrhoea in humans. **Objective:** Compare the prevalence and patterns of distribution of serotypes of *C. jejuni* strains isolated from diarrhoeic patients in Dhaka and in Cape Town during 2000-2002. **Methodology:** Strains were isolated using candle jar. Serotypes were determined following the Penner serotyping scheme, using 60 different reference antisera obtained from the Cape Town University, South Africa. The serotypes of *C. jejuni* isolates in Dhaka were compared with those of the Cape Town isolates. **Results:** Of 181 randomly-selected *C. jejuni* strains from Dhaka and 999 strains from Cape Town were serotyped. Among the serotyped strains by Penner serotyping scheme, 62% were typeable, and 38% of Dhaka strains were untypeable, while 82% were typeable and 18% of Cape Town strains were untypeable. Among the typeable strains, Dhaka strains showed 73% single serotype and 27% multiple serotype, while Cape Town strains showed 65% single serotype and 35% showed multiple serotype. One strain (serotype 41) from Dhaka showed similar reaction of control strain (GBS) obtained from the University of Cape Town, which is usually associated with Guillain Barré Syndrome. It indicates that GBS strains are prevalent in Bangladesh. **Conclusion:** Determination of serogroup is important and useful in patients presenting with aseptic arthritis or Guillain Barré Syndrome after a bout of diarrhoea. An apparent difference in the distribution of serotypes was seen in Dhaka and Cape Town, and this may indicate differences in patterns of infection. A sero-epidemiological study is essential to determine the prevalence and other aspects of *Campylobacter* infection. **Acknowledgements:** The research was supported by the ICDDR,B: Centre for Health and Population Research.

P5 (051)

Estimation of Reducing Sugar in Stool of Diarrhoeal Patients

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Background: Estimation of reducing sugar(s) in stool of diarrhoeal patients is a useful clinical marker. Mono- and disaccharides or their mixtures, which can readily reduce cupric ion in hot alkaline solution, are known as reducing sugars. **Objective:** Find out a simple, cost-effective, and easy methodology for the quantitative estimation of total reducing sugars in diarrhoeal stool. **Methodology:** Supernatant of homogenized stool was used for the estimation of reducing sugar(s) photometrically by the O-Toluidine and glucose oxidase methods. Thin-layer chromatography (TLC) was used for separating and identifying individual sugar(s). **Results:** Estimated sugar(s) by the O-Toluidine method (n=42, mean=96.70 mmol/L, SD=64.40) matched with the presence of reducing sugar(s) (lactose, maltose, sucrose, galactose, glucose, mannose, fructose, xylose, and ribose) identified by TLC. However, those estimated by the glucose oxidase method (n=42, mean=40.74 mmol/L, SD=42.96) only quantified the true glucose because of its substrate specificity. **Conclusion:** Reducing sugar other than glucose produces colour in varying proportions in the O-Toluidine method and could be quantified partially. The O-Toluidine method, with this limitation, may be used for estimating reducing sugar(s) in diarrhoeal stool due to its simplicity, rapidity, and cost-effectiveness. **Acknowledgements:** Clinical Laboratory Branch, Laboratory Sciences Division, ICDDR,B supported this work as a research and development (R&D) work.

Isolation of *Shigella* species is Enhanced by Extended Incubation of Primary Isolation Plates

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Background: Shigellosis is a disease of a significant cause of morbidity and mortality among pre-school children. The disease can be caused by any one of the 4 species of *Shigella,* namely *Shigella dysenteriae*, *S. flexneri, S*. *boydii,* and *S. sonnei*. **Objective:** Evaluate the isolation rates of *Shigella* spp. by extended incubation of primary plates. **Methodology:** Stool or rectal swabs were obtained from patients who attended the Dhaka Hospital of ICDDR,B from 2000 to 2002. All specimens were processed on MacConkey agar (MCA) and *Salmonella-Shigella* agar (SSA) and incubated for 16-18 hours at 35-37 °C. All non-lactose fermenting (NLF) colonies were tested biochemically and identified serologically following standard methods. All primary plates with no visible NLF colonies were reincubated for another 16-18 hours, and plates showing NLF colonies were examined and identified following standard procedures. **Results:** Of 38,243 specimens, 11,577 NLF suspicious colonies were picked, and 3,809 (10.0%) were identified as *Shigella* spp. and 545 (1.4%) as *Salmonella* spp. after 16-18 hours of incubation. Additionally, 194 (4.85%) *Shigella* spp. were isolated from 587 reincubated plates and mostly from SSA plate, but very few from MCA plate. Of the *Shigella* isolates, *S. flexneri* (53.6%) was more frequently isolated, followed by *S. boydii* (24.2%), *S. dysenteriae* (15.0%), and *S. sonnei* (7.2%). **Conclusion:** Extended incubation of conventional stool culture media to enhance *Shigella* isolation has not been evaluated. As this extended period of incubation yielded a good number of additional *Shigella* isolates, the technique is useful for enhancing isolation of *Shigella* spp. Based on the findings of this study, it is recommended that incubation of primary plates should be extended to improve isolation rates of *Shigella* species. **Acknowledgements:** The research was supported by the ICDDR,B: Centre for Health and Population Research.

Effect of Antimicrobials on Shiga Toxin Production and Its *in vitro* Release by *Shigella dysenteriae* Type 1 Strains

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Background: *Shigella dysenteriae* type 1, the only serotype among *Shigella* species, produces Shiga toxin that is involved in haemolytic-uraemic syndrome (HUS). WHO recommends treatment of all bloody diarrhoea cases with antibiotics in those children aged less than 5 years. Antibiotic treatment of these cases is associated with HUS. The effect of antimicrobials on Shiga toxin production and its *in vitro* secretion by *S. dysenteriae* type 1 strain has not been studied. **Objective:** Study the effect of various antimicrobials on Shiga toxin production and its *in vitro* release by *S. dysenteriae* type 1 strain. **Methodology:** Fosfomycin, ciprofloxacin, norfloxacin, and mitomycin were chosen for this study. Shiga toxin titre was measured by RPLA (reverse passive latex agglutination) test kit in *S. dysenteriae* type 1 culture supernatant and bacterial pellets (treated with polymyxin B) at 5, 7, 24, and 48 hours of incubation with or without addition of antimicrobials at a concentration of <MIC50. Bacterial count was also determined at every point of time. The results were compared and analyzed. **Results:** Addition of either fosfomycin (1:256 vs 1:256), ciprofloxacin (1:128 vs 1:128), or norfloxacin (1:64 vs 1:64) did not affect *in vitro* toxin release by the organism when compared with culture without antimicrobials. Only mitomycin caused gradual and sustained increase in toxin titre in culture supernatant (1:256 vs 1:128) and in cell pellet (1:512 vs 1:128), maximally evident after 48 hours. Bacterial count steadily decreased with each drug. **Conclusion:** Only mitomycin caused 2 to 4-fold rise of *in vitro* Shiga toxin production and its release by *S. dysenteriae* type 1 strains, which was more in bacterial pellet than in culture supernatant. Other antimicrobials had no effect on toxin production. **Acknowledgements:** The support of the Indian Council of Medical Research and the Japan Society for Promotion of Science is acknowledged.

Evaluation of Faecal Electrolyte Loss Estimation and Its Correlation with Clinico-biochemical Profile in Children with Acute Diarrhoea

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Background: In developing countries, children aged less than 5 years suffer from 1.6-2.3 episodes of diarrhoea each year. The most common complications seen in diarrhoea are dehydration and electrolyte imbalance. Loss of potassium does not vary much with the underlying pathology, but the loss of sodium, chloride and bicarbonate ions are more in cholera and enterotoxigenic *Escherichia coli*-associated infection This is also related to duration of diarrhoea and purging rate. The principles of oral rehydration therapy have been extensively studied, but controversies remain regarding its electrolyte composition. Very little information is available on biochemical analysis of stools with regard to the newer scenario of diarrhoea management in the last quarter of the century. The present study was done to provide information on exact losses of electrolytes in stools and to compare it with clinical parameters and serum electrolytes so as to evolve a scientific and rational approach in a tertiary-care setting where standard case management of diarrhoea is being provided. **Objective:** Determine the faecal electrolyte losses in children with acute diarrhoea and correlate it with the degree of dehydration and examination of stools and serum electrolytes. **Methodology:** A prospective study was carried out among 84 children aged 0-5 year(s) admitted with acute diarrhoea from 1 October 2000 to 30 September 2001. A detailed history was taken and physical examination was done in all the cases, and paired stool and serum electrolytes were measured at the time of admission. Most (91.7%) children were taking home-made fluids or WHO-ORS orally prior to admission to the hospital. **Results:** There was marked variability in stool composition with wide range of concentrations for each ion. There was no apparent correlation between stool and serum electrolytes. There was no correlation between degree of dehydration and faecal electrolytes. However, there was a marginally significant ($p<0.1$) decrease in serum sodium and chloride with increasing degree of dehydration. With increase in watery consistency of stool, there was a significant decrease in serum potassium levels ($p<0.05$). Also, increase in mucus content of stool was associated with marginally higher loss of stool electrolytes and lower serum levels of potassium and chloride. No correlation was found between purging rate and stool electrolytes, but purging rate was significantly ($p<0.05$) more in the first 1-3 day(s) of diarrhoea than later in the course of illness. Also, purging rate was significantly ($p<0.05$) higher in cholera compared to other aetiologies. All the patients recovered and were discharged from the hospital. **Conclusion:** While treating diarrhoeas, it cannot be assumed that faecal electrolyte concentration in diarrhoeal stools is equal to the mean of published studies since the variability is very large. Knowledge about intake, weight gain, and serum electrolyte composition is more than adequate for appropriate management of acute diarrhoea. ORS and early feeding remain the instrument in decreasing diarrhoeal morbidity and mortality. **Acknowledgements:** The support of Departments of Pediatrics and Biochemistry, Christian Medical College, Ludhiana India, is acknowledged.

Presence of Neutral Fat in Stool and Its Association with Aetiology and Presenting Features of Diarrhoea

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Background: In the small intestine, ingested fats are emulsified and interacted with bile salts forming micelles, which facilitate their transportation to the villous cells. In the enterocytes, they are esterified to neutral fat. Impaired digestion, loss of villous cells, and poor absorption of fat may lead to excessive fat in stools, particularly in children with diarrhoea. **Objective:** Examine presence of neutral fat in stool and assess its association with aetiology of diarrhoea and nutritional status of children aged less than 5 years. **Methodology**: All children (n=7,514) aged 0-59 month(s), enrolled into the diarrhoeal disease surveillance system of the Dhaka Hospital of ICDDR,B during 1996-2001, were studied. Nutritional status, presence of neutral fat in faecal specimens (microscopy of faecal specimens stained with Sudan Red III), and aetiology of diarrhoea (ELISA for rotavirus and faecal culture for bacterial pathogens) in these children were determined. **Results:** Overall, 5,861 of the 7,514 (78%) children—64% (n=3,798) of infants, 25% of 12-23 months old, 8% of 24-47 months old, and 1% of 48-59 months old children—had neutral fat in stool. Neutral fat was more frequently present in rotavirus (91% vs 70%, OR=4.3, 95% CI 3.7-4.9, p<0.001), enterotoxigenic *Escherichia coli* (ETEC) (81% vs 78%, OR=.2, 95% CI 1.1-1.5, p=0.009) and enteropathogenic *E. coli* (EPEC) (83% vs 78%, OR=1.4, 95% CI 1.2-1.7, p<0.001)-associated diarrhoeas compared to other aetiologies of diarrhoea. Children receiving antimicrobial or antidiarrhoeal drugs were more frequently had neutral fat in stool (41% vs 36%, OR=1.3, 95% CI 1.1-1.5, p<0.001). Wasting, stunting, or underweight were not associated with neutral fat in stool. **Conclusion:** Childhood diarrhoea caused by rotavirus, ETEC, or EPEC is associated with neutral fat in stool. The mechanism of steatorrhoea in such children and its nutritional implications need to be investigated. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

P10 (205)

Screening of Infectious Diarrhoea: Evaluation of Faecal Occult Blood Test and Lactoferrin Latex Agglutination Test

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Background: The clinical differentiation between invasive diarrhoea (ID) and non-invasive diarrhoea (NID) is difficult in most cases. A modified faecal occult blood test (FOBT) used to detect occult blood has shown good correlation with faecal leukocytes. Lactoferrin was also used as a marker for faecal leukocytes as reference for an inflammatory process. **Objective:** Examine the diagnostic values of FOBT and lactoferrin latex agglutination test (LT) in distinguishing ID from NID. **Methodology:** The study was conducted at ICDDR,B enrolling 2% systematic sample of all patients registering at its Dhaka Hospital. Isolation of faecal enteropathogens served as gold standard. Stool isolates of *Salmonella, Shigella, Campylobacter, Entamoeba histolytica*, and enteroinvasive *Escherichia coli* were designated as ID group, whereas isolates of *Vibrio cholerae*, enterotoxigenic *E. coli,* and rotavirus were labelled as NID group. **Results:** In total, 594 patients aged 0-80 year(s) were prospectively evaluated from July to November 2002. Of them, 39% were FOBT-positive and 36% LT-positive. 28% were positive for *V. cholerae*, 24% for rotavirus, 15% for *Campylobacter*, 6% for *Shigella*, and 2% for *Salmonella*. *Campylobacter* was the commonest invasive pathogen, whereas rotavirus and *V. cholerae* were the common non-invasive pathogens. Evaluation of FOBT and of LT was done in 465 patients, of whom either a single enteropathogen (n=317) was identified or no pathogen (n=148) could be identified. Of 317 patients, invasive and non-invasive pathogens were isolated from 69 (22%) and 248 (78%) patients respectively. The sensitivities, specificities, PPVs, NPVs, and accuracies of FOBT and of LT were 58%, 64%, 22%, 90%, and 63% and 51%, 64%, 20%, 88%, and 62% respectively. **Conclusion:** The screening tests, such as FOBT and of LT, are not effective in differentiating ID from NID. **Acknowledgements:** This was a collaborative study between ICDDR,B and University of Basel, Switzerland. The study was funded by the University of Basel, Switzerland. The test kits were supplied, free of charge, by Hoffman La Roche, Basel, Switzerland.

GENETICS

P11 (127)

Analysis of Class 1 Integrons among *Salmonella* Isolates from Asymptomatic Carriers

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Background: Integrons play an important role in the spread of antibiotic-resistant genes in bacteria. **Objective:** Investigate the distribution and characterization of class 1 integron among healthy *Salmonella* stains. **Methodology:** Twenty-three strains of *Salmonella* spp. isolated from healthy humans in Guangdong, China, were examined for their susceptibility to 10 common antimicrobials. The presence and genetic content of class 1 integron were examined with specific primers of *intI1* and genes cassettes by PCR and sequencing. **Results:** Four strains—one isolate of *S. Hadar* and three isolates of *S. tshiongwe*—were positive for *intI1* gene. Furthermore, strains yielding a PCR product of *intI1* gene were amplified by primers of in-F and in-B which were set for amplifying the region of antibiotic-resistant genes. Among 4 *intI1*-positive strains, an amplicon of 1,009 bp was yielded in 2 isolates; an amplicon of 1,664 bp was yielded in 1 isolate; and 1 isolate present and two amplicons of 1,009 bp and 1,664 bp. Sequencing analysis revealed that amplicon 1,009 bp harboured gene cassette *aadA2* conferring resistant to spectimomycin and streptomycin, and another amplicon of 1,664 harboured gene *aadA5* and *dfr17* conferring resistant to spectimomycin, streptomycin, and trimethoprim respectively. **Conclusion:** This appears to be the first report of presence of class 1 integron harbouring multidrug-resistant genes among *Salmonella* strains isolated from healthy humans. The study stressed the need for continued surveillance of bacteria from asymptomatic carriers. **Acknowledgements:** The Project was sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

P12 (082)

Common Drug-resistant Loci Present in Both Plasmid(s) and Chromosome of *Shigella dysenteriae* type 1

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Background: *Shigella* has repeatedly demonstrated a tendency to acquire resistance. In the 1940s, *Shigella* acquired resistance to sulpha drugs, in the 1950s to tetracycline and chloramphenicol, and in the 1970s to ampicillin. Recently, fluoroquinolone-resistant *Shigella* has also been reported. **Objective:** Study the drug-resistance pattern of *Shigella dysenteriae* type 1 in relation to plasmid(s) and evaluate chromosomal loci. **Methodology:** Antimicrobial susceptibility tests were performed by the disk-diffusion method. Plasmids were isolated from liquid culture following the procedure of Kado and Liu (1981), and agarose gel electrophoresis was run on 0.8% agarose (Bio-Rad). SDS was used as a curing agent. The curing rate was 0.2-5%. Pulsed-field gel electrophoresis (PFGE) was performed following the standard laboratory protocol for molecular sub-typing of *Shigella* spp. recommended by Pulse Net, the National Molecular Subtyping Network for Foodborne Diseases Surveillance. **Results:** Wild-type strains showed resistance against 10 antibiotics, which contained 6 plasmids ranging from 3 kb to 145 kb. The SDS-cured strains showed 5 different combinations of plasmid(s). However, all the 5 strains were resistant to AMP, BAC, CAR, CHL, PEN, STR, MET, RIF, TMP, and TET. All cured strains were identical in drug-resistance pattern with wild-type strain. PFGE was performed for typing of the plasmid-cured strains with multidrug-resistant wild-type *S. dysenteriae* type 1, which remain unaltered. Plasmidless (cured) strain of *S. dysenteriae* type 1 was unable to grow. **Conclusion:** Similar drug-resistance pattern was observed among wild-type strain and all the plasmid-cured strains. The drug-resistant loci are present in both plasmid(s) and chromosome, which appears as second line of defense against antibiotics. Plasmid(s) were not inserted into the chromosome, as the PFGE pattern of all strains was unaltered. **Acknowledgements:** The financial support of the Council of Scientific and Industrial Research, India, is acknowledged.

P13 (063)

Isolation and Molecular Characterization of *Shigella boydii* Provisional Serovar 16553 from Diarrhoea Patients in Bangladesh

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Background: Bacterial strains that give biochemical reactions typical of *Shigella* species but do not belong to any of the recognized O-serogroups are occasionally designated as provisional serovar after being ratified by the WHO International Collaborating Center for *Shigella*. Here *Shigella boydii* provisional serovar E16553 isolated in Bangladesh has been characterized. **Objective:** Understand precisely the standing of 17 strains of E16553 isolated from stools of diarrhoea patients in Bangladesh and one strain collected each from Sweden and Japan, and characterized biochemically and genotypically in association with specific virulence factor. **Methodology:** Since 1995, 17 *Shigella*-like organisms that did not agglutinate with antisera to any known serotypes of *Shigella* spp. were isolated. These strains were agglutinated with antisrum to reference strain of *S. boydii* provisional serovar E16553. The strains were characterized biochemically and by molecular typing, including plasmid assay, pulse-field gel electrophoresis (PFGE) for strain variation, PCR assay for virulence genes, and Sereny test for invasion property. **Results:** All the isolates were identical in biochemical reaction pattern and had minor antigenic relationship with *Escherichia* coli serogroups O18, O23, O38, and O52 as reported earlier with reference provisional serovar E16553, suggesting their standing as *S. boydii* provisional serovar E16553. All strains harboured the 140-MDa plasmid, had *ipaH* gene, and produced keratoconjunctivitis in the guinea pig eye. Most strains were sensitive to all common antibiotics, except tetracycline. Eight strains were resistant to tetracycline. All the Bangladeshi strains produced a single pattern in PFGE that indicated their evolution from same origin. The PFGE pattern of these strains differed from that of Swedish and Japanese strains. **Conclusion:** It is recommended to change the provisional status of these strains and to include them in the classification scheme of *S. boydii*. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged. Japanese strains provided by Dr. S. Matsushita are gratefully acknowledged.

Analysis of Antibiotic Resistance Mechanisms in *Vibrio cholerae* Strains Isolated in Lao PDR

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Background: Drug susceptibility of *Vibrio cholerae* O1 isolated in Lao PDR has been monitored since 1993. Organisms isolated during 1993-2000 were resistant to streptomycin, while those isolated after 1997 were additionally resistant to tetracycline, chloramphenicol, and trimethoprim-sulphamethoxazole. **Objective:** Analyze the genetic determinants encoding antibiotic resistance, with particular attention to resistance genes in class I integron and SXT constin (a large conjugative element). **Methodology:** Fifty strains recovered from cholera patients from 1993 to 2000 were selected for PCR analysis with primers designed to detect class I integron, SXT constin, and antibiotic-resistant genes. A novel DNA region found in SXT^{LAOS} was cloned into pCR2.1 vector, sequenced, and analyzed. **Results:** Twenty-four of 26 streptomycin-resistant strains, isolated before 1997, contained class I integron harbouring the aadA1 gene cassette. Twenty-four strains, isolated after 1997, contained an SXT constin. Twenty of the latter strains were resistant to chloramphenicol, tetracycline, streptomycin, and trimethoprim-sulphamethoxazole, while 4 strains were susceptible to the antibiotic tested. The antibiotic-resistant gene cluster was found to be deleted in these 4 susceptible strains. Resistance genes encoded in the SXT constins were floR, tetA, strAB, and sulII. A hotspot region of SXT^{LAOS} was sequenced, and two novel open reading frames was identified, showing homology to sO24 (exonuclease) and sO23 (helicase) of the genomic island associated with the multidrug-resistant region of *Salmonella enterica* serovar Typhimurium DT104. **Conclusion:** Analysis of SXT^{LAOS} showed that there is a continuous flux of genes among *V. cholerae* SXT constins which should be carefully monitored.

Cloning and Characterization of Cytolethal Distending Toxin Gene in *Campylobacter coli*

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Background: *Campylobacter jejui* and *C. coli*, which cause gastroenteritis in humans, have been demonstrated to produce a multi-subunit toxin-designated cytolethal distending toxin (*cdt*). Although *C. jejuni cdt* gene has been cloned and sequenced, *C. coli cdt* gene is not yet analyzed. **Objective:** Clone and sequence *C. coli cdt* gene and compare it with *C. jejuni cdt* gene. **Methodology:** The common and specific primers were designed to detect both or either *C. jejuni* and/or *C. coli cdt* genes by PCR. Genomic DNA of *C. coli* was amplified by PCR using degenerated primers, GNW and LPF-D. The PCR product (approximately 1.5 kb) was cloned, sequenced, and used as a probe for southern hybridization after labelled by digoxigenin. A DNA fragment reacted with *cdtB* gene probe was cloned and sequenced. The common and specific primers were designed and tested with 20 *C. jejuni* and 15 *C. coli* strains. **Results:** An approximately 3.0 kb *Hin*dIII fragment reacted with the probe was cloned. Nucleotide sequence analysis of the 3.0 kb fragment revealed that *C. coli cdt*A, *cdt*B and *cdt*C genes were encoded on 774 bp (258 aa), 810 bp (267 aa), and 570 bp (190 aa) respectively. The amino acid sequence homologies of *cdt*A, *cdt*B, and *cdt*C between *C. jejuni* and *C. coli* were 49%, 68%, and 48% respectively. The common primers and specific primers could specifically detect *cdt* gene(s) both or either in *C. jejuni* and/or *C. coli.* **Conclusion:** Nucleotide sequence of *cdtA*, *cdtB* and *cdtC* genes of *C. coli* was analyzed and compared with those of *C. jejuni*. The common and specific primers for *C. jejuni* and/or *C. coli cdt* gene(s) were designed.

Mutagenesis of *Vibrio vulnificus* Using Signature-tagged Transposon

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Background: *Vibrio vulnificus* is a seawater bacterium causing lethal infection accompanying with necrosis and sepsis in humans having hepatic disorder, such as cirrhosis. *V. vulnificus* causes infection through wounds in seashore and through intestines by eating uncooked seafood contaminated with the organisms. Several toxins have been proposed as pathogenic factors of the organisms, but the bacterial factors that are critical for infection of *V. vulnificus* have not been found. **Objective:** Find the genes that are necessary for infection in experimental animals by destroying the genes using transposon. **Methodology:** Using signature-tagged transposon (pUTmini-Tn5Km5), a mutant library was constructed to isolate attenuated mutants for the purpose of searching the pathogenic genes. Sets of the mutants (INPUT) were inoculated intraperitoneally into mice that are overloaded with iron. After 12-24 hours, blood specimens taken from hearts of mice were cultured at 37 °C overnight on LB agar plates containing kanamycin, and survived bacterial cells into blood stream were recovered (OUTPUT). The recovered OUTPUT colonies, and inoculated INPUT cells, were pooled and heated for 100 °C for 5 minutes for preparation of DNA templates. INPUT and OUTPUT templates were subjected to manipulation of Tag by polymerase chain reaction (PCR) using digoxigenin-labelled primers for hybridization to prepare INPUT and OUTPUT probes. **Results:** By dot-hybridization, transposon mutants were found, of which Tag did not hybridize with the OUTPUT probe. One of the less-hybridizing mutants was attenuated completely. The insertion-region was sequenced. The DNA homology in FASTA was searched for, and the region was highly homologous to *V. vulnificus* CMCP6 chromosomal DNA and to a gene for synthesis of lipopolysaccharide. **Conclusion:** A transposon-inserted mutant of *V. vulnificus* was attenuated in the experimental mouse infection model. It is suggested that LPS is responsible for the pathogenicity of *V. vulnificus*-associated infection.

P17 (088)

Characterization of ElTor Choleraphage S20

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Background: Cholera has been a major health problem worldwide. *Vibrio cholerae* is the causative agent of intestinal infection. Vibriophages are able to infect and destroy the organism. Characterization of vibriophages is, thus, important for studying the host genome, for differentiating *V. cholerae* strains, and for phage taxonomy. **Objective:** Characterize ElTor choleraphage S20 by electron microscopy. **Methodology:** High titre phage stock was prepared by the soft agar overlay method. Phage morphology was studied by the negative staining technique. Phage DNA was extracted following the phenol-chloroform method. The basic protein monolayer technique of Kleinschmidt and co-workers was applied to spread the DNA for visualizing under the electron microscope. Partial denaturation of DNA was done using alkali and in presence of formaldehyde. Restriction digestion of DNA with different restriction endonucleases was carried out. **Results:** The phage had a hexagonal head with a short non-contractile tail and, thus, belongs to the *Podoviridae* family according to the International Committee on Taxonomy of Viruses. The phage DNA was linear and double-stranded. The DNA was circularly permuted. The phage DNA was cleaved by restriction enzymes Hae III, Hpa II, Hind III, EcoRV, and Acc I. **Conclusion:** The ElTor choleraphage S20 has a hexagonal head and a short non-contractile tail. The DNA is circularly permuted. Restriction enzymes Hae III, Hpa II, Hind III, EcoRV, and Acc I could cleave S20 DNA. **Acknowledgements:** The financial support of the Department of Science and Technology, Government of India, is acknowledged.

P18 (233)

Rapid Emergence of Multidrug-resistant *Salmonella enterica* Serovar Typhi with Decreased Ciprofloxacin Susceptibility in Bangladesh

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Background: Since 1987, multidrug-resistant (MDR) strains of *Salmonella enterica* serovar Typhi (*S*. Typhi), resistant simultaneously to ampicillin, chloramphenicol, and trimethoprim-sulphamethoxazole, have caused epidemics of severe typhoid fever in Asia and Africa. **Objective:** Study antimicrobial resistance among *Salmonella* enterica serovar Typhi isolates. **Methodology:** Trends in antimicrobial resistance were studied by the disk-diffusion method among 3,132 *S*. Typhi strains and by MIC among 128 randomly-selected isolates (since 1990) from blood cultures of enteric fever cases during 1989-2002, obtained from 3 hospitals in Dhaka, Bangladesh. **Results:** MDR *S*. Typhi, resistant simultaneously to chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole, emerged in 1990, peaked in 1994, declined in 1996, and re-emerged since 2001. Among 128 randomly-selected Typhi strains since 1990, increased ciprofloxacin MICs (0.25-0.38 µg/mL) by E-test were detected in 22 (17%), and strains exhibiting decreased ciprofloxacin susceptibility rose from 8% in 2000 to 46% in 2002. Decreased ciprofloxacin susceptibility was linked to MDR phenotype ($p<0.01$). By disk diffusion, all 22 isolates with increased ciprofloxacin MICs were ciprofloxacin-susceptible and nalidixic acid-resistant, indicating the usefulness of the latter to detect decreased ciprofloxacin susceptibility. **Conclusion:** The re-emergence of MDR *S*. Typhi with decreased ciprofloxacin susceptibility will further complicate therapy of typhoid fever. Nalidixic acid disk diffusion may be a useful tool for surveillance and to guide treatment. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Molecular Epidemiology of *Shigella dysenteriae* Type 1 Strains Associated with Haemolytic-uraemic Syndrome and Other Complications

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Background: *Shigella dysenteriae* type 1 produces Shiga toxin and is associated with complicated form of diseases, such as leukaemoid reaction, haemolytic-uraemic syndrome (HUS), encephalopathy, and septicaemia, which significantly increase morbidity and mortality. Factors that are related to the development of complications in shigellosis caused by *S. dysenteriae* type 1 have yet to be studied. **Objective:** Study whether any particular genetic element(s) are responsible for developing HUS or other complications in shigellosis patients infected by *S. dysenteriae* type 1 strains. **Methodology:** In total, 105 strains of *S. dysenteriae* type 1 isolated from hospitalized patients at ICDDR,B from 1999 to 2001 were analyzed for antibiogram, plasmid profile, ribotyping, pulsed-field gel electrophoresis (PFGE), and ELISA and hybridization for toxin assay. Clinical history of the patients from whom organisms were isolated were obtained from the hospital records. **Results:** Ninety-eight percent of the isolates were resistant to multiple drugs, including ampicillin, trimethoprim-sulphamethoxazole, and nalidixic acid. All the isolates harboured 140, 6 and 2 MDa plasmids. Of 58 strains, 14 (25%), 26 (45%), and 16 (30%) produced 64-200 ng/mL, 201-300 ng/mL, and 301-382 ng/mL of Shiga toxin respectively. Shiga toxin gene located in a single fragment of 380-kb size of NotI digested chromosomal DNA of *S. dysenteriae* type 1 was detected by hybridization with specific probe. PFGE and ribotyping analysis revealed that all the 102 strains were closely related, while remaining 3 strains were different. Analysis of clinical manifestations of the strains revealed that only one strain produced HUS accompanied with renal failure, haemolytic, and bacteraemia. Apart from HUS, one strain produced shock and renal failure associated with leukaemoid stool. Two patients died having a history of shock and did not have any other complications. **Conclusion:** It was noted that the PFGE and ribotyping pattern of these 3 strains was unique and was not reported earlier, indicating the emergence of a new emerging clone of *S. dysenteriae* type 1. No significant correlation was found between clinical manifestation of the disease and genotype of the bacteria. **Acknowledgements:** The study was funded by the United States Agency for International Development (USAID).

T Cell Responses in Blood of Patients with Cholera Due to *Vibrio cholerae* O1

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Background: The adaptive B cell response is known to provide protection after natural cholera infection. **Objective: A**nalyze the involvement of T cells, including cytotoxic T cells, memory T cells, and naive T cells and study the homing potential and kinetics of the response in patients with cholera. **Methodology:** Peripheral blood mononuclear cells (PBMC) were isolated by differential centrifugation on Ficoll-paque from 10 adult patients with acute watery diarrhoea caused by *Vibrio cholerae* O1 and 10 healthy subjects. The cells were stained with fluorescent-labelled cell surface markers (CD45RA, CD4, CD8, CD3, CD19, CD38, CD56, CD25, integrin b7, and CD62L) and analyzed by flow cytometry. PBMCs were also stimulated with membrane proteins of *V. cholerae* (strains T19479 and X25049) for 6 days and then stained with markers to quantify the numbers of proliferating immunocytes. Samples were collected at the acute stage (day 2 post-onset of disease) and at early and/or late convalescence stage of disease (day 7 and 21 post-onset). CellQuest and Flow Jo software were used for acquisition and analysis of data. **Results:** Patients with natural cholera infection responded with increases of memory cells, natural killer cells, activated T cells, and regulatory T cells at the acute stage compared to the healthy controls ($p=0.048-<0.001$), whereas B cells, gut homing B cells, naive T cells, CD4 T cells, and gut homing CD4 T cells increased at convalescence ($p=0.043-<0.001$). Furthermore, after stimulation with *V. cholerae* antigens, CD4 T cells responded with increased proliferation at the acute stage of disease. Up-regulation of CD4 T cell response was also seen during early convalescence, although activation was higher during the acute stage ($p=0.029$). **Conclusion:** The study shows induction of significant T cell responses after natural cholera infection. These responses and especially the increase of gut homing T and B cells appear to be needed to direct the immunocytes back to the intestinal mucosa to protect against future infections. This response may need to be activated after immunization with cholera vaccines to provide long-term protection.

P21 (027)

Isolation, Identification, and Sensitivity Pattern of *Vibrio cholerae* in a Tertiary-care Hospital in Karachi, Pakistan

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Background: Cholera is endemic in Pakistan. An update regarding its frequency, serotype, and antibiotic resistance pattern is required. **Objective:** Isolate and characterize *Vibrio cholerae* strains. **Methodology:** Four years' retrospective data were analyzed in a tertiary-care hospital from 1999 to 2002. Stool samples were inoculated in MacConkey's agar, *Salmonella-Shigella* agar, and TCBS agar. All the isolates were identified using standard microbiological techniques. Antibiotic sensitivity test was done by the Kirby-Bauer disc-diffusion method. **Results:** *V. cholerae* Ogawa was the most common isolate. High level of resistance was observed against nalidixic acid, co-trimoxazole, fosfomycin, and furazolidone. **Conclusion:** *V. cholerae* serotype Ogawa is the commonest enteric isolate, and the organism was resistant to some common antibiotics.

P22 (042)

Serotype Transition of Pandemic Strains of *Vibrio parahaemolyticus* in Vietnam in 1997-1999

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Background: Recent increase in *Vibrio parahaemolyticus*-associated infection in various Asian countries and the United States were attributable to the emergence and pandemic spread of a new clone characterized by O3:K6 serovar, the *tdh*+/*trh*- toxin gene profile, unique arbitrarily primed PCR (AP-PCR) profiles, and positive reaction in GS-PCR. Very high incidence of *V. parahaemolyticus*-associated infection in Khanh Hoa Province, Vietnam, during 1997-1999, was earlier reported. **Objective:** Characterize clinical strains of *V. parahaemolyticus* isolated in the previous study to investigate whether they belong to the pandemic clone and to find a feature(s) of the strains unique to this region. **Methodology:** Test strains were examined by agglutination tests for O:K serotype determination, by PCR methods to detect the *tdh* and *trh* genes and to identify pandemic strains and by AP-PCR and PFGE methods for epidemiological analysis. **Results:** Forty-nine percent of 523 strains were judged pandemic clone as evidenced by the *tdh*+/*trh*- toxin gene profile and GS-PCR+ reaction. Eleven serovars were detected among GS-PCR+ strains with O3:K6 serovar being dominant. AP-PCR analyses demonstrated that all GS-PCR+ strains shared unique profiles. Chronological analysis of the GS-PCR+ strains showed serovar transition from O3:K6 to O4:K68 and then to O1:K25. PFGE analysis also supported the serovar transition and suggested possible order of the emergence of other serovars among the GS-PCR+ strains. **Conclusion:** The study demonstrated that infection by the pandemic clone took place in Vietnam and suggests that diversification of the pandemic strains and their serotype transition may have occurred in the study area. **Acknowledgements:** This research was supported in part by the funds from the Ohyama Health Foundation and Yuasa International Foundation; 4th Advanced Scientific Technology Research Fund from Toyota Motor Corporation; a grant-in aid for scientific research from the Ministry of Education, Science, Sports and Culture, Japan; Swedish Agency for Cooperation with Developing Countries; National Institute of Child Health and Human Development, National Institutes of Health (United States); World Health Organization; Diseases of the Most Impoverished (DOMI) programme (funded by the Bill and Melinda Gates Foundation).

P23 (033)

High Prevalence of *Salmonella enterica* Serovar Typhi-associated Febrile Illness in a Rural Community of West Bengal

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Background: Typhoid fever, caused by multidrug-resistant *Salmonella enterica* serovar Typhi, is endemic in eastern India among infants and children. The organism has also been reported causing epidemic in different parts of India in 1990. **Objective:** Understand the magnitude of typhoid fever problem in rural community of West Bengal, India. **Methodology:** An active weekly surveillance of fever cases was carried out by trained locally resident volunteers. The study area is located in Kalikapur block of South 24-Parganas district, about 35 km away from Kolkata and has a total population of about 28,300. Any fever of more than 72 hours duration without any obvious cause (e.g. ARI, UTI, abscess, etc.) and having no anti-typhoid drug were the criteria for collection of sample. During 14 April-30 June 2003, 52 blood samples were collected from 0-45-year age group. About 5 cc of blood was collected in 50 mL of tryptic soy broth for bacteriological culture as advocated by the WHO Manual of Laboratory Investigation, Geneva (1983). **Results:** Of 52 samples, 7 (13.5%) were positive for *S. enterica* serovar Typhi. All the 7 isolates were sensitive to cefotaxime, tetracycline, gentamicin, norfloxacin, ciprofloxacin, and ofloxacin, but were resistant to ampicillin, chloramphenicol, co-trimoxazole, and furazolidone. Isolation from 0-4-, 5-9-, 10-14-, and ≥15-year age groups was 1 (16.7%), 1 (5.3%), 4 (44.4%), and 1 (5.0%) respectively. Isolation was more frequent, i.e. 4 (33.3%), among 12 febrile cases with 7-13 days history of fever. Similarly, 2 (7.1%) and 1 (14.2%) cases were among 28 and 7 cases sampled with <7 and ≥14 days of fever. Abdomen pain and headache were important presenting symptoms. Two of the 7 cases had vomiting. **Conclusion:** The sensitivity of blood culture was only 50%. So, about one-fourth of all febrile illnesses were typhoid fever. These poor people mainly depend on quacks and private physicians who manage these patients with incomplete and improper treatment which may lead to further complicated situation.

P24 (092)

Antigenic Variability in Serotype 6 of *Shigella flexneri*: Emergence of a New Subserotype

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Background: Different chemical structures of both O-antigen and core structure and different biochemical reactions have documented *Shigella flexneri* type 6 as atypical to other serotypes of *S. flexneri*. Serotype 6 has been found to be associated with acute form of bacillary dysentery and was identified as one of the prevalent serotypes of *S. flexneri* in Bangladesh. **Objective:** Uncover the genetic basis of antigenic variety, thereby re-evaluating the current standing of type 6 in the *S. flexneri* classification scheme. **Methodology:** Sixty-seven strains of *S. flexneri* type 6 were isolated from patients attending the ICDDR,B hospital during 1999-2001. Serotyping was performed using monoclonal and commercially-available antisera. Of the 67 strains, 37 were selected randomly for studying the clonal divergence of strains having different antigenic properties using a variety of techniques, including antibiogram, biochemical characterization, plasmid profiling, pulsed-field gel electrophoresis (PFGE), and ribotyping. **Results:** Of the 67 strains, 36 (54%) were agglutinated only with type factor 6 (designated as 6a), while remaining 31 (46%) agglutinated with type factor 6 and an additional antigenic determinant E1037 (designated as 6b). The sugar fermentation ability, especially in the case of mannitol, showed a clear demarcation with fast and delayed positive reaction between the two groups, 6a and 6b. Plasmid analysis revealed that 6a possessed 2.7 and 1.8 MDa plasmids as common, whereas 2.5 and 1.6 MDa were unique to the strains of 6b. Two PFGE types (A and B) were found among the strains of 6a, while a single type (C) was obtained in 6b showing significant differences from the patterns of 6a. Ribotyping analysis yielded two patterns (R1 and R2) in 6a and a single pattern (R3) in 6b which corroborated with PFGE analysis. **Conclusion:** Overall analysis suggests that *S. flexneri* type 6 strains having two antigenic properties are to be considered as separate subserotypes: 6a and 6b. **Acknowledgements:** The work was funded in part by the Bill and Melinda Gates–Government of Bangladesh Fund of ICDDR,B.

P25 (093)

Clonal Distribution of *Shigella sonnei* in Bangladesh

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Background: In a developing country, isolation of *Shigella sonnei* is a relatively rare event. Although the prevalence of *S. sonnei* in Bangladesh has been reported scantily, no systematic study on its epidemiology in relation with clonality had ever been performed. **Objective:** Get a detailed insight of the epidemiology of *S. sonnei* in Bangladesh. **Methodology:** Of 403 strains of *S. sonnei* isolated from hospitalized patients of ICDDR,B during 1999-2002, 80 were selected randomly for extensive characterization both at phenotypic and genotypic levels using serotyping, antibiogram, biochemical characterization, plasmid profiling, pulsed-field gel electrophoresis (PFGE), and ribotyping. **Results:** Over 60% of the isolates were resistant to nalidixic acid, 89% to trimethoprim-sulphamethoxazole, and 9.5% to ampicillin. More than 4% of the strains were resistant to multiple antibiotics, whereas 4.2% were sensitive to all antibiotics. None of the selected strains produced indole, while all the strains fermented arabinose, mannitol, mannose, trehalose, maltose, and ornithine. Except one, lactose was utilized by all the strains, while raffinose was fermented by 62% of the strains. All the strains were positive for *ipaH* gene, but 54% did not harbour the 140-MDa plasmid. However, most strains contained 5-, 1.8-, and 1.4-MDa plasmid in size. Conjugation experiment suggested that the middle-range plasmids harboured a self-transmissible multiple antibiotic resistance marker. PFGE analysis of *Xba*I-digested chromosomal DNA of representative isolates yielded 5 different types with numerous subtypes. Four different ribotypes were found, and one ribotype was shared by two different PFGE types. **Conclusion:** Overall, it appears that a significant burden of *S. sonnei* prevails in Bangladesh with various clones. This is the first report of the prevalence and clonality of *S. sonnei* in Bangladesh. **Acknowledgements:** This work was funded in part by the Bill and Melinda Gates–Government of Bangladesh Fund of ICDDR,B.

P26 (094)

Molecular Epidemiology of *Shigella dysenteriae* Type 2 Strains Isolated in Bangladesh

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Background: *Shigella dysenteriae* type 1, the most virulent and previously predominant serotype of the dysenteriae group, is now scarce and replaced by other serotypes of *S. dysenteriae,* especially type 2 and type 4. Although outbreaks of shigellosis by *S. dysenteriae* type 2 are relatively rare, its prevalence increased steadily from 1999 to 2002 in Bangladesh. **Objective:** Characterize *S. dysenteriae* type 2 strains extensively at phenotypic and genotypic levels to understand insight of epidemiology of shigellosis incurred by this serotype. **Methodology:** In total, 84 strains of *S. dysenteriae* type 2 were isolated from hospitalized patients of ICDDR,B during 1999-2002. Of these, 67 strains were available for extensive characterization using serotyping, antibiogram, biochemical characterization, plasmid profiling, pulse-field gel electrophoresis (PFGE), and ribotyping. **Results:** Except for arabinose fermentation, all the strains had similar biochemical reactions. More than 60% of the strains were sensitive to commonly-used antibiotics. Only 4.5% (n=3) of the strains were resistant to nalidixic acid, and none of the strains were resistant to mecillinam and ciprofloxacin. All the strains were invasive as demonstrated by the presence of 140-MDa plasmid, Congo red absorption ability, and by the Sereny test performed on representative strains. Plasmid patterns were heterogeneous, but more than 50% of the strains were confined to a single pattern. Middle-range plasmids (90-30 MDa) were present in 31% of the strains, of which all were not resistant to multiple antibiotics, indicating that all the middle-range plasmids were not associated with antibiotic resistance. All the strains were clustered within a single type with 4 subtypes by PFGE, while ribotyping patterns were identical among all the strains. **Conclusion:** The emerging strains of *S. dysenteriae* type 2 in Bangladesh are clonal. **Acknowledgements:** The work was supported in part by the Bill and Melinda Gates–Government of Bangladesh Fund of ICDDR,B.

P27 (101)

Detection of Human Astrovirus Type 8 in Kolkata, India

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Background: Astroviruses are presently gaining importance as viral pathogens, being second after rotaviruses, causing diarrhoea largely among children, occasionally infecting adults and immunocompromised individuals. Astroviruses also cause diarrhoea among various animal species. Eight human genotypes/serotypes exist, besides strains infecting different animal species. The availability of enzyme immunoassay kits and development of reverse transcription–polymerase chain reaction (RT-PCR) have led to marked improvement in laboratory detection of astroviruses in faecal specimens from several countries. **Objective:** Characterize human astroviruses at the molecular level. **Methodology:** Amplified IDEIA^{TM} astrovirus immunoassay (Dakocytomation) was used for initial screening of ast: an attempt to detect astroviruses through preliminary screening of faecal specimens of 242 children with watery diarrhoea, who visited Dr B.C. Roy Memorial Hospital for Children in Kolkata. RNA was extracted from each astrovirus-positive faecal sample; RT-PCR was carried out using the primer pair Mon 348[-] and Mon340[+]; and amplicons of 289 bp further confirmed the presence of astrovirus. Sequence data generated in an ABI 310 automated sequencer were compared with sequences of astroviruses deposited in GenBank database to determine the genotype of astrovirus. **Results:** The initial screening showed the presence of astrovirus in faecal specimens of 22 (11%) children aged less than 2 years. RT-PCR confirmed the presence of astrovirus, as 289 bp amplicon specific for astrovirus was observed. Analysis of the sequence from two 289 bp amplicons showed them as human astrovirus of genotype 8. **Conclusion:** This preliminary study confirms that astroviruses are also an important cause of watery diarrhoea in children in developing countries, and more studies should be undertaken to ascertain their nature in this part of the world. **Acknowledgements:** R. Bhattacharya, Junior Research Fellow, was supported by the Council of Scientific and Industrial Research. The financial support of the Indian Council of Medical Research and the Japan International Cooperation Agency is gratefully acknowledged.

Antimicrobial Susceptibility Patterns of *Helicobacter pylori* in Calcutta, India

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Background: *Helicobacter pylori*-associated infection is extremely common in India as in other developing countries, but few data exist on the susceptibility of *H. pylori* to antimicrobials commonly used in the eradication schedules in this country. **Objective:** Determine the antibiogram and minimum inhibitory concentration (MIC) of *H. pylori* strains from Kolkata, India, for metronidazole, clarithromycin, furazolidone, amoxicillin, and tetracycline. **Methodology:** 40 *H. pylori* strains isolated from gastritis and peptic ulcer patients of Kolkata were examined in the study. MICs to the antibiotics were determined by the agar dilution method. The reference strains of *H. pylori,* namely 26695 and J99, were used as controls. **Results:** Most (87.5%) strains were resistant to at least 8 µg/mL of metronidazole, while only one strain was resistant to tetracycline. All Kolkata strains were highly sensitive to clarithromycin, furazolidone, and amoxicillin. These results differed significantly from the few available reports on drug-sensitivity profile of *H. pylori* from other parts of India, namely Mumbai in the West and Delhi and Lucknow in the North where a high resistance to clarithromycin, amoxicillin, and furazolidone was observed. **Conclusion:** This study confirms the need for appropriate susceptibility testing to determine local/regional trends and to guide empirical treatment. **Acknowledgements:** The financial support of the Department of Biotechnology, Government of India (no. BT/MB/VAP/3/2/98) and Indian Council of Medical Research (no. 5/8-1(161)/*H. pylori*/2000/ECD-II) is acknowledged.

Typhoid Fever in Kolkata, India: A Comparative Evaluation of Hospital and Community-based Surveillance

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Background: Typhoid fever even today continues to be a global health problem, especially in developing countries like India. Very few studies have been conducted so far to estimate the infection rate in hospitalized children compared to that in the community. **Objective:** Evaluate the rate of *Salmonella typhi*-associated infection of hospitalized paediatric children and compare the same from an endemic community. **Methodology:** In total, 194 blood samples were collected from hospitalized children during January 2000-June 2003. During January-June 2003, another 52 blood samples from the peri-urban population in eastern Kolkata were collected from fever cases. **Results:** Of the 194 samples, 17 (8.8%) were positive for *S. enterica* serovar Typhi among hospitalized cases. Of the 52 samples from urban population, 8 (15.4%) cases were positive for the above organism. **Conclusion:** The rate of isolation from the hospitalized patients was much lower compared to that of community-based study. The hospitalized patients may have been treated outside by private practitioners with different antimicrobials prior to their admission, thereby decreasing the rate of isolation of the organism. The importance and utility of community-based study to estimate the disease burden in an endemic area like Kolkata is highlighted.

Serological and Genomic Characterization of a G12 Human Rotavirus Detected in Thailand

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Background: G12 rotavirus has not been detected anywhere in the world since the first detection of a human strain L26 (G12, P1B[4]) in the Philippines in 1990. **Objective:** Perform serologic and genomic characterization of a human rotavirus (strain T152) with G12 specificity isolated from a diarrhoeic patient in Thailand in 2002. **Methodology:** Polyacrylamide gel electrophoresis of RNA, sequencing of the representative genes, neutralization tests, and northern blot hybridization assay were employed. **Results:** The strain T152 exhibited long RNA pattern and subgroup I specificity. The VP7 gene of strain T152 showed the highest identity to G12 prototype strain L26. In contrast, the VP4 gene of the strain T152 showed the highest identity to those of strains with P[9] specificity. The G and P type specificity of strain T-152 was serologically confirmed with G12-specific monoclonal antibodies prepared in this study and a P[9]-specific monoclonal antibody. Northern blot analysis showed that T152 was closely related to strain AU-1 (G3P[9]); 9 RNA segments hybridized to each other between the genes from T152 and AU-1. Gene 5 (NSP1 gene) of T152, which did not hybridize with those of any other strains examined, was 1,652 nucleotides in length and exhibited low identity to those of representative human and animal rotaviruses. **Conclusion:** The strain T152 was a natural reassortant strain with G12P[9] specificity.

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Pathogenic Potential of *Aeromonas* spp. Isolated from Some Biotic Components of an Aquatic Ecosystem

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Background: *Aeromonas* is an autochthonous flora of the aquatic environment. It has been isolated from different components of aquatic ecosystems of Bangladesh. However, very limited information is available about the environmental adaptation and pathogenic properties of *Aeromonas* spp. isolated from different ecological niches in Bangladesh. **Objective:** Study the impact of some physico-chemical parameters of water on the fluctuation of *Aeromonas* spp. density and detect enterotoxigenic potential of the isolates. **Methodology:** *Aeromonas* spp. were isolated from water, aquatic plant and fish samples on MacConkey's agar plate. These samples were collected monthly for one year from a fish farm located in Mirzapur upazila under Tangail district of Bangladesh. Correlation coefficient of *Aeromonas* density with pH, temperature, and conductivity of water was performed using simple statistical method. Standard gene probe assay was performed to detect cytotoxic enterotoxin (act) gene in isolates of *Aeromonas* spp. Culture supernatants of *act* gene-positive and gene-negative isolates were tested for the presence of different virulence-associated factors using different biological assays, such as enterotoxicity test in suckling mice assay, haemolytic activity on blood agar plate, and CAMP-haemolysin test. **Results:** *Aeromonas* density in gills and intestinal contents of fish correlated with pH ($p<0.001$) and water temperature ($p<0.001$). Ten of 32 *act* gene-positive and 2 of 31 *act* gene-negative isolates of *Aeromonas* spp. induced fluid accumulation in SMA. Twenty-five of 31 *act* gene-positive and 27 of 30 *act* gene-negative isolates of *Aeromonas* spp. showed cytotoxicity in vero cell-line. Haemolytic activity (both a and b) was detected in 30 of the 32 *act* gene-positive and 22 of the 30 *act* gene-negative isolates of *Aeromonas* spp. Production of CAM-haemolysin was detected in 14 of 32 the *act* gene-positive and 8 of the 30 *act* gene-negative *Aeromonas* isolates. **Conclusion:** Environmental parameters affect fluctuation of *Aeromonas* spp. in the environment. Moreover, *Aeromonas* strains harbouring *act* gene significantly differed from *act* gene-deficient strains with respect to enterotoxicity in suckling mice assay and haemolytic activity on blood agar plate. **Acknowledgements:** The study was jointly supported by the Swiss Agency for Development and Cooperation (SDC) and the ICDDR,B: Centre for Health and Population Research.

P32 (185)

Analysis of Faecal Leucocytes and Erythrocytes in Infections Due to Different Serotypes or Subserotypes of *Shigella* in Bangladesh

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Background: Shigellosis remains a major health problem globally, and its early diagnosis and effective management are important in reducing morbidity and deaths. **Objective:** Evaluate the usefulness of enumeration of faecal leucocytes and erythrocytes in invasive diarrhoea for making an early diagnosis of shigellosis. **Methodology:** In total, 227 consecutive culture-proven shigellosis patients of all ages and of both the sexes, attending the Dhaka Hospital of ICDDR,B during January 2000-September 2001, were studied. Stool specimen of each patient was examined under microscope and cultured for isolation, biochemical characterization, and serotyping of *Shigella*. **Results:** Patients with shigellosis more often ($p<0.001$) presented with visible blood (37% vs 1%) and mucus (99% vs 78%) in stools when compared with non-*Shigella* patients. Ninety-one percent of *Shigella*-positive patients shed >20 WBC/hpf compared to 74% of non-*Shigella* invasive diarrhoea cases ($p<0.001$), and they were more likely ($p<0.001$) to shed macrophages (>5/hpf) and RBC >20/hpf or >50/hpf ($p<0.001$) than non-*Shigella* patients. Compared to non-*Shigella* invasive diarrhoea cases, presence of >50/hpf of WBC in association with any number of RBC in faecal samples had a modest sensitivity of 67%, specificity 59%, positive predictive value 70%, negative predictive value 56%, accuracy 64%, and positive likelihood ratio of 1.6 in predicting shigellosis. However, when compared with non-*Shigella* patients, presence of >20/hpf of WBC was the best predictor of shigellosis (sensitivity 91%, specificity 88%, positive predictive value 88%, negative predictive value 90%, accuracy 89%, and positive likelihood ratio 7.4). **Conclusion:** Direct microscopical examination of stool specimens for the presence of WBC and RBC is a simple method for diagnosing shigellosis and a cheap alternative for stool culture. However, selection of appropriate cut-off values (number of WBC and RBC/hpf) and comparison group are important in such a screening process while predicting shigellosis. **Acknowledgements:** The financial support of the Swiss Agency for Development and Cooperation (SDC) and the United States Agency for International Development (USAID) is acknowledged.

P33 (314)

Is Perception on 'Safe Sex' of Bangladeshi Young Students Adequate?

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Background: Students are one of the most vulnerable groups likely to be engaged in risky sexual behaviours. It is imperative that they possess adequate level of perception on 'safe sex' (encompassing all sexual activities, single sex-partnering, protected sex, and thus, preventing STDs/HIV/AIDS, etc.). **Objective:** Assess the level of perception on 'key components of safe sex' among young students. **Methodology:** This cross-sectional survey was carried out among 293 students randomly selected from 3 district towns employing verbal autopsy (pre-tested structured questionnaire) during February-April 2002. Data were analyzed using SPSS/Win 11.0. Perceptions of respondents were graded as 'Good', 'Adequate', 'Fair', and 'Inadequate'. **Results:** Of the 293 respondents (47.1% males, 52.9% females, mean age 20.85±1.93 years), although 83.6% heard about the term 'safe sex', 43.2% interpreted 'safe sex' wrongly as 'protected sex'. 43% regarded advantages of safe sex as 'being safe from contracting STDs/AIDS'. They identified commercial sex workers (32%), drug abusers (14%%), and truckers (9%), including 1% of students, as high-risk groups. While the respondents opined that pre- or extramarital sex should be avoided (76.1 and 78.3%, respectively), more males (31%) than females (8.4%) experienced pre- and/or extramarital sex, on average ($p<0.001$), although mentioned as accidental. 33.1% suggested that sex education be introduced at school level and 23.9% at college level in Bangladeshi educational curriculum. **Conclusion:** Since the findings revealed inadequate level of perception on safe sex among the majority of respondents, it is crucial that they, being one of the vulnerable groups for sexual promiscuity but is the backbone of a nation, should be trained on and made aware of safe sex methods/practices. This will help limit STD/RTIs and family sizes (using condoms) and will help avert AIDS, threatened for an ensuing epidemic in Bangladesh. **Acknowledgements:** The study was supported by a small grant from Consultant's House, Moghbazar, and partly, by the Institute of Public Health with part-time manpower and logistic help. The authors are grateful to all physicians/research officers for their dedication and sincerity in collecting data on this very delicate and sensitive issues of safe sex quite successfully.

NEONATAL HEALTH

P34 (007)

Low Birth-weight in Gonoshasthaya Kendra Area: A Survey Experience from Rural Bangladesh

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Background: Low birth-weight (<2,500 g) (LBW) is an important determinant of the chances for a newborn to survive and undergo healthy growth and development. In rural areas of Bangladesh, as 90% of deliveries take place at domiciliary level, weighing them soon after birth poses a considerable challenge. **Objective:** Find out the incidence and predictors of LBW in a rural community of Bangladesh. **Methodology:** A community-based longitudinal study was carried out in Gonoshasthaya Kendra, Savar project area in 2000-2001. Five hundred pregnant women were followed up from their conception to delivery. Data on different maternal, social and anthropometric factors were collected at different points of gestation period. It was possible to record birth-weight of 350 babies within 48 hours of delivery. **Results:** Birth-weight ranged from 1,500 g to 4,600 g with a mean birth-weight of 2,961 g (SD±483.7). Data analysis showed that the incidence of LBW was 24%. Univariate analysis showed that maternal age, education, weight, height, haemoglobin level, parity, history of previous stillbirth, family income, and antenatal check-up were associated with the outcome variable (LBW) at $p<0.2$ level. These exposure variables were then included in the final stepwise multiple logistic regression model. Logistic regression with backward elimination showed that maternal weight, haemoglobin, parity, and family income were important predictors of LBW. **Conclusion:** The incidence of LBW was high in this rural area. Reduction of LBW could be accomplished by selectively targeting modifiable risk factors for those women who are at the greatest risk of delivering a LBW baby. **Acknowledgements:** The financial support of the Bangladesh Medical Research Council is acknowledged.

P35 (045)

Use of a Neonatal Sepsis Risk Score in a Rural Hospital

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Background: The increased risk of newborns to infection is well-recognized. LAMB doctors had been using pre-emptive antibiotic treatment to reduce later need for acute intervention for clinically apparent sepsis. Singh *et al*. ranked factors in a published study (Singh M, Narang A, Bhakoo ON. Predictive perinatal score in the diagnosis of neonatal sepsis. *J Trop Pediatr* 1994;40:365-8) for use in screening neonates. LAMB Hospital has used this system in a modified form since 1995. **Objective:** Describe outcomes for 12 months of using a modified predictive perinatal sepsis score, comprising pre-, peri- and postnatal factors in a rural hospital obstetric unit. **Methodology:** A retrospective descriptive study of application of the modified neonatal sepsis score is presented. Health personnel, using scored risk factors listed on every partogram, made immediate assessment of newborns and manage appropriately. Medical records data from January to December 2002 were examined. **Results:** Of 2,086 live inborn babies, 563 (27%) were diagnosed as at-risk of sepsis and admitted. 15% were observed without starting antibiotics. 6% were started on broad-spectrum antibiotics at birth based on risk score only. 47% of the inborns with high sepsis risk scores had clinical signs. Total neonatal admissions for any reason were 751. Overall mortality was 10% and was also 10% for newborns admitted with sepsis. 60% of admitted infants aged less than 28 days were admitted for score-based sepsis risk or for clinical sepsis. 20% of total admitted infants for sepsis were outborn. The assigned risk score for very low birth-weight increased in August 2002. **Conclusion:** The sepsis score has streamlined the management of at-risk newborns. This tool could be used as a guide for community management, including early referral and initiation of antibiotics before transfer. By raising awareness of risk factors, it could change practices. **Acknowledgements:** The support of LAMB Hospital midwives and medical records staff is acknowledged.

P36 (060)

In a High-risk Community: Are Perinatal Characteristics Reported in Health Surveys and Walk-in Hospitals Comparable?—An Observational Study from Yemen

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Background: Yemen is a least-developing country, with high mortality, poor health standards, deteriorating socioeconomic conditions, and increasing poverty. **Objective:** Compare the perinatal characteristics reported in the 1997 Yemen Demographic and Health Survey (DHS) and hospital records, covering the same 5 years preceding the survey and 5 years after. **Methodology:** This was a retrospective and observational study. Data on total births, proportions of preterm deliveries, low birth-weights (LBWs), delivery complications, and the percentage of prolong/obstructed delivery and excessive bleeding of the total recorded complications were obtained from DHS. It did not report on stillbirths or perinatal mortality rate (PMR). Similar data were obtained from Al-Sabeen, the largest walk-in hospital located in Sana'a, Yemen, for the same 5 years (1993-1997), and 5 years after the survey (1998-2002), including stillbirths and PMR. Taylor series 95% confidence interval (CI) relative risk (RR) was used for comparing data. **Results:** DHS reported 5.7% of home deliveries as preterms compared to 5.8% in hospital for the first 5 years, and 6.1% in the second 5 years (RR 1.02; 95% CI 0.92-1.11, and 1.08; 0.99-1.18) respectively. LBW was 27.6% vs 28.4% (1.03; 0.99-1.07) and 27.7% (1; 0.97-1.04) respectively. Similarly, the percentage of obstructed/prolonged deliveries was 39% vs 40.2% (1.03; 0.98-1.1) and 39.9% (1.03; 0.99-1.07) and excessive bleeding 20.1% vs 21.6% (1.07; 0.98-1.18) and 19.4% (0.96; 0.9-1.03) respectively. Delivery complications of total births were 3 times higher in DHS than in the hospital records, 47.5% vs 17.4% (0.37; 0.32-0.43) and 16.6% (0.35; 0.30-0.41) respectively. In the second 5-year period, the hospital data showed 4% increase in PMR (90 vs 86.7/1,000 births) and 33% increase in stillbirths (56.4 vs 42.5/1,000 births). **Conclusion:** In a community with walk-in public hospitals, poor health standards, deteriorating socioeconomic conditions, increasing poverty, and hospital perinatal characteristics may represent important trend indicators in the community.

Environmental and Other Influences on Severity Patterns among Neonates with Jaundice in Port Harcourt, Nigeria

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Background: Neonatal jaundice is a major paediatric problem and is often associated with serious complications in Nigeria. Earlier reports from Lagos, the industrial capital of the country, Zaria, and other parts of the country indicated that neonatal jaundice was a major contributor to cerebral palsy. Furthermore, neonatal jaundice tended to be more severe in babies born outside hospital. This study was, thus, needed to be undertaken to evaluate the situation in Port Harcourt, the nerve centre of the oil industry in Nigeria. **Objective:** Determine the course of neonatal jaundice among inborn and outborn babies seen at the University of Port Harcourt Teaching Hospital, Nigeria. **Methodology:** A prospective study of neonates with serum bilirubin above 170 micromols/L was carried out from 1 July to 31 December 2002. Social, biological and laboratory data were entered into specially-designed questionnaires. Subsequently, statistical analysis was carried out using the chi-square test, Student's *t*-test and a multiple regression model, and graphs were constructed using the GraphPad Software, Prism 2.01. **Results:** Forty-four of 206 babies had jaundice giving an overall incidence of 21.4%. Eighteen (40.9%) of the 44 babies were inborn, while 26 (59.1%) were outborn. Jaundice was more severe in outborn babies, and the best predictor of kernicterus was the peak serum bilirubin concentration (Partial F-test=5.8830 and the overall F-statistic=20.73, df=6). The case-fatality rate was 34.1%. However, 60% and 40% of deaths occurred among outborn and inborn babies respectively. **Conclusion:** Neonatal jaundice was more severe in outborn babies, the dominant reason for which appears to be considerable delay in presenting to hospital. Probable causes of the delay included ignorance of jaundice on the part of parents and some adverse social and environmental influences. There is, therefore, a need for more vigorous efforts focused at the community level aimed at surmounting some social and environmental barriers and to encourage mothers to present early in hospital as soon as jaundice is noticed. **Acknowledgments:** The authors are grateful to the service laboratories for their support throughout the duration of the study.

Relationship between Antenatal-care Visit of Mothers and Birth-weight of Baby in a Rural NGO Hospital

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Background: Baby born with low birth-weight is vulnerable to infection and death. Low birth-weight is a negative health indicator affecting millions of children and is severe in Bangladesh. Positive pregnancy outcome, such as birth-weight of baby, is directly related to antenatal care (ANC) visit of mother. However, this issue in LAMB Hospital may help for child survival and health development. **Objective:** Find out the relationship between ANC visit of mothers and birth-weight of baby. **Methodology:** In this cross-sectional analytical study, 101 women who had delivered babies at rural setting LAMB Hospital were interviewed. It was conducted during March-June 2003. Mothers who had delivered babies within 37-41 week gestation were interviewed. Before 37 week and after 41 week gestation, delivered twin babies, dead babies, and unwilling to give interview were excluded. Convenient sampling technique was employed for data collection. Data were analyzed and results obtained employing statistical tests. **Results:** Babies born with low birth-weight (24.8%) and birth-weight $\geq$2,500 g (75.2%) showed a significant relationship with ANC visits of mothers (p=0.019). The mean birth-weight of babies of mothers who had no ANC visit (16.8%) was 2494.12$\pm$371.76 g, 1-3 visit(s) (30.7%) was 2698.39$\pm$435.02 g, 4-6 visits (25.7%) was 2,823.08$\pm$442.09 g, 7-9 visits (21.8%) was 2,784.09$\pm$351.31 g, and >9 visits (5.0%) was 3,170.0$\pm$831.11 g (p=0.024). Birth-weight of babies increased with increased ANC visits of mothers. The mean number of ANC visits was 5, and a maximum of 30.7% of the mothers received 1 to 3 visit(s). **Conclusion:** Antenatal care received by mothers has a significant relationship with birth-weight of babies. Therefore, appropriate measure should address immediately. **Acknowledgements:** Approval of the research protocol by the authority of NIPSOM, scholarly guidance by Associate Professor Dr. Md. Amirul Hassan, and facilitation and cooperation of the LAMB Hospital authority for data collection are duly acknowledged.

Newborn Screening in Bangladesh—An Experience of Screening of 16,000 Newborns for Detection of Congenital Hypothyroidism

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Background: Newborn screening for early detection of some selected diseases is an established programme in developed countries for almost 40 years. In many developing countries also, the programme is gaining attention. In Bangladesh, newborn screening is still a new concept. Currently, a pilot project is going on where newborns are screened for the detection of congenital hypothyroidism. **Objective:** Explore the possibility of starting a national programme of newborn screening in Bangladesh. **Methodology:** A few drops of blood are taken from the umbilical cord in a pre-supplied filter paper during delivery of newborns. The blood spots are then dried and sent to the laboratory either by hand or by post for estimation of whole blood thyroid stimulating hormone (TSH) for the detection of congenital hypothyroidism. TSH is measured by immunoradiometricassay (IRMA). A cut-off value of 20 mIU/L is taken as cut-off value. **Results:** The initial attempt of newborn screening in Bangladesh has got positive response from the concerned quarters. Till now, 16,000 newborns have been screened from different parts of the country. Of these, 6 babies have been identified as born with congenital hypothyroidism. **Conclusion:** Newborn screening is a highly ambitious project for a country like Bangladesh. Fund to run such a programme is the biggest constraint. The social pattern where still more than 80% of deliveries are done at home is another big challenge. However, technical capability and also enthusiasm of professionals are there. Now it needs support of the government and other international bodies to start screening babies born at hospitals. **Acknowledgements:** The technical support of the International Atomic Energy Agency and the financial support of the Ministry of Science and Information and Communication Technology, Government of Bangladesh, are acknowledged.

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Trend in Isolation of *Vibrio cholerae, Shigella,* and *Salmonella* in Neonates with Diarrhoea Admitted to ICDDR,B Hospital, Dhaka, Bangladesh

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Background: Enteropathogenic *Escherichia coli* (EPEC) and viruses largely cause neonatal diarrhoea. Very little has been reported about infection due to *Vibrio cholerae, Shigella,* and *Salmonella* spp. in neonates presenting with diarrhoea. **Objective:** Determine the trend in isolation of *V. cholerae, Shigella,* and *Salmonella* spp. in neonates suffering from acute diarrhoea. **Methodology:** The study population included 240 neonates—both male and female—who were admitted with acute diarrhoea and other medical complications to the inpatient department of ICDDR,B Hospital, Dhaka, Bangladesh, in 2001. Their hospital records were reviewed retrospectively. On admission, their rectal swabs or stool specimens were plated directly onto taurocholate-tellurite gelatin agar (TTGA), *Salmonella-Shigella* (SS) agar, and MacConkey's agar for culture of *V. cholerae*, *Shigella, and Salmonella* spp. **Results:** A single enteric pathogen was detected in 71 (29.5%) neonates, and multiple pathogens were detected in 12 (5%) neonates. Enteropathogens were identified as follows: *V. cholerae* O1 in 42 (17.5%), *Shigella* spp. in 22 (9.1%), *Salmonella* spp. in 8 (3.3%), *Aeromonas* spp. in 9 (3.7%), and *Hafnia alvei* in 2 (0.8%) neonates. Stool culture of other neonates revealed no organism. Serotypes of isolated *V. cholerae* included ElTor Inaba (37), ElTor Ogawa (1), non-O1 (1), and O139 (3). Isolates of *Shigella* spp. included *Shigella flexneri* (17), *S. dysenteriae* type 2-12 (3) and *S. sonnei* (2). Isolated *Salmonella* spp. included group B, C, D, E, and G. **Conclusion:** The findings of the study suggest that *V. cholerae* and *Shigella* infection may be common in neonates which is of public-health importance for creating awareness among physicians about management strategy of neonatal diarrhoea. **Ackowledgements:** The research was supported by the ICDDR,B: Centre for Health and Population Research, which is supported by countries and agencies which share its concern for the health problems of developing countries.

P41 (307)

Hospital-based Maternal and Perinatal Death Audit: A New System Promising Improved Care for Mothers and Newborns

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Background: Bangladesh has one of the highest maternal and perinatal death rates. To reduce this high burden, a hospital-based maternal and perinatal death audit system is being piloted at the Department of Obstetrics and Gynaecology of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. **Objective:** Review systematically all maternal and perinatal deaths in the ward, find out the causes of deaths and risk factors for death, and look for solutions that reduce maternal mortality ratio (MMR) and perinatal mortality rate (PNMR). **Methodology:** The piloting hospital will maintain a birth register to record all deliveries, and all perinatal and maternal deaths will be recorded in two different registers where delivery details, primary and final causes of perinatal and maternal death, and avoidable risk factors will be documented separately. Data from these registers will, then, be fed into a computer software called PPIP (Perinatal Problem Identification Program), and the software will calculate PNMR and MMR of the hospital using appropriate formula. **Results:** Proper auditing of perinatal and maternal deaths in the hospital cannot only save newborn lives by averting avoidable deaths, it can also identify the risk factors perpetuating such deaths in the hospital facilities. Therefore, through analysis of audit findings, the service providers can easily identify health system deficiencies that need to be addressed to avert perinatal and maternal deaths in hospital settings. **Conclusion:** Proper implementation of the PNDA system is likely to enhance accountability of service providers and to improve health system performance in the country. **Acknowledgements:** The assistance of the Saving Newborn Lives, Bangladesh Programme is greatly acknowledged.

P42 (318)

Evaluation of Skills of Community Health Workers to Recognize and Manage Sick Neonates in the Community

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Background: A community-based neonatal health intervention project is being implemented in Sylhet, Bangladesh. One of the two models being tested utilizes the services of community health workers (CHWs) to educate families on birth and newborn care, deliver healthcare services, including recognition and management of neonatal infection in the communities. They use a clinical management algorithm adapted from the IMCI algorithm based on the findings of the WHO Young Infant Study. **Objective:** Measure skills of CHWs in assessing and managing sick newborns in low-resource community settings. **Methodology:** CHWs received 5-week training with extensive focus on newborn care, including management of sepsis. The training included clinical case presentation in a tertiary-level hospital. Post-training evaluation assessed the performance of trained CHWs on the use of the algorithm and case management of sick newborns in comparison to trained physicians (gold standard). The assessment consisted of hospital-based and community-based components. **Results:** Each CHW assessed 18 cases—10 in hospital and 8 in the community. Each case comprised 3 components: clinical evaluation, classification, and management. In the hospital, 35 of 40 CHWs passed the test demonstrating adequate knowledge and skill. Five CHWs who failed made accurate clinical assessment and disease classification and passed all components after retraining. All CHWs passed the community assessment. **Conclusion:** It was evident from the assessment exercise that, if adequately trained and exposed to clinical cases, a cadre of community-level workers with education around tenth grade will have adequate clinical skills to assess and manage neonates with serious sicknesses. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and Saving Newborn Lives Initiative (SNL) of Save the Children–USA through a grant from the Bill and Melinda Gates Foundation, is acknowledged.

P43 (038)

Breast-feeding Practices of Mothers of Infants: A Study in a Tertiary-care Facility

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Background: Exclusive breast-feeding (EBF) practice plays an important role in optimum growth and development during the early part of life. It improves the nutritional status and reduces the chances of contracting infection, thereby reducing morbidity and mortality. Mother's perception and practice is the key factor facilitating EBF. **Objective:** Assess the extent of practice of EBF and factors facilitating it among mothers. **Methodology:** In a cross-sectional study, 800 mothers were interviewed using a pre-designed and pre-tested questionnaire during November 2002-April 2003 at the Immunization Clinic of S.C.B. Medical College, Cuttack, India. Prior to the interview, informed verbal consent was obtained from mothers. The definition of EBF as per the ongoing reproductive and child health (RCH) programme was followed in the present study. Data were analyzed by chi-square test and percentage. **Results:** 652 (81.5%) mothers were from urban locality and the rest from rural areas; 120 (15%) belonged to higher socioeconomic status, 400 (50%) were from middle socioeconomic status, and the rest belonged to lower socioeconomic status. Fifty-one (6.37%) subjects did not breastfeed at all. Of those who had practised breast-feeding (749), 440 (58.74%) resorted to EBF and the rest 309 (41.2%) to non-EBF. Colostrum feeding was practised by 361 (48.2%) infants; of them, 289 belonged to the EBF group and 72 to the non-EBF group. Breast-feeding was initiated by 204 (27.23%) infants within one hour, 433 (57.81%) within 24 hours, and 112 (14.95%) after 24 hours of delivery. The percentage of children who started breast-feeding within one day of birth did not vary much in regard to the residential status or the type of assistance during delivery. Mother's socioeconomic and educational status favourably influenced the early initiation of breast-feeding. **Conclusion:** The practice of EBF was only 58.74%, which needs further improvement by suitable focused intensive information, education, and communication activity. **Acknowledgements:** Cooperation extended by the staff of the immunization clinic is acknowledged.

P44 (097)

Distribution and Impact of Nutritional Status among Children Admitted with Severe Pneumonia to a Rural Hospital at Matlab, Bangladesh

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Background: Pneumonia, the severe form of acute respiratory infection (ARI), is the leading cause of childhood mortality in developing countries, including Bangladesh. However, the relation of this disease with nutritional status is not well-addressed. **Objective:** Assess the distribution and impact of nutritional status of children admitted with severe pneumonia. **Methodology:** The study was conducted at the rural treatment centre of ICDDR,B: Centre for Health and Population Research at Matlab. An ARI surveillance had been ongoing since 1999 among all children aged less than 5 years in a defined population of about 110,000. Community Health Research Workers (CHRWs) detected children with pneumonia during their monthly home visits following the WHO algorithm. Data were analyzed from children, aged 2-59 months, admitted to the Matlab hospital, with severe pneumonia during January-December 2000. **Results:** During the study period, 436 children were admitted with predominance of male admission (63% vs 37%, $p<0.05$). The mean z-score of height-for-age (HA), weight-for-age (WA), and weight-for-height (WH) was -1.48, -2.00, and -1.35 respectively. About 34% of them were stunted, 51% were underweight, and 28% were wasted. Most (71%) children admitted were associated with wheezing. Seventy-six percent of pneumonia without wheeze was contributed by infants (2-11 months) compared to 27% with wheeze ($p<0.05$). Stunted children (HA $\leq$-2 z-score) without wheeze had longer duration of hospital stay and fast breathing compared to non-stunted children ($p<0.05$). **Conclusion:** Pneumonia without wheeze is more severe if the child is stunted. Also, younger children suffer more with pneumonia without wheeze while older children with wheeze.

P45 (108)

Impact of Periodic Deworming with Albendazole on Nutritional Status of Children in an Urban Slum of Kolkata

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Background: Intestinal helminths are a worldwide problem, especially among children of developing countries. Nutritional status of children with worm infestation may be adversely affected particularly if their food intake is marginal in quality and quantity. **Objective:** Study the impact of periodic deworming on the nutritional status of children. **Methodology:** A double-blind, placebo-controlled, randomized, community-based intervention trial was undertaken in Tiljala slum area of Kolkata. In total, 700 children aged 24-60 months were enrolled. Baseline demography was followed by nutritional assessment (weight-for-age) of the children. Albendazole/placebo was administered twice at 6 monthly interval; the study children receiving 400 mg of albendazole in liquid form in vitamin B complex base and the control group children receiving only vitamin B complex, in identical form and taste. Measurement of weight was done once before albendazole/placebo administration, twice thereafter (at 3 monthly interval) and the fourth, 3 months after the second dose of albendazole/placebo administration. Stool samples were collected from a sub-sample of the study children and examined for presence of ova before and every 3 months after deworming. **Results:** Analysis of proportional weight gain of the children at the end of one year showed a weight gain of >10% in 45% of the study children and 30% of the control children ($p<0.01$, CI 1.08-1.66). Examination of stool samples revealed a significant reduction in *Ascaris* load only in the albendazole-treated group in all 3 monthly screening. **Conclusion:** This study showed that periodic deworming with albendazole not only reduces the worm load of the children in the community but also plays an important role in improving their nutritional status.

Wheat Bread-baking Flour Enriched by Vitamin-Mineral-added 'KAP Complex No.1'

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Background: Iron deficiency became an important social problem in Uzbekistan and one of the global problems for the last 10-15 years. Medical-demographic investigations in the Republic of Uzbekistan showed that 61% of children and 60. 4% of mothers suffer from iron-deficiency anaemia. It is recommended to work out and accomplish programmes for fortification of iron products in regions where more than 40% of people suffer from anaemia. **Objective:** Determine the nutrition value of wheat bread-baking flour enriched by preparations of iron and vitamins, that is vitamin-mineral-added 'KAP Complex No.1'. **Methodology:** Bread-baking flour enriched by vitamin-mineral-added 'KAP Complex No.1' was the material of investigation. Appearance index of enriched flour was defined by GOST-9404, moisture by GOST-27650, protein by method of Kjeldal, fat by Sokslet, carbon and energy value by calculation methods, Vit B_1 by GOST 29138, Vit B_2 by GOST 29139, Vit B_3 by GOST 29140, and iron by GOST 26928. **Results:** Wheat bread-baking flour of first sort and flour 'Uzbekistan' were enriched with vitamin-mineral-added 'KAP Complex No.1'—making of soft flour of 'local' sorts or from soft flour mixed with more than 30% of flour of 'local' sorts, which corresponds to demand of GOST by appearance and physical-chemical indexes, and without irrelevant smells and taste, not mould, not sour, and not bitter. Researching of the nutrition value of the enriched flour showed that there are 10.6% of protein, 1.3% of fat, 67.1% of carbons, and 331 kcal of energy value. The enriched flour was rich in vitamins and microelements: 2.0 mg/kg–Vit B_1, 3.0 mg/kg–Vit B_2, 10.0 mg/kg–Vit B_3 and 1.5 mg/kg–folium acid, 50 mg/kg of elemental electrolytic iron, and 22.0 of mg/kg zinc. Technical conditions were worked out, confirmed by the Ministry of Public Health and GOST standard of the Republic of Uzbekistan: "The wheat bread-baking flour enriched by vitamin-mineral-added 'KAP Complex No.1'." **Conclusion:** In present time, flour enriched by iron elements and vitamins is produced in researching regions (Karakalpakistan, Horezm, Fergana, Jizzak, and Tashkent). **Acknowledgments:** The financial support of the Second State Tashkent Medical Institute is acknowledged.

Study on Chemical Properties of Fats of Some Varieties of Fatty Fish of Bangladesh

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Background: There are about 144 varieties of fish in Bangladesh. Fish oil is characterized by w-3 long-chain fatty acids, making it beneficial for people with heart diseases. However, no study on the nature of fish fat has been done so as to conclude the beneficial aspects of particular fish. **Objective:** Identify the beneficial aspects of high-, medium- and low-fat fish usually consumed in Bangladesh. **Methodology:** Three categories of fish as high-fat fish, pangas and hilsha, medium-fat fish as rui and sarputi, and low-fat fish as boal and bele were analyzed for content and type of fat. Dorsal and ventral portions of each type of fish were separated and homogenized, and lipid fraction was collected. Chemical properties as iodine value, saponofication value, etc. were done for the degree of unsaturation and chain length of fatty acids. **Results:** Analysis of the nature of muscle from the 3 categories of fish revealed that the lower the fat content, the higher the moisture content and vice-versa—the moderately high iodine value of lean fish might be due to the presence of PUFA which is beneficial for cardiac health. Lean fish had 0.94-1.44% of total fat, medium-fat fish had 4.9-7.4% of fat, and high-fat fish had 11.05-11.25% of fat. Fat contents of the 3 classes of fish were lower in the dorsal than in the ventral portions. The high iodine value of lean- and medium-fat fish was due to the presence of unsaturated fatty acids. However, high-fatty fish, such as hilsha and pangas, showed contrasting results. The fat of pangas revealed more short-chain and saturated fat with low molecular weight fatty acids which are less beneficial for health. On the other hand, the fat of hilsha contained a considerable amount of unsaturated long-chain fatty acids which are capable of reducing high serum lipids. **Conclusion:** Increased intake of hilsha and low-fat fish is recommended for people who are vulnerable to cardiac problems. Limited intake of pangas is also recommended.

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Correlation of Maternal Anthropometry with Low-birth-weight Babies

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Background: Birth-weight of less than 2.5 kg is termed as low birth-weight (LBW). LBW is associated with high infant mortality and morbidity and growth faltering. Incidence of LBW is high (about 45%) in Bangladesh. The causes of LBW besides maternal malnutrition and maternal care have not yet been analyzed. **Objective:** Find out whether maternal characteristics, such as age, weight, stature, body mass index (BMI), anaemia, etc., affect birth-weight. **Methodology:** Fifty mothers having low-birth-weight babies and an equal number of mothers having normal-weight babies were randomly selected from the maternity ward of Dhaka Medical College Hospital and Addin Hospital in Dhaka city. The babies were weighed soon after birth. A structured questionnaire was used, and medical reports were collected. Anthropometry of mothers was taken using appropriate tools. **Results:** 56% of LBW deliveries occurred to mothers aged 15-20 years. 64% of LBW babies had their mothers' weight below 40 kg. In contrast to only 10% in mothers of normal-weight babies, stunted mothers (less than 145 cm) also delivered higher number (44%) of LBW babies. Higher BMI was associated with higher LBW of babies, contributed by oedema. **Conclusion:** Maternal weight, height, and BMI have positive effect on birth-weight. Greater care is needed for teen-age pregnant mothers to upgrade their nutritional status.

Exploring Deaths among Adolescents to Find Ways to Improve Quality of Life

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Background: BRAC, a national development organization, showed that, in 34 upazilas, the crude death rate was 2.6 per 1,000 population. The health intervention of BRAC is a combination of preventive, curative and rehabilitative health services, which are also actively collaborating with different government health programmes. As part of the BRAC health programme, the nutrition initiative was developed in 1991 and is continuing till date. As part of its programme activities, information on all deaths is recorded. An analysis of these records was done for the period from March to December 2002. **Objective:** Collect death-record and information about events leading to death and conduct verbal autopsies to formulate policies and to guide the programme towards rationale interventions. **Methodology:** All deaths are recorded in all the 34 upazilas, where there is a nutrition programme. Of the 34 upazailas, 15 were randomly selected to record all events of death and specific verbal autopsy as and when required through interviews with a household member by the Community Nutrition Promoters. **Results:** The highest percentage of deaths (41%) occurred in the age group of 60+ years, followed by those aged less than 5 years (29%). Results of analysis of only total adolescent deaths showed that the major cause of death was suicide (21%), the second major causes were PUO and cancer (9%), and then accidental death (8%) and snake bite (3%). The highest cause of adolescent death in both boys and girls was suicide, followed by accidental deaths in boys and pregnancy-related deaths in girls. Among the obstetric causes of death, the major cause was eclampsia (36%) and postpartum haemorrhage (36%). **Conclusion:** The community should be motivated to encourage newly-wed couples not to have their first child before women are 20 years of age or have a body mass index of more than 20. Maternal death, especially if the pregnant girl is an adolescent, should be investigated. Communication channels and media that are most capable of reaching and influencing the target groups should be user-friendly, culturally and socially appropriate, practical, brief, relevant, technically corrected, and in colloquial language. Last but not the least, improvements in general living conditions, healthy lifestyle, particularly with regard to diet, sanitation, and other public-health measures, preserving fitness and preventing morbidity, and reducing mortality at all ages are necessities.

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Exploring Events Leading to Deaths among Children to Find Ways to Improve Quality of Life

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Background: BRAC, a national development organization, showed that, in 34 upazilas, the crude death rate was 2.6 per 1,000 population. The health intervention of BRAC is a combination of preventive, curative and rehabilitative health services, which are also actively collaborating with different government health programmes. As part of the BRAC health programme, the nutrition initiative was developed in 1991 and is continuing till date. As part of its programme activities, information on all deaths is recorded. An analysis of these records was done for the period from March to December 2002. **Objective:** Collect death-record and information about events leading to death and conduct verbal autopsies to formulate policies and to guide the programme towards rationale interventions. **Methodology:** The numbers of all deaths are recorded in all the the 34 upazilas, where there is a nutrition programme. Of the 34 upazilas, 15 were randomly selected to record all events of death and specific verbal autopsy as and when required through interviews with a household member by the Community Nutrition Promoters. **Results:** Male children aged less than 5 years died more than females (52% vs 48%) of the same age. Female deaths occurred more in the age group of 6-35 years, after which male deaths occurred more. Of the total deaths, 29% occurred in children aged less than 5 years, among which more than 50% were infants. Among children aged less than one year, 54% of deaths occurred in the first 28 days of life. Among neonatal deaths, 66% died within the first week. 48% died in the first day. 10% of deaths occurred in the first hour of their life. The first 24 hours of a child's life was very critical for survival. **Conclusion:** Community health workers should receive training to teach family members on methods to detect pneumonia using two simple signs, the breathing rate, and chest in-drawing. Furthermore, to prevent child malnutrition, the course of only breast-feeding for 180 days of a child's life should be inspired as it not only provides special nutritional benefits to the infants but also protects against diarrhoea, cough, and cold, and other life-threatening illnesses, including measles, diphtheria, and whooping cough. Communication channels and media that are most capable of reaching and influencing the target groups should be user-friendly, culturally and socially appropriate, practical, brief, relevant, technically corrected, and in colloquial language. Improvements in general living conditions, healthy lifestyle, particularly with regards to diet, sanitation and other public-health measures, to preserve fitness and prevent morbidity and reduce mortality at all ages are necessities.

Breast-feeding Status of Infants Belonging to Upper Socioeconomic Strata

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Background: The importance of exclusive breast-feeding in early infancy and its benefits on child health are well-recognized. WHO and UNICEF recommend exclusive breast-feeding during the first 6 months of life. Studies on breast-feeding patterns among the low-socioeconomic groups in India have demonstrated very low exclusive breast-feeding rates. However, there is very little literature available from India on the breast-feeding patterns of affluent women in the country. To implement any national programme, it is important to assess the breast-feeding status of infants belonging to affluent populations in the country. **Objective:** Study the breast-feeding patterns of women belonging to the upper socioeconomic strata from the western part of Delhi and gain insights into reasons for following a particular breast-feeding pattern. **Methodology:** The study was carried out in two phases: the first phase was quantitative, whereas the second phase used qualitative research techniques. In phase I, 50 mother-infant pairs were enrolled randomly from private wards of hospitals and nursing homes in West Delhi. Infants were enrolled within 3 days after birth and followed up for 16 weeks by 4 weekly household surveillance to assess their breast-feeding status. Data were collected using two pretested questionnaires. The breast-feeding status of infants was categorized as exclusive, predominant, partial, and non-breastfed at each of 4 weekly household visits during 3-16 weeks of age. **Results:** The results showed that exclusive breast-feeding rates were the highest during the first 4 weeks after which they declined during the next 12 weeks. The rates of exclusive breast-feeding were, however, very low at birth because of administration of prelacteal feeds at birth. Predominant breast-feeding was common, and weaning started early in life. Ninety-four percent of the infants were, however, breastfed at 16 weeks of age. Sex, parity, mode of delivery, age of mother, education or occupation of parents, religion, or type of family showed no association with the breast-feeding status of the infant. In phase II, two focus-group discussions, each consisting of 5-7 breast-feeding women, were conducted to gain insights into knowledge and practices relating to breast-feeding. Customs and traditions clubbed with lack of proper information on advantages of exclusive breast-feeding were the main factors affecting breast-feeding practices. **Conclusion:** Findings of the study suggest that there is an urgent need for increasing awareness among women regarding the benefits of exclusive breast-feeding, and programmes must be directed towards this issue.

Fall of Hilsha Production and Its Impact on Nutritional Status of the People of Bangladesh

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Background: Hilsha is the national fish of Bangladesh. Its unique taste and nutritional value have made it the most popular fish not only in Bangladesh but in many parts of the world too. Production of hilsha has been reduced tremendously over the years causing a negative impact on the nutritional status of the Bangladeshi population. **Objective:** Find out the cause and effects of the fall of hilsha production and possible remedies to increase the production. **Methodology:** This is an analytical study. Based on the Yearbook of Bangladesh Statistics and with data from the Department of Fisheries and information from newspapers and magazines, the study was done. According to the yearbook, the production of hilsha fish in major rivers has been declining tremendously. After about 50% fall of the production from 77,325 tons in 1990-1991 to 44,672 tons in 1990-1991, the production increased in a gradual manner up to 1995-1996 (61,289 tons). But thereafter the production has been continuously declining, and in 1999-2000, it was only 49,786 tons. Although data for the current year could not be collected, it can easily be guessed that the production has further declined to a great extent for which the price of hilsha fish is beyond the reach of common people. **Results:** If the estimated population in 1989-1990 was 120 million while the hilsha production in major rivers was 77,325 tons, the per-capita consumption was 0.64 kg. And if the estimated present population is 130 million and the hilsha production is estimated to be 40,000 (based on the declining proportion) tons from the major rivers, the per-capita consumption stands at 0.30 kg, which is 106% less than the consumption rate as was 12 years ago. The major cause of this production fall is the current net using which about 125,000 metric tons of jatka (hilsha fry) are caught every year. If the weight of a piece of jatka is assumed to be 100 g and had it not been caught until it gains 1 kg of weight, the total weight of this huge quantity of jatka would be about 1.25 million tons, the total price of which would be 31,250,000,000 taka (@ 250 taka per kg). Along with other reasons, the tremendous fall of hilsha production might be a major cause of malnourishment of the people of Bangladesh, while hilsha seemed to be the most nutritious fish in Bangladesh containing the highest range of 200-400 kilo calorie per 100 g of fish along with high rate of energy (1,100-1,700/g), calcium (180-310), protein (21-38/g), and fat (19-35/g). **Conclusion:** It is obvious that if meeting the nutritional demands of Bangladeshi children is not cared for, about 60% of whom are malnourished, the nation will have a heavy burden of malnourished people in the future, and the present fragile economy will be bent to be broken.

Effects of Rice Consumption on Obesity of Korea Elementary Students

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Background: The food-consumption pattern in Korea has been changed since 1960s when industrialization for economic development was started. The trend for decreasing rice consumption has been revealed. **Objective:** Demonstrate the benefit of consuming rice for the Korean population to encourage rice consumption. **Methodology:** In total, 357 elementary male and female school students aged 9-13 years, divided into two groups—221 normal (±10%) and 136 obese (over 20%)—were included in this study. Nutritional screening tests were based on the anthropometric measurements and biochemical assessments. Food intake was collected by a 3-day record method. **Results:** The average rice intake of the normal-weight group was 172.2 g, and 166.7 g for the obese group. Rice intakes were not significantly different between the two groups. The average rice intake per kg body weight of the normal group was significantly higher than that of the obese group. Levels of height, weight, body mass index (BMI), and atherogenic index were negatively correlated with average rice intake per kg body weight of subjects. Levels of high-density lipoprotein cholesterol (HDL-C) were positively correlated with average rice intake per kg body weight of subjects. Levels of body weight, BMI, atherogenic index, blood glucose, triglyceride, and blood cholesterol were positively correlated with obesity rate. Levels of HDL-C were negatively correlated with obesity rate. The average energy and fat intake, including animal fat of the normal-weight group, was significantly lower than that of the obese group. The average energy intake from rice of the normal-weight group was significantly higher than that of the obese group. **Conclusion:** The results showed that the proper amount of rice intake beneficially affects for child health and maintaining normal body weight.

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Prevalence of Anaemia and Iron Deficiency among Young Children with Acute Diarrhoea in Bhaktapur

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Background: Iron deficiency is one of the leading nutritional deficiencies in the world, particularly in developing countries. Iron deficiency in young children is associated with impairment of cognitive performance, behaviour, and motor development. However, the detection of iron deficiency may be difficult due to infections, which are common in developing countries. **Objective:** Estimate the prevalence of anaemia and iron deficiency and its association with socioeconomic and anthopometric parameters of young children presenting with acute diarrhoea. **Methodology:** Blood samples of 1,419 children were analyzed for haemoglobin and plasma ferritin that had diarrhoea and were included in a clinical trial to assess the efficacy and effectiveness of zinc in Bhaktapur, Nepal. 0.5 g/dL was added to the cut-off of haemoglobin to adjust for the altitude of Bhaktapur (1,400 m), and anaemia was defined as haemoglobin <11.5 g/dL. Iron deficiency was defined as plasma ferritin level <g/L among children without elevated C-creative protein (i.e. <10 g/L). Iron-deficiency anaemia was considered when anaemia was associated with low ferritin level. **Results:** The mean haemoglobin concentration was 11.1 g/dL, whereas the mean ferritin level was 16.1 g/L. The prevalence of anaemia was 60.0% (95% CI 57.5-62.6). Iron deficiency was found in 39.5% of the children (95% CI 36.5-42.4). The prevalence of iron-deficiency anaemia was 26.4% (95% CI 23.4-29.4) in which 46% were infants. **Conclusion:** The prevalence of anaemia among children presenting with acute diarrhoea was high. However, iron-deficiency anaemia explained only less than half of the total anaemia prevalence, suggesting that other causes of nutritional anaemia may be prevalent in this population.

P55 (170)

Homestead Food Production Increases Consumption of Micronutrient-rich Foods

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Background: Micronutrient deficiency is still a widespread problem in Bangladesh, Nepal, and Cambodia. The bioavailability of vitamin A from plant foods is lower than previously assumed and, therefore, the intake of vitamin A from animal food products has to be promoted to meet nutritional requirements. **Objective:** Assess the impact on consumption practices of pilot projects that integrated animal husbandry with home-gardening activities. **Methodology:** Data were collected from 3 pilot projects in Bangladesh, Nepal, and Cambodia in 2001 and 2002 at baseline (n=544) and follow-up 10-12 months later (n=551). Data were obtained through household interviews. **Results:** The data showed that the number of eggs consumed by children and women of target households during the previous week had significantly increased. The consumption of chicken liver had significantly increased over 10-12 months following the baseline. The percentage of liver that was obtained from the own homestead production had also increased. Income that was generated by selling produce from their homestead food production was used for purchasing other high-quality and micronutrient-rich foods, including animal source foods. **Conclusion:** The data from the 3 pilot projects suggest that homestead food production increases both quantity and diversity of consumption of micronutrient-rich foods, especially eggs and liver. The increased consumption of micronutrient-rich foods can improve the intake of micronutrients, including vitamin A. Homestead food production plays an important role to improve household food security and to meet nutritional requirements of micronutrients. **Acknowledgements:** The contribution of data collectors and the households, participated in the pilot projects, is very much appreciated. The financial support of the United States Agency for International Development (USAID) is gratefully acknowledged.

Assessment of Nutrition and Health Status in Slums of Dhaka, Khulna, and Chittagong, Bangladesh

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Background: Rapid urbanization in Bangladesh is fuelling a growth in urban poverty, particularly in slums where the quality of life is extremely poor. This raises concern for nutrition and health of slum dwellers who live in extreme poverty and face a daily struggle to meet their basic requirements for food and access essential healthcare services. **Objective:** Assess the nutritional status and prevailing health conditions of slum dwellers in 3 slum areas. **Methodology:** Data were collected by the Helen Keller International and the Institute of Public Health Nutrition's Nutritional Surveillance Project from 7,509 urban slum households in Dhaka, Khulna, and Chittagong in 2002. **Results:** The proportion of urban slum households with an energy intake of <1,805 kcal/person/day, indicating 'extreme' poverty, varied considerably within the year and between the cities: it was the highest in Chittagong (45-58%), followed by Khulna (40-50%) and Dhaka (29-41%). The prevalence of stunting and underweight in children aged 0-59 month(s) was 'very high' in the surveyed slums throughout the year. Maternal chronic energy deficiency (body mass index <18.5 kg/m^2) ranged from 18% to 21% in Dhaka slums, 25% to 35% in Khulna slums, and 35% to 41% in Chittagong slums, indicating 'serious' food insecurity. The prevalence of diarrhoea among children aged 6-59 months was the highest in Chittagong slums (7.3%), followed by Dhaka (5%) and Khulna (1.5%). **Conclusion:** The findings show that child and maternal nutritional status in these slums is discouraging and varies between slums and within the year. To improve household food security, direct nutrition interventions are needed, together with interventions to improve access to healthcare, water and sanitation, employment opportunities, credit facilities, and food assistance, specifically addressed to people living in urban slums. **Acknowledgements:** The contribution of data collectors and the households, participated in the survey, is very much appreciated. The financial support of the United States Agency for International Developments (USAID) is gratefully acknowledged.

P57 (172)

Nutritional Status of Young Children and Their Mothers in Chittagong Hill Tracts, Bangladesh

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Background: The Chittagong Hill Tracts (CHT) has distinct geographical characteristics, ethnic diversity, and agricultural, dietary and cultural practices compared to the rest of Bangladesh. After signing the peace accord in 1997, CHT has become a focus of development efforts, and information on the nutrition and health situation of its people is needed. **Objective:** Assess the nutritional and health status of children and mothers in CHT. **Methodology:** The survey was conducted by the Helen Keller International and the Institute of Public Health Nutrition's Nutritional Surveillance Project in May-June 2000. A stratified multi-stage sampling design was used for selecting a total sample of 1,760 households with at least one child aged 0-59 month(s) from the 3 districts in CHT. **Results:** The prevalence of underweight (56%) and stunting (48%) in children aged 0-59 month(s) was 'very high' according to the international criteria, while the prevalence of chronic energy deficiency in mothers (body mass index <18.5 kg/m^2) indicated 'serious' food insecurity (43%). Poor complementary feeding practices, inadequate sanitation facilities, and a wide gender disparity in education were also of serious concern. **Conclusion:** In CHT, malnutrition is a very serious problem, both among children and mothers. Progress towards better nutrition and health requires a combination of policies and programmes to provide direct nutritional interventions to those who are malnourished, improve household food security, create a healthy environment, and increase access to basic health and social services. Special efforts are needed to deliver these interventions to the community people who live in remote areas and to adapt strategies to the unique needs of the different ethnic groups. **Acknowledgements:** The contribution of data collectors and the households, participated in the survey, is very much appreciated. The financial support of the United States Agency for International Developments (USAID) is gratefully acknowledged.

DAY 2: MONDAY, 8 DECEMBER 2003

11:30 am-12:30 pm: **Poster Session 2** (Venue: Lobby)

DIARRHOEAL DISEASE 2

P58 (124)

Control of Diarrhoeal Diseases in Afghanistan: A Case Study

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Background: The safe water system (SWS) consists of a bottle of 0.5-1% sodium hypochlorite solution to disinfect water at the point of use, along with a home water storage vessel to prevent water recontamination, and behaviour change communications to improve hygiene practices. Studies have confirmed that children in homes using SWS had 30-60% fewer episodes of diarrhoea. Population Services International (PSI) has social-marketed over 5 million bottles of water treatment solution globally. In 2002, PSI received funding from USAID to launch a Control of Diarrhoeal Diseases Programme, including SWS, in Afghanistan. **Objective:** Increase adults' healthy behavioural practices that result in prevention and improved management of diarrhoea in their children through: household water-quality improvement, hygiene practices, e.g. hand-washing, and rational home management of diarrhoea episodes. **Methodology:** Quantitative and qualitative operations-research methodologies are being used for understanding the barriers to hygiene-related behaviour change in target populations. PSI started the social marketing of *Clorin* water purification solution in Kabul in June 2003, with over 30,000 bottles sold to-date in the Kabul province alone. Nationwide distribution of *Clorin* and a water storage vessel will commence in the last quarter of 2003. Generic hygiene-promotion messages are aired on radio and TV. PSI has trained over 400 health workers to reach 25,000 mothers of small children through neighbourhood meetings. **Results:** An increase in healthy behavioural practices to prevent and manage diarrhoea is being demonstrated through changes from baseline to endline in KAP surveys and through sales and communications-rich data. **Conclusion:** SWS bridges the water and sanitation infrastructure gap in Afghanistan, providing an affordable, safe, and simple way to improve household water-quality. **Acknowledgements:** The support of USAID, CDC, UNICEF, WEDC, Afghan Ministry of Health, and NGO partners is acknowledged.

Knowledge Attitude, and Practice of Mothers of Children, Aged Less Than 5 Years, about Diarrhoeal Disease and Its Management: A Study in Urban Slum Area of Cuttack City, India

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Background: Diarrhoeal diseases are the second most common cause of mortality and morbidity among children, aged less than 5 years, of the developing world. The mainstay of prevention and control centres around the level of awareness among caregivers, particularly mothers. **Objective:** Assess knowledge, attitude, and practice of mothers of children, aged less than 5 years, regarding different aspects of diarrhoeal disease. **Methodology:** A cross-sectional study was undertaken in an urban slum from November 2002 to April 2003. 10% (n=150) of the total households were selected by systematic random sampling. Information was collected from mothers having one or more child(ren), aged less than 5 years, in a pre-designed, pre-tested schedule by door-to-door survey. **Results:** Of the 150 mothers, 82% attributed watery stool as a sign of diarrhoea, 72% increased frequency, 63% had mucoid stool, and 55% had blood in stool. 18% were ignorant about any one of signs of diarrhoea. To treat diarrhoea, 49% of the mothers mentioned about fluid therapy, 40% antibiotics, and 11% homeopathy, etc. 89% knew about oral rehydration solution and home-available fluids. About causes of diarrhoea, 38% ascribed polluted food, 15% unhygienic condition, 9% lack of nutrition, 7% seasonal change, and 12% worms. 81% opined that diarrhoea is preventable. The suggested preventive measures were as follows: using boiled drinking-water (84%), proper cooking/feeding (53%), hand-washing with soap (29%), use of sanitary latrines (33%), and clean environment (18%). Healthcare-seeking behaviour was as follows; no advice (11%), visiting hospital (45%), consulting pharmacist (21%), traditional healers (18%), and private doctors (5%). 12% of the subjects did not seek any advice. Dietary modification was practised by 79% of the mothers. 19% stated that breast-feeding should be stopped. **Conclusion:** There still exist some gaps among mothers in understanding diarrhoea. It needs to be addressed by intensified information, education and communication activities. **Acknowledgements:** The help and guidance of staff and faculties of the Jobra Health Centre are acknowledged.

P60 (030)

Impact of Training on Mothers of Infants in Controlling Diarrhoea

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Background: Diarrhoea is a very common disease of infants and if it is not checked may cause death of children. About 20.1% of deaths of infants is reported in Kanpur only. Lack of awareness among mothers of infants about the control measures against this disease is the main reason of child mortality. **Objective:** Evaluate the impact of training on mothers. **Methodology:** Training-cum-demonstration camps were organized in rural areas of Kanpur jointly by College of Home Sciences of C.S. Azad University of Agriculture & Technology, Kanpur and Navnirman Women Welfare Society, Kanpur. Three-stage random sampling was adopted for selection of blocks, villages, and mothers of infants. Kalyanpur and Patara blocks were selected. From each block, 5 villages were selected. From selected 10 villages, 150 mothers of infants were selected randomly. Training was organized in each village, and selected mothers from each village were trained. **Results:** 85 mothers had some knowledge about oral rehydration solution (ORS). Of these, 26 had heard of ORS through TV/radio programmes and 13 through contact with medical staff. Only 18 mothers could prepare ORS efficiently before training. The knowledge level of trained mothers improved significantly by exposures of calendars. Age had no significant relationship, whereas education and income had significant relationship with knowledge gained.

L-Histidine-supplemented Rice-ORS Reduces Diarrhoeal Stool in Cholera Patients: A Double-blind, Randomized Study

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Background: L-histidine, an amino acid, is known to inhibit cholera toxin-stimulated intestinal secretion of fluids and electrolytes in animals. **Objective:** Evaluate the therapeutic effects of L-histidine in reducing intestinal fluid loss in patients with cholera. **Methodology:** In a double-blind trial, 126 adults with infection due to *Vibrio cholerae* were given a rice-based ORS (CeraLyte-90) (n=64) or L-histidine mixed with CeraLyte-90 at a concentration of 2.5 g/L (n=62). CeraLyte-90 or L-histidine-mixed CeraLyte-90 was given orally as required to correct dehydration. In addition, all patients received ciprofloxacin, 500 mg 12 hourly for 72 hours. Fluid intake and output were monitored 8 hourly for 72 hours. **Results:** L-histidine significantly ($p<0.05$) and consistently reduced stool volume during 32-64 hours of treatment compared to the control group; mL/kg, 32-48 hours: 11.5±6.9 vs 18.8±16.0; 40-48 hours: 6.7±4.4 vs 11.5±9.7; and 56-64 hours: 6.3±5.8 vs 7.8±4.1. An overall stool reduction of 22% was observed during the entire course of illness. There was a significant reduction of unscheduled intravenous infusion and ORS intake in the histidine group compared to controls. The total duration of illness was significantly shorter in the L-histidine group compared to controls (hours, mean±SEM: 42.7±1.7 vs 47.0±1.8, $p<0.05$). No side-effects of L-histidine were observed. **Conclusion:** It is concluded that L-histidine reduces stool volume and duration of illness in cholera and could be a useful and safe therapeutic adjunct to increase success rate of standard therapy of cholera with ORS plus antibiotic. **Acknowledgements:** The financial support of the Cytos Pharmaceuticals, USA, is acknowledged.

P62 (119)

Efficacy and Safety of a Sucrose-based Hypoosmolar Oral Rehydration Salt Solution in Adults and Older Children with Cholera: Clinical Trial

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Background: A randomized clinical trial was conducted in children with acute non-cholera diarrhoea to evaluate a hypoosmolar oral rehydration salt (ORS) solution containing sucrose in place of glucose. Results of this unpublished study indicated that a sucrose-based hypoosmolar ORS might be highly absorption-efficient compared to WHO-UNICEF ORS. **Objective:** Evaluate whether a sucrose-based hypoosmolar ORS solution is more absorption-efficient and safe in adults and older children with cholera compared to standard WHO glucose-based ORS solution which is mildly hyperosmolar. **Methodology:** The study was conducted in the Infectious Diseases Hospital, Kolkata. In a double-blind, randomized, controlled clinical trial, 130 male patients aged 10-55 years were randomly assigned to receive either sucrose-based hypoosmolar ORS or WHO-ORS after initial rehydration with intravenous ringer's lactate solution. Both the groups received doxycycline (300 mg) as a single dose. Serum sodium was measured at the time of admission and on recovery. **Results:** In total, 130 adult male patients were evaluated for the study, of which 100 were positive for *Vibrio cholerae*. Of these, 52 received hypoosmolar sucrose-based ORS, and 48 cases received standard WHO-ORS. Patients of both the groups were comparable with respect to age, pre-admission duration of diarrhoea, frequency of stool before admission, initial body weight, serum sodium level on admission, and intravenous fluid requirement. The patients who received sucrose-based hypoosmolar ORS had statistically significantly shorter duration of diarrhoea, lesser stool output, and lesser ORS consumptions compared to the patients who received WHO-ORS. **Conclusion:** Sucrose-based hypoosmolar ORS is superior to WHO-ORS in the treatment of cholera in adults and older children.

Single-dose Ciprofloxacin is as Effective as 12-dose Erythromycin in Treatment of Childhood Cholera

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Background: Cholera is primarily an infection of children in endemic areas. Single-dose antimicrobial therapy has been found to be effective in the treatment of cholera in adults; however, that has not been possible in children. **Objective:** Compare efficacy of a single-dose ciprofloxacin with the standard, 3-day erythromycin therapy in childhood cholera. **Methodology:** Children, aged 2-15 years, with diarrhoea of $\leq$24 hours, severe dehydration, and dark-field stool microscopy positive for *Vibrio cholerae* were enrolled in the Dhaka and Matlab Hospitals of ICDDR,B. An informed consent was obtained from their parents/guardians. They were randomly assigned to receive either a single 20-mg/kg dose of ciprofloxacin or 12.5 mg/kg of erythromycin every 6 hours for 3 days. Children were hospitalized for 5 days, stool volume was measured every 6 hours, and stool culture was performed daily. Clinical and bacteriological failure was defined, if watery stools continued and if *V. cholerae* 01 or 0139 were isolated respectively after study day 2. Children were followed up 10-14 days and 4-6 weeks after discharge. **Results:** In total, 180 children were enrolled; 18 were excluded from analysis for various reasons. Therapy was clinically successful in 47/78 (60%) of ciprofloxacin- and 46/84 (55%) of erythromycin-treated patients (p=NS). Children receiving ciprofloxacin were less likely to vomit and had fewer stools and lesser stool volume ($p<0.05$ for both the comparisons) than those receiving erythromycin. Bacteriological failure was more frequent in ciprofloxacin-treated patients ($p<0.001$). Arthropathy was not observed in any patient. **Conclusion:** Single-dose ciprofloxacin is safe and clinically as effective as 12-dose erythromycin therapy for the treatment of childhood cholera and is associated with less vomiting. **Acknowledgements:** The support of the Bayer Pharmaceutical, USA and the New England Medical Center, Boston, USA, is acknowledged.

P64 (049)

Antimicrobial Resistance Trends of *Shigella* spp. Isolated during 1994-2000 in Malaysia

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Background: The emergence of drug resistance in *Shigella* spp. has been observed over the years. However, in Malaysia, data of resistant patterns in *Shigella* spp. are lacking. **Objective:** Determine the antimicrobial resistance patterns of Malaysian *Shigella* spp. **Methodology:** In total, 331 clinical strains of *Shigella* isolated during 1997-2000 from sporadic cases of endemic shigellosis in Malaysia were studied. *Shigella* spp. were identified by biochemical and serological tests at the Institute for Medical Research, Kuala Lumpur, Malaysia. Antimicrobial susceptibility tests were determined by the Bauer-Kirby disk-diffusion method. Antibiotic disks of 6 antimicrobial agents, such as ampicillin, tetracycline, streptomycin, kanamycin, chloramphenicol, and trimethoprim-sulphamethoxazole were tested. **Results:** 33.2% of all *Shigella* isolates tested showed full sensitivity, while 76.8% were highly resistant to at least one antibiotic. Overall, 63.4%, 54.7%, 45.9%, 43.5%, 39.9%, and 0.6% of *Shigella* spp. were resistant to streptomycin, tetracycline, ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, and kanamycin. *Shigella flexneri* was more resistant than other *Shigella* spp. A high rate of resistant to chloramphenicol was observed only in *S. flexneri*. All *S. dysenteriae* type 2 tested were resistant to streptomycin only. Sixteen resistant phenotypes were defined. Resistant phenotype PXV ($Amp^rSxt^rTe^rS^rC^r$) was the most prominent phenotype in *S. flexneri* (37.6%), whereas the prevalence of resistant phenotype PX ($Sxt^rTe^rS^r$) was the highest in *S. sonnei* (29.0%). **Conclusion:** The endemicity and prevalence of high resistance rates of *Shigella* spp. necessitate better control measures. The use of antimicrobial agents in empiric therapy should be reviewed annually to prevent misuse or excessive use of existing antimicrobial agents. **Acknowledgements:** The work was supported by the research grant IRPA 06-02-03-0750 from the Ministry of Science, Technology and Environment, Malaysia.

Distribution of *Shigella* Serotypes among Hospitalized Patients in Bangladesh and Their Antimicrobial Resistance

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Background: Shigellosis continues to be endemic among the disadvantaged populations. Antimicrobial therapy is indicated to reduce morbidity and deaths. However, it is often complicated by emergence of multiple drug-resistant *Shigella*. **Objective:** Document the isolation of *Shigella* spp. and their seasonal variations and antimicrobial resistance in Dhaka, Bangladesh. **Methodology:** This cross-sectional study was conducted at the Dhaka Hospital of ICDDR,B from January 2000 through September 2001. Fresh stool specimens were colleted for microscopic examination and culture for isolation of *Shigella.* Serogrouping and antibiotic sensitivity of the isolates were also done. **Results:** In total, 389 patients were enrolled. *Shigellae* were isolated from 227 (58%) patients, of whom 144 (63%) were children aged less than 5 years. *Shigella flexneri* (n=122; 54%) was the most frequently-isolated species, followed by *S. dysenteriae* (n=45; 20%), *S. boydii* (16%), and *S. sonnei* (10%). Among *S. flexneri,* 29 (24%) were 2a, 23 (19%) 2b, 21 (17%) type 1 (1a, 1b, and 1c), 16 (13%) 3a, 13 (11%) 4X, and 9 (7%) type 6. Among *S. dysenteriae* serogroup, *S. dysenteriae* type 2-7 were more frequently isolated (n=27; 60%), followed by *S. dysenteriae* type 1 (n=8; 18%) serotype. None of the *Shigella* strains was resistant to mecillinam or ciprofloxacin. Resistance to nalidixic acid was most frequent among *S. dysenteriae* type 1 (100%). *S. flexneri* was most frequently resistant to trimethoprim-sulphamethoxazole (74%), followed by ampicillin (66%) and nalidixic acid (34%). Among various *S. flexneri* serotypes, *S. flexneri* 2a was more frequently resistant to nalidixic acid than the other subserotypes (69% vs 13%, $p<0.001$). Isolation of *Shigellae* followed a bimodal distribution with the highest isolation during the hot summer and monsoon (March-July) months (number of isolates ranged from 20 to 25) and in winter (January, n=28). **Conclusion:** Systematic monitoring is needed to identify most-prevalent serotypes and to detect changes in the prevalence and antimicrobial resistance pattern. **Acknowledgements:** The financial support of the Swiss Agency for Development and Cooperation (SDC) and the United States Agency for International Development (USAID) is acknowledged.

Hypernatraemia as a Complication of Diarrhoea: Presenting Features and Outcome of Treatment

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Background: Hypernatraemia (serum sodium ≥150 mmol/L) is a serious complication of diarrhoea. However, little is known about its magnitude and associated morbidity and mortality, particularly from developing countries. **Objective:** Investigate the magnitude of hypernatraemia in diarrhoea patients and assess associated morbidity and deaths. **Methodology:** Records of all patients, admitted to the Dhaka Hospital of ICDDR,B, with diarrhoea and hypernatraemia from March 2001 to March 2002 were reviewed, and analysis of relevant information entered onto personal computer using structured templates was done. **Results:** In total, 7,212 patients were admitted to the longer-stay wards of the hospital, of whom 371 (5.1%) had admission hypernatraemia, and records of 312 (84%) of them were available for review. The median age of the patients was 5 months; nearly all had watery diarrhoea of median 3 days duration, all received ORS at home, and 55% were admitted during the first quarter of the year. Only 6.4% (19/299) of the infants were exclusively breastfed, 83% and 88% of the patients presented with fever and cough respectively in addition to diarrhoea, >50% had pneumonia, and 42% had severe underweight and/or bipedal oedema. Of the 312 patients, 59% had mild (serum sodium <160 mmol/L), 25% had moderate (serum sodium 160-169.9 mmol/L), and 16% had severe hypernatraemia (serum sodium ≥170 mmol/L). The overall case-fatality rate (CFR) was 15% and that for mild, moderate and severe hypernatraemia was 9%, 18%, and 35% respectively ($p<0.0001$). **Conclusion:** Hypernatraemia occurs in about 5% of patients admitted to the hospital with diarrhoea and associated problems, and it was more common during winter. Since hypernatraemia is not uncommon and is associated with high CFR, identification of its risk factors for prevention, early diagnosis, and efficient management is critically important. **Acknowledgements:** The study was supported by the Government of Japan and the United States Agency for International Development (USAID).

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Clinical Features, Complications, and Outcome of Critically Ill Hospitalized Children with Diarrhoea in Urban Bangladesh

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Background: In developing countries, many children attend hospitals with complications of diarrhoeal diseases, and there is a lack of information on aetiology of diarrhoea, clinical features, and outcome of such patients. **Objective:** Examine clinical features, including complications, laboratory findings, and outcome of critically ill children aged less than 5 years, admitted to hospital with diarrhoea and associated complications. **Methodology:** In this prospective study, all children aged less than 5 years, directly admitted to the Special Care Unit (SCU) of Dhaka Hospital of ICDDR,B from January 1999 through June 2003, were enrolled. Clinical and laboratory information and outcomes of patients were recorded and analyzed. **Results:** Over 7,000 critically ill patients were admitted to SCU during the study period, and 708 of them (from neonates to 80 years old) were enrolled (every tenth) into the study, of whom 584 (82%) were children aged less than 5 years. Of them, 76% were infants, 19% were aged 12-35 months, and the remaining 5% were aged 36-59 months with equal sex distribution. Neonates more often attended with shorter duration (<3 days) of diarrhoea (70% vs 52%, p=0.02) and less vomiting (68% vs 82%, p=0.04) than 1-11 month(s) old infants. Some and severe dehydration was present in 49% and 24% of the patients respectively. Although stunting was equally distributed, severe underweight and wasting were more frequently observed (41% vs 26%, p=0.002, and 8% vs 3%, p=0.01 respectively) among 6-11 months old infants compared to those aged 0-5 month(s). Sixteen percent of the study children had serum sodium ≤24 mmol/L, while another 16% had serum sodium that ranged from 125 to 129 mmol/L. Overall, 44% of the study children presented with hypokalaemia (potassium <3.5 mmol/L), and 8% had severe hypokalaemia (<2.0 mmol/L). About 12% had hypernatraemia (sodium >150 mmol/L, and another 12% presented with hyperkalaemia (>5.5 mmol/L). Forty-six percent had severe acidosis (serum TCO_2 <10.0 mmol/L). About 15% presented with hypoglycaemia (<3.0 mmol/L). Seventy percent had raised serum creatinine level >62.0 µmol/L. *Escherichia coli* (28%), *Pseudomonas* spp. (24%), and *Streptococcus pneumoniae* (20%) were common blood isolates. Overall, the case-fatality rate was 13%, and 14% of them had a positive blood culture, notably *Pseudomonas* spp. and *S. pneumoniae*. *Vibrio cholerae* O1 (31%) and *Shigella flexneri* (22%) were the most frequently-isolated bacterial enteric pathogens. **Conclusion:** Estimation of biochemical parameters of critically ill patients for prompt identification of problems, better diagnosis of disease, and early initiation of therapy is important. Laboratory investigations for isolation of organisms, particularly in blood, are critical in averting deaths due to bacteraemia or sepsis. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

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Risk Factors of Death among Children Presenting with Diarrhoea and Hypernatraemia

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Background: Hypernatraemia (serum sodium of ≥150 mmol/L) is a complication of diarrhoea associated with higher mortality. Identification of risk factors for mortality among children with hypernatraemia might help reduce deaths. **Objective:** Investigate risk factors of death among children with diarrhoea-associated hypernatraemia. **Methodology:** Records of all patients admitted to the longer-stay ward of Dhaka Hospital of ICDDR,B with diarrhoea and hypernatraemia between March 2001 and March 2002 were reviewed. In total, 7,212 patients were admitted, and 371 (5.1%) of them had admission hypernatraemia. Records of 312 of these patients were available for review, and relevant information from these records were entered onto computer using structured templates for analysis. **Results:** The case-fatality rate (CFR) among hypernatraemic children was 15%, and 38% of those who died had admission serum sodium of ≥170 mmol/L compared to 12% of those who survived ($p<0.0001$). Eight of 23 children who died and 40 of 86 survivors had a fall in serum sodium that exceeded 10 mmol/L per 24 hours ($p=0.3$). A higher proportion of children with a fatal outcome had admission respiratory distress (87% vs 62%, $p=0.001$) and convulsions (15% vs 5%, $p<0.01$) compared to the survivors. Bipedal oedema, hypoglycaemia (blood glucose <3 mmol/L), and hyperglycaemia (blood glucose >11 mmol/L) were significantly ($p<0.05$) less frequent among the survivors. Logistic regression analysis identified admission serum sodium ≥170 mmol/L (OR 2.9, 95% CI 1.3-6.9), bipedal oedema (OR 3.0; 95% CI 1.3-7.3), hypoglycaemia (OR 10.4, 95% CI 1.9-55.6), and respiratory distress (OR 7.5; 95% CI 2.0-27.5) to be independently associated with deaths. **Conclusion:** Hypernatraemic diarrhoeal children with a serum sodium of ≥170 mmol/L, bipedal oedema, hypoglycaemia, and respiratory distress are at higher risk of death. Presence of such factors needs to be carefully considered in managing hypernatraemia in children with diarrhoea. **Acknowledgements:** The financial support of the Government of Japan and the United States Agency for International Development (USAID) is acknowledged.

P69 (302)

Identifying Risk Areas for Childhood Diarrhoeal Deaths: Use of a Health Risk-assessment Tool

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Background: Diarrhoea still remains the leading cause of death among children, aged less than 5 years, in Bangladesh accounting for more than 230,000 deaths annually. However, little attempt has been made to determine the level of risks for childhood diarrhoeal deaths. **Objective:** Identify the districts that have a high level of public-health risks for childhood deaths from a diarrhoeal outbreak in the country, using a risk assessment tool. **Methodology:** Using representative data from secondary sources, a weighted health risk index (HRI) has been developed for determining vulnerability to childhood deaths from diarrhoeal diseases. The work has been carried out by the Bangladesh Center for Health Emergency Preparedness and Response during October 2002-March 2003. Using selected health and nutrition indicators as proxies for public-health risks, HRI has been computed from a risk-categorization scale that has assigned differential weightage to each of the health and nutrition indicators selected for this index. **Results:** Based on the prevailing health and nutrition situation in the districts, HRI has been able to measure, for each district, the level of public-health risks for childhood deaths from diarrhoeal diseases. The districts have also been ranked in accordance with the index score. **Conclusion:** The application of this risk-assessment tool can, prospectively, identify high-risk areas for childhood deaths from a diarrhoeal outbreak in the country. The tool offers promising opportunities for alert and response in terms of targeting resources for emergency response to diarrhoeal outbreak in the country in a way that can maximize public-health benefits through reducing deaths and minimizing risks to child health. **Acknowledgements:** The financial support of the World Health Organization, Bangladesh Country Office is acknowledged.

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Impact of Women as Providers and Co-Managers of Community Clinics

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Background: Women's use of formal health services is generally low in Bangladesh, while use of rural medical practitioners is common. Barriers to use of formal health services include lack of female providers and inconsistent implementation of a client-centred (specifically women-friendly) approach. **Objective:** Explore women's and children's use of community clinics staffed by all-female health workers and managed by a committee, including 2/3 women with leadership experience. **Methodology:** LAMB Project has facilitated development of 9 union-level Health Care Centres (HCCs) in Dinajpur and Rangpur districts staffed by women trained in general medicine and obstetric first-aid. Usage of general clinics for a 12-month period was analyzed by gender, plus use of obstetric services at clinic and village levels. Presence on the management Health and Development Committee (HDC) of experienced village health volunteers (VHVs) and women's group leaders is outlined. **Results:** Union clinic usage by female adults was 70%. 58% of children were males. Antenatal clinics were the busiest section, contributing to 70% of mothers making 3 or more antenatal visits. Use of safe delivery units is increasing. 83% of referred high-risk or complicated mothers presented for hospital delivery in 2002. VHV attendance at 63% of home deliveries, supported by strong referral ties to SDUs and LAMB contributed to an average perinatal mortality rate of 38 per 1,000 births. Women members of HDC have definite problem-solving roles. Only one trained community health assistant position has changed hands since training and services began in 1999. **Conclusion:** Having trained, experienced women with strong links and commitment to other women in the community involved with services and management may increase acceptability and continuity of services. Selecting and training local women may improve acceptability of public-health services. **Acknowledgements:** LAMB thanks partners PLAN International and DFID.

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Caring for Orphan Children of People Living with AIDS Lessens Burden of Social Pandemic

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Background: Children aged less than 15 years among detected people living with AIDS (PHA) in Bangladesh are only 17. Of them, around 10 have become orphan due to early death of their parents. Although, many of them are negative, they would also be abandoned totally from family and even from society. This poses tremendous challenges not only to healthcare and community systems but a silent burden to the society that would cope with responding to the pandemic. **Objective:** Reduce the effect of social stigma and discrimination of PHA and their children through continuous counselling, advocacy, and rehabilitation interventions. **Methodology:** Proper documentation of PHA, their children and family members are being made through visits and follow-ups by peer counsellors. Opportunistic infections, one of the major threats, are also being treated. For this, confidentiality is a major factor for both patients and their family members. Continuous counselling and rehabilitation plan for them and their children are being considered. Self-advocacy and establishment of rights of PHA for care, treatment, and rehabilitation are growing up. **Results:** Appropriate voluntary testing and counselling (VCT), follow-up, and care facilities at various levels are being set up. High-risk behaviour community is being motivated for VCT, follow-up, tests, and treatment in a confidential manner and are introduced with different skills training, jobs facilities, and income-raising activities. National policy and strategy on care and support for PHA and their family members are being revised. **Conclusion:** Hope, after proper implementation of revised national policy and action plans within next 3 years, a maximum number of orphan children of PHA will be rehabilitated.

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Satisfaction of Parents on Services Provided at an Under-five Clinic in a Non-government Rural Hospital

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Background: Under–five clinic is a clinic which provides promotive, preventive, curative and rehabilitative health services for children aged less than 5 years. Most of them are located in rural areas of Bangladesh, and the poor people of rural Bangladesh use those facilities. NGOs or government-NGO collaboration mainly operate the clinics. Some non-government missionary hospitals are also operating those clinics with the same intention. Now, it is important to assess the satisfaction of clients on the services provided by those clinics to know the quality of services or the expectations and needs of parents of those children. **Objective:** Assess the satisfaction of parents with services provided at an under-five clinic in a selected non-government rural hospital. **Methodology:** A descriptive cross-sectional study was conducted at the Under-Five Clinic of LAMB Hospital from March to June 2003. The study population was one consenting parent, either father or mother, of children attending the clinic. Selection was by non-probability purposive sampling. A pre-designed questionnaire was used for collecting information on accessibility, competence, timing, fairness and courtesy, availability of services, delivery system, and costing. Data were collected by face-to-face interviews at the exit point of the clinic, after exposure to every component of the service. **Results:** Of 109 parents surveyed, accessibility, competence, cleanliness, behaviour, and availability of different services were satisfactory for over 80%. Opening and closing time was satisfactory for 78% and 76% respectively. Waiting time for consultation was satisfactory for over 70%; drug-dispensing and pharmacy time for investigations was satisfactory for 67% and 53% respectively. Satisfaction with costs of different treatment components varied from 50% to 70%. **Conclusion:** A further study on waiting time and on treatment costs is recommended. Opening and closing time should be revised. Healthcare delivery can be modified according to parental needs/expectations. This study would also apply to other hospitals of rural Bangladesh. **Acknowledgements:** The research was conducted as part of the course requirements for an MPH degree at the National Institute of Preventive and Social Medicine, Dhaka.

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From ‘Working for’ Towards ‘Working with’ Community

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Recognition of the importance of community involvement and sectoral cooperation in health and social services, formalized in Alma-Ata Declaration in 1978, was reinforced at Riga meeting held at the mid-point between Alma-Ata and the year 2000. Then at the 20th Anniversary of Alma-Ata Declaration, it was declared that ‘Primary Health Care is Everybody’s Business’. Health does not exist in isolation. Health cannot be defined only as an outcome of ‘medical care’ but also as one of ‘social action’; the responsibility of disease prevention and health promotion rests not only on governments, but also with non-government organizations, the community, and individuals. The major accomplishment to date may be that there is growing realization in the health sector that community involvement is more than a political right—it is absolute necessary. Lessons have learned during the implementation of disease-specific vertical programmes, such as CDD, ARI, and EPI; first, integrated and holistic approaches to childcare are needed, and second, the need for community involvement has become evident. IMCI is a broad integrated strategy with an overall objective contributing to reducing child morbidity and mortality in developing countries. And one of the IMCI components, ‘Community IMCI’, which is now broadening as an entry point for child-focused community development initiatives. Community IMCI/child health is an integrated approach to the promotion of key family and community practices that have impact on: child growth and development, disease prevention, home care for sick, malnourished children, and care-seeking behaviour and compliance with advice and treatment. The presentation addresses, in brief, the development of community IMCI, operational aspect of community/IMCI, key strategies, and tools available for implementation of C/IMCI. The paper draws some successful programme examples on working together for IMCI with the community in various countries which had substantial benefits on programme outcomes.

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Status of Selected Child Health Indicators Generated Through Two Different Systems in Bangladesh

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Background: Every year, the Ministry of Health and Family Welfare (MoHFW), Government of Bangladesh, collects extensive population-based data identical to national census and special surveys under the rubric of Geographic Reconnaissance (GR). The quality of data is never validated adequately and remains underutilized. **Objective:** Compare selected indicators for child heath generated through GR and health and demographic surveillance system during 2000-2002. **Methodology:** Health and family-planning workers of MoHFW in Abhoynagar sub-district of Jessore and Mirsarai sub-district of Chittagong district collected GR data on deaths over the previous year. During the same period and in the same area, trained female interviewers routinely visited 13,998 households on a quarterly basis and collected data on births and deaths for 13,285 married women of reproductive age (MWRA) under a health and demographic surveillance system administered by the Health Systems and Infectious Diseases Division (HSID) of ICDDR,B. **Results:** In Abhoynagar, the neonatal mortality rates (NNMRs) were 10.8 from GR and 30.2 from HISD in 2000-2002. The postneonatal mortality rate (PNMR) was 10.3 and 10.5, and the infant mortality rate (IMR) was 21.1 and 40.7 respectively. In Mirsarai, NNMRs were 12.6 from GR and 35.4 from HISD in 2000-2002. PNMR was 7.1 and 16.2, and IMR was 19.7 and 51.6 respectively. **Conclusion:** GR substantially underestimated death rates especially for neonates. A one-year recall period results in under-reporting of neonatal and postneonatal deaths potentially leading to inappropriate or suboptimal implementation of programmes. If GR data continue to be used for local-level planning and management, modification in frequency of collection, enhanced training of field staff, and professional supervision will be needed. **Acknowledgements:** The funding support of the United States Agency for International Development (USAID) for maintaining the health and demographic surveillance system is acknowledged.

Do Participatory Health Interventions Reduce Disparity in Child Immunization? Experience from a Project in Chakaria, Bangladesh

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Background: Despite tremendous progress in childhood immunization coverage in Bangladesh, socioeconomic disparity in coverage rates still persists. It has been of interest to know whether the disparity can be reduced with the existing delivery system of immunization. **Objective:** Report results of an evaluation study on the impact of a community-based participatory health intervention programme on the reduction of socioeconomic disparity in immunization coverage in Chakaria, a remote coastal area of Bangladesh. **Methodology:** The project incorporated a quasi-experimental design in which 3 unions, the lowest administrative unit, that mobilized for health as intervention and other two unions considered as referent. Before (1994) and after (1999) intervention, study design was used for assessing the socioeconomic dependence of the change in immunization coverage among children aged 12-23 months. Multiplicative logistic regression model was used for analyzing data. In total, 2,753 children were included in the study; of them, 1,121 were from intervention, and 1,632 were from the referent community. **Results:** Intervention had significant effect ($p<0.01$) in increasing immunization coverage over time. The odds of increasing vaccination were 1.9 times higher in the intervention community than that in the referent community between 1994 and 1999. The inequity gap between 1994 and 1999 remained similar in the referent community and reduced in the intervention community. Intervention effect was mostly among children of the lower socioeconomic group. **Conclusion:** It is possible to reduce the socioeconomic gap in immunization coverage through the existing delivery system if the villagers can be involved at the various stage of the programme. **Acknowledgements:** The study was conducted under the auspices of the Chakaria Community Health Project of ICDDR,B, which has been supported by Swiss Red Cross.

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Healthcare Seeking for Childhood Diseases: Does Provision of High-quality Service Matter?

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Background: Worldwide, most child deaths are attributable to acute respiratory infection (ARI) and diarrhoea, which are treatable with quality medical care. Hence, targets of child-survival programme can be achieved through improving the quality of health services. **Objective:** Compare the current status of childhood (under 5 years of age) sickness care-seeking in Matlab, an ICDDR,B service area, with the government service area and evaluate the effect of high-quality services. **Methodology:** The study used child-health information collected by the Health and Demographic Surveillance System (HDSS) from Matlab in 2001. Community Health Research Workers (CHRWs) collected this information during their monthly visits in Matlab. The ICDDR,B service area has sub-centres, and Matlab Hospital other than public and private health facilities, thus, can be considered a proxy of high-quality service in terms of supply, attendance of staff, and supervision. In this study, different aspects of prevalence and sickness care of childhood diarrhoea and ARI have been observed for both the areas, and comparison was made accordingly. **Results:** The prevalence of diarrhoea was similar in both the areas. But from childhood sickness care perspective, there existed a marked difference between the two areas. Use of oral rehydration solution to manage diarrhoea was significantly ($p<0.001$) higher in the ICDDR,B service area. In the government service area, around half of diarrhoea patients were treated by village quacks, whereas in the ICDDR,B service area, the similar number of patients was treated by field-workers/health auxiliaries. In the case of ARI, use of antibiotics to treat pneumonia and the practice of seeking hospital/physicians' help were significantly higher ($p<0.001$) in the ICDDR,B service area. In contrast, the people of the government service area tended to seek traditional healers' or village quacks' help. **Conclusion:** HDSS data demonstrate that, in terms of childhood disease management, provision of high-quality services actually contributes to better disease management and improves healthcare-seeking behaviour.

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Urban Poverty and Healthcare among Street and Slum-based Children of Dhaka City

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Background: Marie Stopes Clinic Society (MSCS) has been providing reproductive health services to the vulnerable population of urban areas in Bangladesh with separate programmes for floating and slum dwellers, and 35% of the total services are general health and child health. Over the years, MSCS has found itself catering to a large extent to the needs of children health (basic) among this target group. **Objective:** Enable access to delivery of quality health services and improve child health status among the urban poor. **Methodology:** MSCS strategy to make health services accessible to the poor and vulnerable entails satellite mobile service-delivery vans for floating population (7 in Dhaka city and 2 in Chittagong) and mini-clinics in slum areas (22 in Dhaka city itself). The services are given by trained doctors and paramedics and include child-survival initiatives, EPI, and BCC (focusing on diarrhoea, iodized salt, immunization among other things) components. The services are low cost and free (Red Card), and subsidies are provided. MSCS has partnerships with RADDA-MCH FP Centre and Ad-din Hospital for their child-nutrition programmes and has a strong referral linkage with Shishu Hospital. **Results:** MSCS child health services, along with general health, cover the biggest percentage of services which is 35%. Surveys done among homeless showed that the most common health problems are fever (54%), cold/coughs (34%), and diarrhoea (10%), while the rest had skin problems, eye problems, etc. Lack of knowledge on immunization procedure and location of hospitals also featured on the list for the low number. At present, 20% of the total clients are children in the programmes. With free services from the Family Red Card Scheme, 25% of children are getting the services who could not afford it before. **Conclusion:** Enabling access to free and low-cost services that are on the door steps of clients, MSCS is also working to improve the health status of the vulnerable children and hard-core poor of Dhaka city. Through addressing the poorest of the poor, it is hoped that an improved health status will also help them find improved livelihood and decrease their poverty status. **Acknowledgements:** The support of the Department for International Development (DFID), UK and the Marie Stopes International (MSI) is acknowledged.

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Quality for Delivery of Selected Essential Service Package by an Urban Primary Healthcare Clinic in the Public Sector

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Background: Most public sector primary healthcare (PHC) facilities in urban areas are characterized by a limited range of poor-quality services and weak support system. Although the Government of Bangladesh is committed to provide quality services under the essential services package (ESP) at PHC level but its modalities were not clearly spelled out. Provision of integrated service-delivery, supportive supervision, and a user-friendly job-aid (disease-management protocol) could be conducive in improving some aspects of quality of care for ESP services at urban PHC clinics. **Objective:** Assess the effectiveness of a job-aid in improving the quality of service-delivery in an urban PHC clinic for selected ESP services (ARI and diarrhoea of children aged less than 5 years and child immunization). **Methodology:** A component was looked at under an umbrella intervention 'Delivery of ESP from Urban Areas' by introducing a user-friendly job-aid to rectify providers' practices in a quasi-experimental study. The job-aid was standardized according to the national guidelines, and specific modules were developed. All the providers were given training on its use. The effectiveness of the job-aid was evaluated after 18 months through observation of 40 client-provider interactions using checklists. In addition, service statistics had been collected to see the diagnosis and treatment patterns over times. **Results:** Technical components of quality of care, such as history-taking, necessary physical examinations, appropriate management, patient appraisal, and screening for additional needs, were done in most cases by all types of providers. Diagnosis became more protocolized, and rational drugs were prescribed. The average interaction time was increased from 1-2 minute(s) to 4-5 minutes. However, some deficiencies were still observed in inquiring of co-infection while managing ARI and diarrhoea cases. **Conclusion:** Use of protocol can help improve the diagnostic and management practices of providers at PHC level. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and collaborative partner of Government of Bangladesh and Dhaka City Corporation is acknowledged.

Perception on 'Acne' and Its Psychosocial Consequences in Bangladeshi Adolescent Girls/Young Women

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Background: Acne is a common skin disease, often causing cosmetic problem among adolescent girls/women, erupting mostly in face. It is important that young women possess adequate perception on acne, particularly on its treatment/management, instead of breaking down psychosocially. **Objective:** Study acne-related morbidity and its psychosocial consequences and assess level of perception on its key issues of adolescent girls/women. **Methodology:** This cross-sectional survey was carried out among 68 adolescent girls/young women from a randomly-selected college in Rajshahi town, using verbal autopsy, in 2002. Perceptions of the respondents were graded as 'adequate' or 'inadequate' using SPSS/Win 11.0. **Results:** Nearly 78% of the respondents reported that acne increases during winter. Small spots/rash in facial region were observed in 85.3% of the respondents. Although 40% received antibiotics and/or external cream (23.5%), 27% sought Ayurvedic treatment. Their perception level indicated that 38% had no idea on gross aetiology of acne, 27.9% wrongly perceived that mental stress cause acne, 14.7% blamed constipation, 22.1% mentioned menstrual/sexual problems, and 41.2% opined that pills/condoms cause acne. While 86.8% had confusion if oily cosmetics increase acne, 35% misbelieved that certain foods do. However, 97.1% knew it correctly that touching/scratching acne pimple(s) is harmful. 47.1% wanted to see a doctor/dermatologist right when acne appeared, although 40% hesitated to do it unless it worsens. Gross misconceptions about treatment of acne prevailed among them, and 23.2% did not rely on it. Several psychosocial consequences emerged in >33% of adolescent girls/young women due to scarring, partly disfiguration, and failure to effort treatment, including false beliefs/prejudice on acne. **Conclusion:** Since the majority of the respondents had misconceptions about acne and related morbidity, it is important that they should be made aware of various key components of acne, particularly of its latest effective treatment/management, which will help these young women shake off prevailing false beliefs/superstitions, thus relieving them from psychosocial stress. **Acknowledgements:** The work was supported by a small grant from Consultant's House, Moghbazar and, partly, by the Institute of Public Health with part-time manpower and logistic help. The authors are grateful to all physicians/research officers for their dedication and sincerity in collecting data on this very delicate and sensitive issues of safe sex quite successfully.

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MCH Intervention and Causes of Infant Mortality in Rural Bangladesh during 1988-2001

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Background: ICDDR,B: Centre for Health and Population Research has set up a hospital in Matlab and 4 treatment centres, has trained its community health workers in management of diarrhoea and pneumonia, and informed mothers about the availability of services to improve child's survival. Little is known about the long-term effects of the ICDDR,B interventions on mortality, particularly from diarrhoea and pneumonia. **Objective:** Examine the cause-specific mortality of late neonates and postneonates (age: 7 days-11 months) in the ICDDR,B and the government areas in Matlab during 1988-2001. **Methodology:** The study used mortality data collected by the Matlab Health and Demographic Surveillance System (HDSS) during 1988-2001. HDSS field workers detect death and record signs and symptoms leading to death asking mothers during their monthly home visits, and a medically-trained person reviews the death information and assigns the underlying causes of death. The ICDDR,B area is a proxy for the provision of high-quality and low-cost services compared to the government area. Cause-specific mortality rate (per 1,000 livebirths) and proportion of deaths are partitioned by area and period to exhibit the effects of the ICDDR,B intervention on mortality from diarrhoea and pneumonia in 1988-2001. **Results:** All-cause mortality rate was 49 in the government area in 1988-1991, and the rate was 28% lower in the ICDDR,B area. While the rate declined by more than 33% in both the areas by 1997-2001, diarrhoea mortality declined by 56% and pneumonia mortality declined by 17% over the years. **Conclusion:** The greater decline in diarrhoea mortality turned pneumonia as a number one killer of late neonates and postneonates even in the ICDDR,B area. The pneumonia-control programme needs to be strengthened in both the areas. **Acknowledgements:** The support of the ICDDR,B: Centre for Health and Population Research is acknowledged.

Maternal Education, Socioeconomic Status, and Gender as Determinants of Treatment-seeking Behaviour in Gaibandha, Bangladesh

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Background: A multitude of factors affect treatment-seeking behaviour in rural Bangladesh where both western and traditional modes of healthcare are available. **Objective:** Examine the association between maternal education and socioeconomic status (SES) on treatment-seeking behaviour for pregnancy-related and infant morbidities, and the effect of child gender on treatment-seeking behaviour for infant illness. **Methodology:** JiVitA Bangladesh is a Maternal Health and Nutrition Project, implemented by the Johns Hopkins University under the National Integrated Programme for Health and Population of Ministry of Health and Family Welfare, Government of Bangladesh. The JiVitA study is following 113,060 married women of reproductive age for incident pregnancies in 19 unions of rural Gaibandha and Rangpur districts. Since the study's onset in July 2001, 34,700 pregnant women have been enrolled. Consenting women are interviewed at the time of enrollment and at 3 months after pregnancy outcome, during which past 7-day morbidities and treatments sought and woman's socioeconomic status are assessed. For livebirths, a 3-month postpartum interview assesses the infant's history of illnesses and treatment-seeking, if any, for the most severe episode. Two maternal morbidities (high fever and lower abdominal pain) and two infant morbidities (fever and diarrhoea) were analyzed by woman's educational status, number of cows owned, and the number of days' worth of rice stored in the woman's household at the time of interview. Infant-treatment data were also examined by child gender. **Results:** Approximately, 50% and 10% of women with a history of high fever and abdominal pain in the previous week respectively had sought treatment. Most women who sought treatment outside the home went to a kabiraj, a practitioner of homeopathic or ayurvedic medicine, a medicine shop, or a village doctor. About 90% of women whose children had fever or diarrhoea sought medical help of some kind. Girls were 1.4 times ($p<0.02$) more likely than boys to receive no treatment for fever or diarrhoea. Girls were more often treated by a kabiraj or with homeopathic/ayurvedic professionals, whereas boys were taken to a medicine shop or village doctor. Boys were almost twice as likely to be taken to an MBBS doctor/clinic for fever, and 1.6 times more likely for diarrhoea. Boys were taken to a hospital 2-3 times more than girls for fever and diarrhoea. Treatment-seeking behaviour for maternal and infant illness did not vary with maternal education or SES, except that better-educated women were more likely to go to an MBBS doctor or clinic. **Conclusion:** A woman's SES does not seem to affect her treatment-seeking decisions. Better-educated women are more likely to visit an MBBS doctor or clinic. Infant boys are more likely to receive treatment than infant girls and are more likely to receive a higher quality of healthcare. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID), the Bill and Melinda Gates Foundation, the Micronutrient Initiative, the Sight and Life Research Institute, and the Government of Bangladesh is acknowledged.

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Home-based Delivery Cards—Is It an Effective Way to Obtain Birth Information? A Feasibility Study

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Background: Obtaining accurate information about complications surrounding childbirth has been a major challenge for the public health community working in developing countries where a majority of births take place at home. **Objective:** Evaluate the feasibility of a home-based delivery card (HDC) completed by family members for obtaining information on labour and delivery occurring at home. **Methodology:** JiVitA Bangladesh is a Maternal Health and Nutrition Project, implemented by the Johns Hopkins University under the National Integrated Programme for Health and Population of Ministry of Health and Family Welfare, Government of Bangladesh. The JiVitA study is following 113,060 married women of reproductive age for incident pregnancies in 19 unions of rural Gaibandha and Rangpur districts. Since the study's onset in July 2001, 34,700 pregnant women have been enrolled and visited weekly by locally-hired and trained female workers called Field Distributors. After field pre-testing for 50 deliveries, HDC was finalized and distributed to 2,452 pregnant women at their 28th week gestation. HDC contains 10 simple questions that ask the respondent to write down the day, month, and time of day corresponding to the onset of labour pain, water-breaking, pushing, birth, placental delivery, breast-feeding initiation, and the number/type of births. A field worker explains how HDC is to be completed and encourages the woman to have a literate person present at the time of birth to complete it. After a pregnancy outcome, Field Distributor collects this card, irrespective of its completion status. **Results:** Of 2,452 cards distributed, 56% were returned completed, 29% were not filled out, 9% were lost, and the remaining 6% of women were not met before their outcome. In an analysis of 1,833 HDCs returned to date, there was no difference in the location of delivery between those who completed their HDC and those who did not. Other factors, such as parity, type of birth attendants, and wantedness of pregnancy, also did not significantly influence HDC completion. In a regression analysis, the pregnant woman's education level was significantly associated with an increased likelihood of card completion, although husband's education was not. Nearly twice as many husbands in households with completed cards were private or government service workers. Households with at least one clock were more likely to complete HDC (OR=1.44, $p<0.01$). Other SES indicators were also positively associated with HDC completion. **Conclusion:** An HDC can be used for collecting birth information, although limitations exist with respect to its use. Almost half of women who were given HDC failed to complete it, suggesting that there were barriers to its successful completion. Confirming initial findings from HDC pre-testing, two essential prerequisites for successful card completion are basic literacy of the person(s) attending birth, and the availability of a timepiece in the home at the time of delivery. Providing more instructions for a literate relative or birth attendant who will be present at the time of delivery may enhance successful completion of HDC. Provision of a low-cost digital wristwatch and a pencil/pen might also increase compliance. Given the apparent feasibility of this method, plans are presently underway to test the comparability of data obtained through HDCs with conventional interview-based methods. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID), the Bill and Melinda Gates Foundation, the Sight and Life Research Institute, and the Government of Bangladesh is acknowledged.

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Marriage, Fertility, and Births in Northern Bangladesh

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Background: Several studies in Bangladesh have documented the seasonal variation in marriages, conceptions, and births. The reason(s) for the seasonality of these demographic events are not well-understood, and many hypotheses have been postulated, ranging from agricultural demands on the workforce to contraceptive availability and access. **Objective:** Describe the seasonality of marriage, contraception, conception, and births among newly-married women in the northern rural district of Gaibandha over a period of 18 months of population surveillance. **Methodology:** JiVitA Bangladesh is a Maternal Health and Nutrition Project, implemented by the Johns Hopkins University under the National Integrated Programme for Health and Population of Ministry of Health and Family Welfare, Government of Bangladesh. The JiVitA study is following 113,060 married women of reproductive age for incident pregnancies in 19 unions of rural Gaibandha and Rangpur districts. Since the study's onset in July 2001, 34,700 pregnant women have been enrolled and visited weekly by locally-hired and trained female workers called Field Distributors. Every 5 weeks, the Field Distributors complete a surveillance for incident pregnancies and newly-married women entering the study area. To date, 10,278 newly-married women have been enrolled into the JiVitA-1 trial. Data from these enrolled women have been used for looking at seasonality of marriage, conception, and pregnancy outcomes, including livebirths, stillbirths, miscarriages, and premature termination of pregnancy. Distribution of contraceptive use among newly-married women has also been analyzed. **Results:** The mean age of newly-married women was 16 years. Of 9,708 newly-married women on whom data were available (94%), life table analysis revealed that 25% of the cohort became pregnant by the end of their 6^{th} month after marriage, and 50% by the 13^{th} postnuptial month. A peak in marriage was seen in July, followed by a spike in conception in August. Miscarriages and menstrual regulations (MRs) follow shortly after with a peak in October. Two peaks in stillbirths were observed in October and May (perhaps artifactual as n=34). Births spike from May to July. In 33% of couples, both husband and wife expressed a desire to have delayed the current pregnancy. Despite this, within families where neither partner wanted pregnancy, 87% were not practising any method of contraception. **Conclusion:** The pattern of seasonality of marriage, conceptions, and births seen in Gaibandha are comparable to findings from Matlab in southern Bangladesh and Haryana, India. The study community is predominantly agrarian, so seasonality of marriage, conception, and birth may be explained in part by the 'Boro' and 'Aman' rice-sowing and harvesting seasons. A substantial proportion of women become pregnant shortly after their marriage, yet a significant number of these are unwanted. This may represent an unmet need for family planning among newly-married couples in this population. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID), the Bill and Melinda Gates Foundation, the Sight and Life Research Institute, Micronutrient Initiative, and the Government of Bangladesh is acknowledged.

Causes of Deaths among the Elderly in Rural Bangladesh

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Background: In 1998, the Government of Bangladesh (GoB) introduced an 'Old Age Allowance' to support needy people retiring at the age of 58 years. People aged 60 years and above comprise 6.8% of the population in Bangladesh and are projected to reach 16% in 2050. They are not a focus of the health service-delivery system, and their healthcare needs are not well-known. **Objective:** Assess the causes of death among people aged 58 years and above. **Methodology:** Trained female interviewers routinely visited 6,953 households quarterly to record births and deaths under a health and demographic surveillance system administered by the Health Systems and Infectious Diseases Division of ICDDR,B in Abhoynagar/Keshobpur sub-districts of Jessore district from 1983 to 2002. Verbal autopsy was collected for 3,468 deaths, of which 1,324 deaths were among people aged 58 years and above. **Results:** Of the 1,324 deaths, 19% occurred to 58-64 years old people, 39% in 65-74-year age group, and 42% in 75 years and above. A consistent increase from 18% during 1983-1987 to 31% during 1998-2002 has been observed. Fifty-four percent of deaths occurred to males. Among females, 85% were widowed. The major causes of death among the 58-64-year age group were cardiovascular diseases (21%), followed by respiratory diseases (13%), senility (7%), liver disease (7%), malignancy (6%), gastrointestinal disorders (6%), and neurological problems (5%). The causes were almost similar in the 65-74-year age group. Senility (51%), cardiovascular diseases (16%), and respiratory diseases (9%) were the main causes of deaths among people aged 75 years and above. **Conclusion:** Non-communicable diseases are becoming major causes of death among people aged 58-74 years in rural Bangladesh. Senility diagnosed as a cause of death needs more improvement for better understanding. The impact of old age allowance on daily living and healthcare practices of this segment of the population should be studied. **Acknowledgements:** The funding support of the United States Agency for International Development (USAID) for maintaining the health and demographic surveillance system is acknowledged.

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Clinical Presentation, Complication, Treatment, and Outcome of Salmonellosis in Bangladeshi Hospitalized Patients

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Background: Salmonellosis is a common illness in many developing countries, including Bangladesh. **Objective:** Evaluate clinical features, antimicrobial susceptibility, and outcome of *Salmonella*-associated infections. **Methodology:** In this prospective study, all consecutive patients admitted to the Dhaka Hospital of ICDDR,B with culture-proven (blood or faecal) *Salmonella*-associated infections during July 1997-December 1999 were studied. Clinical and laboratory information, complications, treatment, and outcome of patients were recorded and analyzed. **Results:** In total, 500 patients aged 26 days to 80 years, with culture-proven salmonellosis were studied, of whom 447 (89.4%) were aged £5 years, and 53 (10.6%) were aged less than 5 years. Isolation of *Salmonella* Typhi, *S.* Paratyphi A, *S.* Paratyphi B, *Salmonella* group B, and other *Salmonella* from stool was 9.0%, 0.4%, 1.9%, 53.2%, and 35.4% and from blood was 28.1%, 0.4%, 1.2%, 7.6%, and 62.7% respectively. A higher proportion of *S.* Typhi-infected children were febrile (n=28, 67%), had some dehydration (n=115, 46%), shorter duration (1-3 day(s)) of diarrhoea (n=162, 32.4%), watery stool (n=168, 33.6%), and presence of mucus and/or blood in stool (n=332, 66.4%). Peripheral blood WBC counts were within normal range in 28.3% (n=99), and leucopenia and neutropenia were present in 5 (1.4%) and 53 (15.1%) cases respectively. In X-ray investigations, lung infiltrations and dilated intestinal loops were present in 241 (69.7%) and in 161 (46.5%) cases respectively. Only 2.0% of the salmonellosis cases required referral to other hospitals for better case management due to pneumonia, hepatitis, and severe colitis. All isolates were susceptible to ciprofloxacin and ceftriaxone. **Conclusion:** Further studies are needed in other areas of Bangladesh and in other developing countries to better understand clinical presentation, complications, and drug-susceptibility patterns of salmonellosis.
Acknowledgements: The research was supported by ICDDR,B.

Epidemic Due to *Shigella dysenteriae* Type 1, Changing Patterns of *Shigella* Species, and Their Antimicrobial Susceptibility in Urban Bangladesh, 1980-2002

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Background: Shigellosis remains endemic in developing countries, where periodic epidemics due to mutiply-resistant *Shigella dysenteriae* type 1 also occur leading to high case fatalities. **Objective:** Study changes in distribution of the species and antimicrobial resistance patterns of *Shigella*. **Methodology:** Secondary analysis of data of the diarrhoeal disease surveillance system of the Dhaka Hospital of ICDDR,B was undertaken. The system enrolls a systematic sample of all patients for monitoring changes in sociodemography and records aetiology of diarrhoea, antimicrobial resistance of bacterial enteric pathogens, and emergence of newer pathogens. **Results:** All 4 species of *Shigella* were prevalent in urban Bangladesh, but *Shigella flexneri* was the most common, followed by *S. dysenteriae*. No *S. dysenteriae* type 1 strain was isolated among surveillance patients during 2001-2002. Two distinct peaks of *S. dysenteriae* type 1 were observed in 1984 (n=3,925) and 1993 (n=5,175). In 1984, 98% of *S. dysenteriae* type 1 isolates were resistant to tetracycline, 84% to trimethoprim-sulphamethoxazole (TMP-SMX), 84% to chloramphenicol, and 10% to ampicillin. Resistance to ampicillin increased to 52% in 1985. In 1993, all (100%) epidemic *S. dysenteriae* type 1 isolates were resistant to nalidixic acid, 98% to TMP-SMX, and 95% to ampicillin. Only 2% were resistant to mecillinam. Among *S. dysenteriae* type 1 strains, resistance to nalidixic acid increased from 5% in 1986 to 80% in 1990, and 100% in 1993. Resistance of *S. flexneri* increased from 4% in 1986 to 60% in 2002. All *S. flexneri* strains are currently susceptible to mecillinam and ciprofloxacin. **Conclusion:** Systematic monitoring of the changes in the distribution of various species and serotypes of *Shigella* and their antimicrobial susceptibility are important for clinical management of patients. Increasing resistance to commonly-used drugs and periodic occurrences of epidemics due to *S. dysenteriae* type 1 resistant to commonly-used drugs require identification of newer effective drugs. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

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Diagnosis of Pneumonia in Severely-malnourished Children with Diarrhoea

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Background: The WHO guidelines for diagnosis of pneumonia in non-severely malnourished children are based on age-adjusted respiratory rates (RR) and chest wall in-drawing; however, it recommends making a diagnosis of severe pneumonia in severely-malnourished children with cough, disregarding RR, and their early referral to hospital for confirmation of diagnosis by chest X-ray. **Objective:** Describe the presentation and respiratory symptomatology of severely-malnourished children with diarrhoea and look for findings that best predict the concurrent presence of pneumonia. **Methodology:** Pneumonia was diagnosed in severely-malnourished children, aged less than 5 years, with diarrhoea using the WHO guidelines, and by an investigator using history and physical examination findings at enrollment and 6 and 48 hours later, when their oxygen saturation was measured by pulse oxymeter. Serum electrolytes and chest X-ray were performed at enrollment and 48 hours. The investigator, a pediatrician, and a radiologist made independent X-ray diagnosis. **Results:** 126 of 139 of enrolled children were eligible for analysis. All had dehydration and history of cough. WHO diagnosis of pneumonia remained unchanged at 6 hours. However, the investigator's diagnosis increased from 87% to 91% (p=NS), and diagnosis by these two methods significantly differed (p<0.001). There was moderate reproducibility of investigators' diagnosis at different time points. Respiratory grunts, nasal flare, and chest in-drawing significantly reduced at 6 hours (p=0.03, <0.001, and 0.001 respectively), and oxygen saturation at 48 hours improved from that on admission (98 vs 97%; p=0.001). There was wide inter-observer variability in X-ray diagnosis of pneumonia among the investigator, independent pediatrician, and radiologist. **Conclusion:** The WHO guidelines overestimate pneumonia in malnourished children. A clinician can make a diagnosis by a carefully-performed physical examination. Pulse oximetry appears to be useful in diagnosing pneumonia in severely-malnourished children; however, an optimal cut-off value needs to be defined.
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Comparison of Trained and Untrained Doctors' Knowledge on WHO-recommended Standard Case Management of Acute Respiratory Infections

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Background: Thana Functional Improvement Pilot Project (TFIPP) was piloted in Bangladesh during 1995-2000, which proved to be effective in running the rural health centres (THCs) efficiently than others. It is important to evaluate the difference in knowledge on WHO-recommended standard case management (SCM) of acute respiratory infections (ARIs), between the TFIPP-trained and the untrained physicians. **Objective:** Assess and compare the level of knowledge between ARI-trained and untrained TFIPP's physicians on WHO-recommended SCM of ARI in rural Bangladesh. **Methodology:** With prior consent, data on selected variables pertaining to WHO-set SCM of ARI were collected from randomly-selected 120 Medical Officers from all the 55 THCs (Thana Health Complexes) under TFIPP in September 1998 using a pre-tested structured questionnaire. Data were analyzed using SPSS/Win 10.5. **Results:** Around 44% of physicians (53/120) had prior training on ARI/pneumonia. Trained physicians (52/53) were aware of the importance of child's age [0-2 month(s), 2-12 months, 1-5 year(s)] significantly more than untrained (29/38) ones ($p<0.001$). WHO classification of ARI (very severe disease-VSD, severe pneumonia–SPn, pneumonia–Pn, and no pneumonia–NPn) was more correctly followed by trained (43/53) than untrained (35/67) physicians ($p<0.001$). Trained physicians were more efficient (47/67) in diagnosing pneumonia without a stethoscope, as suggested by WHO, than untrained physicians (34/67), ($p<0.00$). Cases of Pn/SPn were diagnosed more correctly by assessing age-specific fast breathing (33/53 vs 14/53), and chest in-drawing (33/53 vs 14/53), respectively, by trained and untrained physicians ($p<0.00$, for both). Knowledge on WHO-recommended therapeutic intervention for SPn, Pn, and NPn was more correct among trained (50/53) than among untrained (45/67) physicians ($p<0.001$). Maltreatment with antihistamines was seen significantly more among untrained (30/67) than trained (8/53) physicians ($p<0.001$). **Conclusion:** Knowledge of TFIPP physicians having prior training on ARI was significantly higher than their untrained counterparts, showing a positive impact of training to ensure better diagnosis and case management of ARI accurately. **Acknowledgements:** The study was supported by the Associations of Cooperation of International Health, Kumamoto and Kumamoto National Hospital, Kumamoto, Japan, and partly logistic support/manpower from the Institute of Public Health, Dhaka. The authors are grateful to all physicians working at TFIPP THCs for all of their sincere efforts in making the study a success.

Pharmacokinetic Profile of Once-daily Dosing of Gentamicin in Malnourished Children with Diarrhoea and Pneumonia

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Background: Very little is known about pharmacokinetics of once-daily dosing of gentamicin in malnourished children. **Objective:** Evaluate and compare pharmacokinetics of a daily large single-dose versus conventional daily triple-dose regimen in malnourished children with diarrhoea and pneumonia. **Methodology:** This is part of a study for evaluation of efficacy and safety of once-daily dosing of gentamicin in malnourished children aged 6 months-5 years with diarrhoea and pneumonia. They randomly received gentamicin 5 mg/kg/day once daily (OD) or the same amount in 3 divided doses (TD) intramuscularly, in addition to ceftriaxone 75 mg/kg/day. In a sub-sample, pharmacokinetics of gentamicin in 59 children of OD and 43 of TD regimen respectively were studied in hydrated state. On second day of the study, blood was collected immediately before (trough value) and at 1 hour (peak value), 3 hours, 5 hours, 8 hours, and 24 hours from patients in OD regimen and immediately before and at 1 hour in TD regimen after administration of gentamicin. Serum concentrations of gentamicin were assayed by fluorescence polarization immunoassay (Abbott Laboratories, Tdx). **Results:** The mean±SD of serum gentamicin concentrations in OD were (in mg/L) trough: 0.23±0.1, peak at 1 hour: 11.7±4.1, at 3 hours: 4.4±1.2, at 5 hours: 2.08±0.9, at 8 hours: 1.01±0.6, at 23 hours: 0.24± 0.1; and in TD, trough: 0.45±0.2, peak at 1 hour: 4.7±1.8. The serum peak level of gentamicin in OD was significantly higher ($p<0.001$), and the trough level in OD was significantly lower ($p<0.001$). **Conclusion:** The significantly higher serum peak and lower trough values suggest probability of better antibacterial efficacy and less toxicity of once-daily dosing of gentamicin compared to conventional daily triple-dose regimen. **Acknowledgements:** The research was supported by the United States Agency for International Development (USAID), Washington. ICDDR,B acknowledges with gratitude the commitment of USAID to the Centre's research effort.

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HIV/AIDS in Children: The Scenario in North India

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Background: While the incidence of HIV/AIDS among children is increasing in western and southern India, its exact prevalence in North India, especially in clinical settings, remains unknown. **Objective:** Assess the magnitude of HIV/AIDS in children and study the clinical profile of children with proven HIV/AIDS. **Methodology:** In total, 400 women attending antenatal clinic and 134 children either suffering from chronic diarrhoea, failure to thrive or disseminated tuberculosis (n=84), or having parental HIV/AIDS (n=50) were included in the study. ELISA and rapid/simple tests for HIV were carried out in all. **Results:** Only one women (0.25%) of 400 antenatal women was reactive to HIV ELISA. Of the 134 children screened, 10 (7 males and 3 females) were positive for HIV. All children were symptomatic. The mean age at presentation was 4.4 years. All of them had acquired infection through the perinatal route. Clinical manifestations included weight loss, pyrexia, chronic diarrhoea, upper and lower respiratory tract infections, lymphadenopathy, and hepato-splenomegaly. Diagnosis of tuberculosis was confirmed in 4 children. AIDS defining illnesses, such as HIV encephalopathy, recurrent bacterial infections, and cryptococcal diarrhoea, were also seen. **Conclusion:** Although the prevalence of HIV is still low among antenatal women, symptomatic AIDS is not uncommon among children, especially among offsprings of known HIV/AIDS parents. Overall, HIV/AIDS infection still seems to be evolving in North India.

Transfusion-transmitted Infections among Bangladeshi Blood Donors: How Much Risky Is It for Transfusion?

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Background: Transfusion-transmitted infection (TTI) is a potential threat to global public health, particularly in countries like Bangladesh, where risky blood transfusion from professional donors remains a common practice. The magnitude of TTIs among blood donors yet remained largely unexplored in several countries, including Bangladesh. **Objective:** Study the prevalence of common TTIs (HBV, HCV, HDV, syphilis, HIV) among professional and voluntary blood donors in Bangladesh. **Methodology:** Blood from 100 professional (mean age [±SD] 30.2±4.14 years) and 110 voluntary (mean age [±SD] 23.6±3.72 years) donors were screened for HBV, HCV, HDV, *T. pallidum,* and HIV markers at the immunology department of BIRDEM using standard serological/immunological methods. Data were analyzed employing SPSS/Win 10.5 using appropriate statistical tools. **Results:** The HBV and HCV markers were found significantly more among professional than among voluntary donors in terms of HBsAg ($p<0.0001$), anti-HBc total ($p<0.0001$), and anti-HCV ($p<0.001$). Moreover, 14% of the professional and none from the voluntary donors had co-infection with HBV and HCV. 13% and 17% of the HbsAg-positive professional and voluntary donors respectively was positive for anti-HBe. However, none of the professional or voluntary donors were positive for HDV and HIV (on LIA). **Conclusion:** The higher prevalence of HBV and HCV was observed among professional donors. This has serious public-health implications since they sell their blood and are the most vulnerable group for contracting TTIs. **Acknowledgements:** The study was supported by the Bangladesh Medical Research Council through GoB's HPSP, funded through WHO and World Bank. The authors are grateful to all physicians/immunologists at DMCH/ IPH and BIRDEM, Dhaka, for all of their individual and collective apparoach and expertise, and also thank patients and their guardians for thier pain-staking patience and sincere cooperation in collecting blood samples/data.

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Effect of Modification of Specific Behavioural Risk Factors on Recurrent Episodes of Diarrhoeas in Children with HIV Infection

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Background: Diarrhoea is a major problem in seropositive children associated with wasting, cachexia, and high morbidity and mortality. Organisms responsible for diarrhoea in HIV-infected children are mostly transmitted via contaminated food and water. Specific behavioural risk factors in the family, which predispose a child for recurrent episodes, if modified, reduce recurrent infection. **Objective:** Study the effect of modification of specific behavioural risk factors on current epidemics of diarrhoea in children with HIV infection. **Methodology:** In total, 50 patients, aged 6 months-10 years, attending the paediatric HIV clinic, presenting with recurrent episodes of diarrhoea, were enrolled in this prospective study. Behavioural risk factors relating to feeding patterns, defecation habits, hand-washing practices, storage and source of drinking-water, use of oral rehydration solutions, home-available fluids, mode of washing utensils, etc. were noted. Modification of these risk factors made in the form of hand-washing with soap and water or ash rather than mud ($p<0.000001$), straining of drinking-water by cotton cloth or boiling water, storage of water in clean metal pots than earthen pots, cleaning of utensils with detergents or ash rather than mud. Nutritive food supplements of low cost were advised. Patients were followed up for one year. **Results:** Modification of the existing risk factors in the family within the available means and resources resulted in 60% reduction in the recurrent episodes of diarrhoea ($p<0.000001$), and it had a protective value ($p<0.000001$). **Conclusion:** Although it may not be possible to cure diarrhoea in a HIV-infected child, still overall awareness about small sociocultural habits in the family and modification of the existing behavioural pattern are effective, simple interventional strategies to minimize recurrent diarrhoeal episodes in immunocompromised children.

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Molecular Characterization of HIV-1 Circulating in Eastern and Northeastern India

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Background: HIV/AIDS pandemic is now a growing concern in India. 4.58 million people are currently infected, as estimated by the National AIDS Control Organization. The majority are infected through heterosexual transmission, while in northeastern states, infection initially occurred through sharing of needles in injecting drugs. Although considerable work is continuing in the areas of prevention and intervention, data on molecular characterization of HIV-1 are still limited. Information on genetic make-up of circulating HIV-1 strain is needed for developing and/or testing any vaccine candidate. **Objective:** Characterize genetically the envelope gene of HIV-1 isolated in eastern and northeastern India. **Methodology:** To determine the HIV-1 subtypes, heteroduplex mobility assay (HMA) was done. The C2-V3 region of the envelope gene was amplified by specific primers and was allowed to form heteroduplexes with subtype-specific reference amplicons. These were analyzed by polyacrylamide gel electrophoresis. Heteroduplex, showing the fastest mobility, indicated the closest subtype. Subsequently, sequencing and phylogenetic analysis were done using different software packages. **Results:** Of 54 samples studied from Kolkata, the majority was of subtype C. Of these, 27 were of subtype C3, 7 of C2, and 1 of C1, while 18 remained non-typeable. The majority of 25 samples studied from Manipur revealed subtype C, followed by subtype Thai B. Regarding gag subtyping of 30 samples from Kolkata, 14 were of subtype C4, 7 of C3, and 1 of C5, 1 of C2, and 7 remained non-typeable. **Conclusion:** The majority of HIV-1 strains in eastern and northeastern India were of subtype C with non-typeables forming a separate cluster. In Manipur, subtype Thai B prevails along with subtype C. **Acknowledgements:** The work was supported by the Indian Council of Medical Research.

Paediatric HIV/AIDS and Antiretroviral Therapy: Prospects in a Developing World

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Background: Port Harcourt Teaching Hospital, a tertiary health facility in the Niger Delta Region of Nigeria, currently providing services for HIV-infected adults. There has been an influx of adults and children infected with HIV into the treatment programme. Treatment for children is yet to be introduced at the national level. Different schools of thought exist as to when to initiate treatment for HIV-seropositive children: start as soon as the test shows that the child is positive or wait till all maternal antibodies are deemed to have been destroyed and the child shows evidence of infection. There are, however, no studies to indicate the most appropriate time to start treatment in resource-poor countries considering the cost of care and the low coverage of the PMTCT programme. This study reviews the prospects of antiretroviral treatment for children to assist policy-makers to decide on the best time to initiate treatment for children. **Objective:** Determine the prospects and outcome of paediatric antiretroviral drug treatment in Nigerian children. **Methodology:** This was a prospective study based at the University of Port Harcourt Teaching Hospital, Nigeria, from January 2002 to August 2003. All children with signs, suggestive of HIV/AIDS, were screened usingstandard ELISA techniques, and those who tested positive were included in the study. In addition to biosocial variables, CD4 counts, weight-for-age z-scores (WAZ) were obtained for each child. Data were analyzed relative to treatment outcome in each child. **Results:** One hundred and forty-six children aged 0.11-168 month(s) (mean age–21.8 months) were studied. 75% of them were aged less than 24 months. 15.1% belonged to socioeconomic classes 1 and 2. Of mothers who were tested, 25% were already showing signs of AIDS, but only 17% were being treated with antiretroviral drugs (ARV). The mean WAZ was -2.662, and the mean duration of symptoms before presentation was 65 days for diarrhoea, 91 days for oral thrush, and 79 days for fever. The mean CD_4^+ T lymphocyte count was less than $200/mm^3$. **Conclusion:** Late presentation and advanced disease were important contributors to poor outcome. Furthermore, the finding that the vast majority of the positive cases were aged less than two years appears to suggest that the major route of infection was vertical transmission. Therefore, strengthening the Prevention of Mother to Child Transmission of AIDS (PMTCT) Programme would go a long way in improving outcome in children with HIV/AIDS. **Acknowledgments:** The authors are grateful to the National Action Committee Against AIDS (NACA) of the Federal Government of Nigeria for making the drugs available.

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Marginal Costs of Integrating Child Nutrition Services into ESP Delivery System

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Background: Severe malnutrition (weight-for-age) affects about 16% of weaning-age children in Bangladesh and an estimated one of 4 children in urban slums. Over the last few years, ICDDR,B scientists have, therefore, extended their experience with the management of severe malnutrition in hospitalized children to outpatient management by primary-care clinics. This was piloted at 3 urban NGO clinics from March 2001 through March 2003. Results of this intervention suggest that integration of this approach into the routine child healthcare component of Essential Services Package (ESP) is feasible and effective in achieving recovery in the majority of children suffering from severe malnutrition. However, decisions by concerned programme managers and policy planners on the scaling up of this model to other NGO clinics require further understanding of its cost implications and effectiveness. Keeping this in mind, a cost-analysis exercise was designed and conducted. **Objective:** Assess the marginal costs of the intervention with regard to provider and client costs. **Methodology:** The 'ingredients' approach was employed to calculate the provider and client costs. For the estimation of provider cost, all severely-malnourished children enrolled during the 25-month intervention period were considered. In addition, a sub-sample of 67 severely-malnourished children was followed by the study team to assess the client cost and related socioeconomic variables. **Results:** The estimated total marginal cost of the provider was US$ 19,230, corresponding to average cost per enrolled severely-malnourished child of US$ 41 or US$ 66 per child rehabilitated from severe underweight. The average client cost per severely-malnourished child recovered in the sub-sample was only US$ 4. **Conclusion:** The intervention on community-based protocolized management of severely-malnourished children is cost-efficient. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

Assessment of Nutritional Status of the Elderly in Dhaka City

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Background: The number of senior citizens is gradually increasing in Bangladesh. They are suffering from different diseases and malnutrition and are becoming burden of the society. **Objective:** Assess socioeconomic condition, dietary and disease patterns, and the nutritional status of the elderly in Dhaka city. **Methodology**: A cross-sectional study on nutritional status of the elderly was conducted in 6 locations of Dhaka city from July 2002 to August 2003. Two areas were selected from each of 3 upazilas of Dhaka city by simple random sampling. In total, 204 elderly aged 60 years and above were selected. Socioeconomic, disease pattern, and dietary information were collected using a pre-tested questionnaire. Anthropometric measurements, such as, height, weight, and body mass index (BMI) were done using standard procedures. Data were analyzed using the SPSS programme. **Results:** The average monthly income, total expenditure, and food expenditure of households was Tk 12,656, Tk 10,545, and Tk 5,572 respectively. About 48.5% took more than 3 kinds of foods per meal. Loneliness was very common among 75.0% of the elderly. The majority (51.0%) had gastrointestinal problems. About 54.2% of the young elderly (aged 60-69 years) and 43.0% of the old elderly (aged 70 years and above) had normal nutritional status ($p=0.03$). The majority (51.2%) of females had normal nutrition than their counterparts (46.7%) ($p=0.5$). In terms of normal nutrition, the literate elderly (50.6%) was better than the illiterate elderly (48.7%) ($p=0.019$), while the non-working elderly (55.1%) were better than the working elderly (44.3%) ($p=0.2$). Over-weight was alarmingly high (30.4%) among the households spending more than Tk 4,000, while it was 8.7% among those spending less than Tk 4,000 ($p<0.001$). Rice, vegetables, small and big fish, pulses, bread, milk, and potato were commonly consumed and preferred food items of the elderly. **Conclusion:** The nutritional knowledge and practice and also special care for senior citizens should be ensured to improve their nutritional status. Developmental policies and programmes should be taken for the welfare of the elderly. **Acknowledgements:** The support of the Bangladesh National Nutrition Council, Ministry of Science and Information and Communication Technology, and College of Home Economics, University of Dhaka, is acknowledged.

Feeding Pattern and Weight Gain during First 3 Months of Life for a Group of Urban Bangladeshi Infants

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Background: Although exclusive breast-feeding is advocated worldwide, breastmilk complemented with water or milk/milk-based product during the early infancy is the usual mode of infant feeding. **Objective:** Compare weight increment between predominantly breastfed babies and mixed-fed babies over a 3-month postpartum period. **Methodology:** A retrospective cohort study was conducted among 98 infants who were delivered at the Dhaka Medical College Hospital. They were grouped into two based on their feeding regime. Forty-nine 'predominantly breastfed' infants (received breastmilk with or without complement of water) were compared with 49 'mixed-fed' infants (received additional milk or milk-based products). Records of weekly feeding history and body-weight measurements at a 4-week interval were compared. **Results:** The mean birth-weight of the predominantly breastfed infants and mixed-fed infants was 2.83□0.48 kg and 2.93□0.56 kg respectively, and they were not significantly different. But the predominantly breastfed infants gained significantly more weight than the mixed-fed group ($p<0.05$). The mean increment in weight between birth and at 12 week's time for the groups was 2.42□0.46 kg (predominant breastfed) and 2.19□0.61 kg (mixed-fed) respectively. Furthermore, in a subgroup analysis, it was noted that, at 3 months time, there was no significant difference between the low-birth-weight and the normal-birth-weight babies in the predomenent breastfed group, indicating a catch-up growth by low birth-weights near to the level of normal birth-weights. **Conclusion:** Gain in weight of the predominantly breastfed and mixed-fed infants differed in the first 3 months of life. The predominantly breastfed infants grew better than the mixed-fed infants did. Low-birth-weight babies in the predominant breastfed group were protected from growth faltering.

Assessment of Iron-supplementation Activities among Pregnant Women in an Upazila of Bangladesh

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Background: Pregnancy is probably the greatest physiological challenge to the human body, and there is no doubt that haemoglobin values are reduced in pregnancy. Worldwide, anaemia is a major cause of morbidity and mortality, particularly in developing countries. Correction of anaemia continues to be an insurmountable challenge. Many antenatal care programmes distribute iron supplements to pregnant women in Bangladesh, which is the best way to prevent iron-deficiency anaemia. Therefore, it is important to know how the supplementation activities are going on among pregnant women, especially at the grassroots level. By proper and adequate distribution of iron supplements and with improving of patients' compliances to it, maternal deaths due to severe anaemia can be reduced. Thus, the results of this study might help improve strategies for anaemia-prevention programme. **Objective:** Find out the coverage and compliance and source of iron supplementation among pregnant women. **Methodology:** A cross-sectional descriptive study was conducted among pregnant women in Sreepur upazila of Gazipur district. Data were collected for 3 months (16 March-June 2002). The sample size was determined using the formula:

$$n = \frac{z^2pq}{d^2} \quad \text{and} \quad nf = \frac{n}{1 + \frac{n}{N}}$$

In total, 236 pregnant women of the study population were selected for collection of needed information. Data were collected through face-to-face interview using a semi-structured pre-tested questionnaire. **Results:** Of the 236 pregnant women, 95 (40.3%) received iron from any source. Of them, more than one-third (38%) complied with the iron therapy at the time of interview. Pregnant women with formal education and working in garments had significantly high coverage and compliance rate compared to those who were illiterate or had non-formal education and the women who were housewives ($p<0.05$). Of the pregnant women, 72.9% had up to 3 antenatal clinic visits, 9.3% had 4-5 visits, and 17.8% did not have any visit at all. Regarding the reason of not receiving iron supplementation in current pregnancy, nearly half (45%) complained of long distance and 33% financial incapabilities as barrier in reaching the health centre, and the rest (22%) were not at all aware of taking iron tablets. **Conclusion:** The iron-supplementation activities among the pregnant women of Sreepur upazila of Gazipur district are very unsatisfactory. Poor coverage and compliance rate need to be addressed on a priority basis. **Acknowledgements:** The authors express gratitude to the chairman and members of the "Dissertation Committee" of NIPSOM. Special gratitude is to Dr. Rowshon Ara Begum, Assistant Professor, Department of Nutrition and Biochemistry, NIPSOM, as guide.

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Prevalence of Plasma Retinol Levels and Dark-adaptation Thresholds among Pregnant Women in Yemen

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Background: Vitamin A deficiency and associated nightblindness during pregnancy have been considered by the World Health Organization to be of public-health importance, especially in developing countries and, thus, warrant assessment and control programmes. **Objective:** Determine the prevalence of plasma retinol levels and dark-adaptation thresholds among pregnant women in western Yemen. **Methodology:** A survey was conducted in western Yemen involving 150 pregnant women from 15 villages selected randomly within 3 underserved districts. Phlebotomy and dark-adaptometry testing was conducted simultaneously using standard procedures to determine plasma retinol values and dark-adaptation threshold. Mother's age, gestational period, and parity were also recorded. **Results:** The mean plasma retinol concentration of subjects was 36.69±14.81 microgram/dL with 23.4% having values less than 30 microgram/dL and 9.5% having values less than 20 microgram/dL. The mean pupillary threshold was -0.609±0.436 log candela/m^2 with 91.3% having values above the IVACG-proposed cut-off level of -1.11 log candela/m^2. No significant correlations were found for both the indicators to either mother's age, gestational period, or parity. **Conclusion:** Vitamin A deficiency is a public-health problem among women of childbearing age in western Yemen and warrants intervention programmes of vitamin A supplementation to lactating women and dietary fortification and diversification in the region. The dark-adaptation assessment tool employed in the survey was shown to be a promising screening tool for nightblindness and, thus, vitamin A status among at-risk populations in developing countries. **Acknowledgements:** The financial support of the Micronutrient Initiative is acknowledged.

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Mothers' Intervention Reduces Chance of Micronutrient Deficiencies among Children Aged Less Than 5 Years

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Background: Early child development and promotion of growth requires vitamin A, iron, and iodine. Micronutrient malnutrition remains highly prevalent among children, aged less than 5 years, in developing countries. **Objective:** Introduce behavioural change communication guidelines to reduce micronutrient malnutrition among 0-59 month(s) old children. **Methodology:** This intervention study was conducted during January-May 2003 in 4 thanas of Dhaka city. Mothers with children from 222 intervention and 210 non-intervention households were selected. Informal guidelines on nutrition and micronutrients for 3 hours were given to intervention groups for 3 consecutive months. Monthly weight monitoring of children and appropriate management was done. A pre-designed and pre-tested questionnaire was used for data collection from both the groups at the 4th month. **Results:** Severe, moderate and mild nutritional status by weight-for-age median was identified in 37% of children in the intervention and 58% of children in the non-intervention groups ($t=-0.081$, $p<0.003$). All intervention children had normal nutritional status by mid-upper arm circumference (MUAC). The non-intervention children had 44.0% normal, 0.6% moderate, and 54.8% severely-low nutritional status ($t=-1.041$, $p<0.001$). Vitamin A deficiency was found in 7.2% and 12.4% ($t=0.789$), iron deficiency in 13.1% and 13.8% ($t=0.320$), and iodine deficiency in 1.6% and 0.5% ($t=0.602$) of children in the intervention and non-intervention groups respectively. Mothers' understanding level of micronutrient malnutrition of children, aged less than 5 years, was good in 65% and 22% and knowledge and perception to prevent diarrhoea by feeding vitamin A-rich foods in 54.4% and 4.8% of the intervention and non-intervention respondents respectively. Understandability about iron and iodine deficiency was found in 73.0% and 33.8% and in 71.2% and 14.8% of mothers of the intervention and non-intervention groups respectively. **Conclusion:** Nutritional behaviour of the intervention mothers was changed significantly. Nutrition education for mothers towards reduction of micronutrient malnutrition in children should be reflected in plans and programmes. **Acknowledgements:** The financial support of the Ministry of Science and Information and Communication Technology, Government of Bangladesh, is acknowledged.

Obesity Ringing the Bell

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Background: Recently, quite a high number of overweight/obese children are seen in different schools, particularly in English medium private schools. Obesity leads to chronic illnesses, such as cardiovascular diseases, hypertension, maturity onset diabetes, etc. Anecdotal observation shows that a good amount of resources are already being drained in the management of these illnesses. These are also causing premature deaths often leading to a national loss. In a country where undernutrition is still a grave problem, the extent of obesity among school-going children is not known. **Objective:** Assess the nutritional status of children from well-to-do families attending English medium schools in Dhaka. **Methodology:** A pilot study was conducted in 4 English medium schools in Dhanmondi. Mothers of 38 children from Play Group to Class V were interviewed. Height, weight, date of birth of children, their eating habits, and leisure-time activities were recorded. Family-history of chronic illnesses were also recorded. Anthropometric measurements of mothers were also taken. **Results:** 23.69% of children were obese, while 71% had adequate weight-for-age. 28% had some kind of teeth problems. 71% had family history of one or more disease(s) of affluence. The commonest leisure-time activity was television-watching (92%), while the second commonest activity was playing indoors (63%). 63% of mothers were either obese or overweight. **Conclusion:** While a firm conclusion cannot be drawn from such a small-scale pilot study, it definitely points to ominous sign that the country is drawing towards double burden of malnutrition. It merits a larger well-designed study to guide the future health and nutrition policy and programmes in Bangladesh.

Therapeutic Use of Zinc during Diarrhoea Improves Physical Growth of Bangladeshi Children

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Background: Routine supplementation of zinc has been shown to improve physical growth of children in zinc-deficient population. The effect of therapeutic use of zinc during diarrhoea on physical growth is not known. **Objective:** Assess the impact on physical growth of therapeutic use of zinc during diarrhoea in children aged less than 5 years. **Methodology:** The service areas of 30 Community Health Workers (CHWs) in Matlab field site of ICDDR,B were allocated to intervention or comparison by stratified random selection. During November 1998-November 2000, caretakers of 3-59-month old children with diarrhoea in both intervention and comparison clusters were offered oral rehydration solution (ORS), feeding advice, and referral care for each diarrhoea episode. The caretakers of these children in the intervention clusters were, in addition, offered zinc syrup with the advice to give 5 mL (20 mg of elemental zinc) per day for 14 days irrespective of the duration of diarrhoea episode. Weight and height of children, who were aged 6-11 months at the beginning of the study, were measured at an interval of every 2 months. **Results:** Children in the intervention clusters (n=66) had significantly higher weight gain than children in the comparison clusters (n=59) irrespective of nutritional status at baseline (3.35 kg±0.55 in intervention vs 3.17 kg± 0.59 in control; p=0.01). Similarly, children in the intervention clusters had significantly higher height gain than children in the comparison clusters (17.15 cm±1.69 in intervention vs 16.60 cm±2.15 in control; p=0.03). **Conclusion:** Therapeutic use of elemental zinc during diarrhoea has positive impact on physical growth of children. **Acknowledgements:** The study is supported by the Johns Hopkins Family Health and Child Survival Cooperative Agreement and ICDDR,B cooperative agreement with funding from the United States Agency for International Development (USAID).

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Supplementation of Drink Fortified with Multiple Micronutrients among Rural Adolescent Girls: Some Preliminary Findings

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Objective: Determine the efficacy of multiple micronutrient-mix in improving micronutrient status and growth among adolescent girls in Bangladesh. **Methodology:** The double-blinded, randomized, placebo-control trial was conducted for one year. In total, 1,044 adolescent girls, aged 11-19 years—522 in each group—were randomly selected from 54 BRAC Non-formal Adolescent Schools in Sherpur district. One group received fortified powder-drink, while the other received identical placebo. A serving of 200 mL containing iron (7 mg), vitamin A (1,296 IU), iodine (75 mg), and other micronutrients was provided daily for 12 months to each individual in school setting. Assessments were made at baseline and at the end of the sixth and twelfth month. **Results:** At baseline, no significant difference was observed between the groups in terms of age, socioeconomic, anthropometric (BMI: p=0.56 and underweight p=0.32), blood haemoglobin (p=0.28), and food consumption indicators. After 6 months of the supplementation, there was a significant difference in blood haemoglobin level (p=0.00) between the groups. In contrast, no significant difference was observed between the groups in BMI (p=0.15) and prevalence of malnutrition (p=0.10). However, there was an increased trend in reduction of malnutrition and BMI in the same group that had better haemoglobin level. **Conclusion:** Six-month supplementation of multiple micronutrient drink is effective in reducing anaemia in adolescent girls. There is a potential chance to effectively reduce malnutrition with one-year supplementation. **Acknowledgements:** All study adolescents and their families, school-teachers, and *Shasthya Shebikas* (health volunteers). The Micronutrient Initiative, Canada, for providing fund, and the Procter and Gamble Company, USA, for supplying the micronutrient-mix.

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Growth and Nutritional Status of Children with Epilepsy on Ketogenic Diet: A Preliminary Prospective Long-term Study

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Background: The ketogenic diet is one of the most effective therapies for intractable seizure for the paediatric population. Although the beneficial effects of the diet are well-known, potential sub-optimal growth and nutritional risks of the diet have been evaluated by limited research articles only. The 4-month earlier study of the authors has demonstrated that the nutritional status and linear growth of children on the ketogenic diet can be maintained. **Objective:** Investigate the impact of two types of ketogenic diets on children's growth and nutritional status over a longer period. **Methodology:** Participants included 19 children on the classic ketogenic diet and 6 children on the MCT (medium-chain trigylceride) ketogenic diet. All children had failed at least two anticonvulsant. Weight, height, and biochemical indices were obtained at pre-diet, 6 months, 12 months, 18 months, and 24 months of the study. **Results:** In classic ketogenic diet groups with 19 participants, there were, on average, 2.4 cm (2.6%) of height increase and 0.9 kg (0.05%) of weight increase at 6 months on the diet; 5.3 cm (6.2%) of height increase and 1.2 kg (0.1%) of weight increase at 12 months with 11 (58%) participants; 4.6 cm (4.7%) of height increase and 2.5 kg (0.17%) of weight increase at 18 months with 5 (26%) participants; and 8.1 cm (8%) of height increase and 3.8 kg (0.24%) of weight increase at 24 months with 2 (11%) participants. In MCT ketogenic diet group with 6 participants, there were, on average, 1.3 cm (1.3%) of height increase and 1.2 kg (0.08%) of weight decrease at 6 months on the diet since some children were over-weight pre-diet; 3.1 cm (3.1%) of height increase and 1.19 kg (0.02%) of weight decrease at 12 months with 5 (83%) participants; 6 cm (6%) of height increase and 1.54 kg (0.09%) of weight increase at 18 months with 4 (67%) participants; and 9.7 cm (9.6%) of height increase and 2.2 kg (0.14%) of weight increase at 24 months with 3 (50%) participants. All biochemical indices, including serum albumin, calcium, magnesium, zinc, vitamin E, and phosphate remained within normal range for both the diet groups. Two subjects had low ferritin level and continued on iron supplement. Six subjects had low ferritin level pre-diet and improved their ferritin level during the diet. **Conclusion:** This preliminary findings demonstrated that the nutritional status and growth on the ketogenic diet might be maintained. **Acknowledgements:** Susan Cohen, Research Assistant, is acknowledged for her hard work during the study.

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Mid-day Meal in Schools and Its Effect on Improving Nutrition of School Children in Sri Lanka

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Background: It is an accepted norm that some form of food be provided for children in Sri Lanka's school to maintain their nutrition levels, although what it should contain is contested. **Objective:** Evaluate the effects of lunch packets containing rice and curry provided for school children in grade 2 by their parents on child's weight and general health vis-à-vis fortified biscuits provided by the school through the Department of Education. **Methodology:** In 4 backward districts of Sri Lanka, a random sample of 2,510 grade 2 school children was obtained for the study. Data were collected using an interview schedule that was developed after a formative study on food intake of children in schools in the said districts. **Results:** The results showed that those students who ate rice packets brought from home together with the biscuits given by the school were better-off in their weight compared to those who did not get such packets from home but ate only the biscuits. Those who did not eat the biscuits but had only the rice packets from home were equally better-off with regard to their body weight. This finding has proved true irrespective of the student's socioeconomic class, sex, and being a rural or urban student. **Conclusion:** Provision of lunch packets containing rice and curry can help improve body weight and general nutrition of young school children in backward districts of Sri Lanka. **Acknowledgements:** The financial support of the Presidential Task Force is acknowledged.

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Food Safety in Bangladesh: How Safe is Our Food?

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Background: Food safety is essential to maintain nutrition, combat food/waterborne diseases, maintain food quality, and stop food adulterations, being rampant in Bangladesh. **Objective:** Highlight the current status of food safety and occurrence of food-specific adulteration in Bangladesh. **Methodology:** Findings of national data from analysis of samples collected randomly from different parts of Bangladesh on food adulteration status and findings on HACCP methods on street-vended foods were reviewed. **Results:** Around 51% of food samples tested at the Institute of Public Health was adulterated to different degrees. Almost similar to 2001, reports from 2002 revealed that 100% of samples of butter oil and Banaspati dalda and 90% of samples of condensed milk/sweet-meats were adulterated. Moreover, while ghee and honey were adulterated up to 72.3%, cow's milk was adulterated by 57.2%. However, almost all of the 52 samples of street-vended foods were highly contaminated with various pathogenic bacteria and/or fungi. On the contrary, latest data (2002) on contamination of drinking-water, both chemically and bacteriologically, showed promising, compared to the last few years' data revealing only 3.2% and 8.2% respectively. **Conclusion:** There is a need for strengthening the food-safety programme in Bangladesh and improving existing food-testing laboratories and updating existing laws/regulation in the country, including proper implementation. **Acknowledgements:** Active cooperation of all the physicians and technologists related to this study, including the sanitary inspectors, is acknowledged.

VACCINE

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A Successful Vi Polysaccharide Vaccination Campaign among Children and Adults: Results of the DOMI Cluster Randomized Vi Effectiveness Trial in Hechi, Guangxi, China

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Background: Typhoid fever is a major cause of morbidity with an estimated global prevalence of 30 million cases and 600,000 deaths per year. Vaccination of high-risk populations is considered to be the most promising strategy for the control of typhoid fever. The Vi vaccine has been targeted by the DOMI Programme for accelerated introduction into public-health programmes. **Objective:** Determine the protective effectiveness of Chinese-produced Vi polysaccharide vaccine following routine administration in a 1-dose schedule, estimate the cost-effectiveness of Vi vaccination, monitor the safety of Vi vaccine following administration through a mass-vaccination campaign, and estimate the logistic feasibility of a mass-immunization campaign. **Methodology:** An open, cluster-randomized effectiveness trial in Hechi city, China, has been conducted. A target population of 118,558, aged 5-60 years, was divided into 107 clusters which were randomly allocated to receive Vi typhoid vaccine or meningitis A vaccine. A mass-vaccination campaign took place from 8 April to 12 May 2003. The population will be followed for 2 years using passive detection of typhoid fever cases. **Results:** In total, 92,481 people were vaccinated; the vaccine coverage was 78%. Seventy-one adverse events were documented, but none of them was serious. Eight needle-stick accidents were reported. A random sample of vaccinees was interviewed to estimate the private cost of vaccinations. A representative sample of sera was collected for immunogenicity testing. **Conclusion:** The first Vi mass-vaccination campaign, targeting adults and children, was conducted successfully in Hechi city. Several needle-stick accidents occurred despite carrying out of safe injection practices. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

P108 (243)

The Collaborative IVI-DOMI/NICED Project in Kolkata

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Background: The National Institute of Cholera and Enteric Diseases has undertaken a project on cholera and typhoid fever in Kolkata, India, in collaboration with the International Vaccine Institute's Diseases of the Most Impoverished (DOMI) programme. **Objective:** Quantify the burden of cholera and typhoid fever and prepare a field site for an efficacy trial of the killed oral bivalent whole-cell cholera vaccine and an effectiveness trial of the polysaccharide Vi typhoid vaccine. **Methodology:** The study site is in an urban slum. A complete census was conducted from December 2002 to March 2003. Surveillance for cholera and typhoid fever was started in April 2003. Private and institutional costs of cholera and typhoid fever, willingness to pay for cholera and typhoid fever vaccines, and behaviours relating to these diseases will also be studied. Existing published and unpublished data from India are also being collected and analyzed to get a country-wide measure of the burden of these diseases. **Results:** Analysis of the census data showed that the total population enumerated was 57,099 individuals. The enumerated individuals lived in 10,960 households with a mean of 5.2 individuals per household with an average number of 1.5 rooms per household. The median monthly household income was INR 3,000 or US $67. The study population was stable with a mean of 36 years of residence in the same household. During the past 4 and a half months of surveillance, the estimated annual incidence of stool culture-positive cholera was 1.8/1,000 population/year, blood culture-positive typhoid fever was 1.4/1,000 population/year, and blood or serology-positive typhoid fever was 6.3/1,000 population/year. **Conclusion:** The preliminary data suggest that cholera and typhoid fever are highly endemic in this impoverished area in Kolkata. This project will provide important information towards achieving the overall goals of the DOMI programme. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

Vi Vaccine Effectiveness Demonstration Project Karachi: Pilot Phase Disease Surveillance Preliminary Results

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Background: Typhoid fever is endemic and accounts for a major proportion of acute febrile illness among children and adolescents in Pakistan. However, little objective data are available from prospective population-based studies in representative populations. **Objective:** Estimate the incidence of *Salmonella typhi* in urban slums of Karachi, Pakistan, by clinical, microbiological and serological screening methods. **Methodology:** A census was completed in 2002 to identify the target population. Suspected typhoid cases were enrolled through a prospective community-based domiciliary disease surveillance system. Criteria for enrollment were age (2-16 years), history of fever $\geq$3 days, and permanent residence in the target community. **Results:** In the pre-vaccination phase, from January 2002 to September 2003, 3,425 suspected typhoid cases were registered. The mean age of patients registered was 7 ($\pm$4) years with 43% aged 2-5 years, 34% aged 6-10 years, and 23% aged >10 years. The mean duration of fever was 5 days for registered patients. 88% were febrile at the time of presentation with a mean temperature of 38 °C. Previous history of any medication was present in 34% of registered patients. The majority of those with prior history of medication were not able to identify the medication taken. The incidence of *S. Typhi* based on blood cultures was 2.6/1,000/year. Based on the slide agglutination Widal test, the incidence of typhoid was 23.4/1,000/year. **Conclusion:** Typhoid fever is an endemic disease in urban slum setting of Pakistan with an annual incidence of 26/10,000 among 2-16-year old children based on results of blood culture. However, serology data indicate that the actual incidence may be higher than indicated by blood culture alone. A field trial to test Vi vaccine effectiveness is underway to accelerate the introduction of Vi vaccine into public-health programmes. **Acknowledgements:** This work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the Aga Khan University, Pakistan and International Vaccine Institute, South Korea.

P110 (242)

Introduction of Vi Polysaccharide Typhoid Vaccine in Hue City, Central Vietnam: An Evaluation of Feasibility, Public Acceptability, Effectiveness, and Cost-effectiveness in Students

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Background: Licensed typhoid fever vaccines have been available for the last decade, but have not been widely introduced in public-health programmes in the endemic countries. In Vietnam, decision-makers require better information for the accelerated introduction into public-health programmes of the Vi vaccine, targeting school-age children. A cluster-randomized trial to evaluate feasibility, public acceptability, effectiveness, and cost-effectiveness of Vi typhoid vaccine in students in Hue, Vietnam, is scheduled to start in November-December 2003. **Objective:** Evaluate feasibility, public acceptability, effectiveness, and cost effectiveness of Vi polysaccharide typhoid vaccine. **Methodology:** An initial house-to-house *de jure* census was conducted in Hue in 2001 and updated in 2003. The disease surveillance system relies on patients (school-age children) attending the health facilities (government and private) to capture the majority of episodes of prolonged fever (≥3 days) in the community. Mortality information, for school-age children, was collected monthly through commune health centres. Causes of death were assessed using a verbal autopsy. **Results:** A census in August 2003 found that 286,754 individuals were living in 56,157 households in the catchment area for the trial. The median monthly household income in 2001 was 1 million dong (~US$ 65). 89% of 72,990 children in the target age-group plan to attend school in 2003-2004. 57% of children with fever for more than 3 days sought care in the government facilities and 35% in the private sector. Surveillance for typhoid fever for school children started in December 2001. Private physicians were included in the surveillance system in July 2002. Between July 2002 and August 2003, 4,944 blood samples were obtained and cultured. Seasonal variation in fever and typhoid fever was observed. During the 14-month observation period, the annual incidence rate of blood culture-positive typhoid fever was 0.25/1,000 population/year. Serological testing is likely to increase the rate. No deaths due to typhoid fever were detected. In addition to the effectiveness of the Vi vaccine, several social science aspects of the disease, including private and institutional costs, willingness to pay for a vaccine, and perceptions and behaviours relating to the disease are also being studied. **Conclusion:** This trial will provide Vietnamese policy-makers with important information about the costs, logistic feasibility, and acceptability of school-based Vi immunization. While the low incidence of culture-proven typhoid may prevent statistically precise estimates of vaccine protection in this population, meta-analysis of the aggregate of cluster-randomized Vi trials conducted by the DOMI programme in China, India, Pakistan, and Vietnam will be adequately powered to provide these estimates. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

DAY 3: TUESDAY, 9 DECEMBER 2003

12:30 pm-1:30 pm: **Poster Session 3** (Venue: Lobby)

DIARRHOEAL DISEASE 3

P111 (039)

Study of Bacterial and Viral Enteropathogens among Children, Aged Less Than 5 Years, with Acute Diarrhoea in Cuttack, India

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Background: Infectious diarrhoea continues to be a significant cause of morbidity and mortality in the paediatric age group throughout the major part of the world, despite the better understanding of causative agents and advances in diagnostic and therapeutic approaches. In relation to the tropical belt, 15-40% of all deaths among children, aged less than 5 years, are diarrhoea-related. **Objective:** Find out the prevalence of enteropathogenic organisms with reference to diarrhoeagenic *Escherichia coli* and rotavirus among hospitalized diarrhoea cases aged less than 5 years. **Methodology:** A prospective case-control study was conducted for 15 months from July 2001 to October 2002 at Microbiology Department of S.C.B. Medical College & Hospital, Cuttack, India. In total, 100 hospitalized diarrhoea cases and 30 control cases aged less than 5 years were studied. Stool samples were collected, and bacteriological isolation and identification done. Isolated *E. coli* were preserved and sent to NICED, Kolkata, for further characterization by multiplex PCR. Duplicate stool samples were also stored at -20 °C and screened for rotavirus by RNA electrophoresis technique at NICED, Kolkata. **Results:** Sixty-four (64%) of 100 *E. coli* strains were isolated from cases and 14 (47%) of 30 from control group. Fourteen (21.8%) of 64 *E. coli* were found to be diarrhoeagenic by PCR, 12 (85.6%) EaggEC, and 2 (14.3%) ETEC. Among 14 isolated *E. coli* of the control, none was diarrhoeagenic by PCR. 34 (34%) of the 100 cases and 1 (3.3%) of the 30 controls were positive for group A rotavirus; 82.3% showed long e-type (electropherotype) and 17.6% short e-type pattern. In 5 cases, both *E. coli* and rotavirus were isolated. Other enteropathogens were found in 21 (21%) of the 100 cases and 3 (10%) of the 30 controls which included *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Candida albicans*, *Citrobacter freundii*, and *Enterococcus faecalis*. On seasonal analysis, rotavirus was more common during winter, whereas diarrhoeagenic *E. coli* (EAggEC) was more common during rainy season. **Conclusion:** In the study, EAggEC (14%) and group A rotavirus (34%) were two common enteropathogens. **Acknowledgements:** The authors are grateful to Dr. T.N. Naik and Dr. T. Ramamurthy of National Institute of Cholera and Enteric Disease, Kolkata, for the support provided for conducting the investigations. Thanks are also to the staff of Rotavirus and *E. coli* sections for their technical assistance.

P112 (050)

A 5-year Analysis of Bacterial Pathogens Associated with Diarrhoea in Children in National University of Malaysia Hospital

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Background: In the National University of Malaysia Hospital, no established investigative data are available on the aetiologic agents of infectious diarrhoea in children. **Objective:** Investigate the bacterial pathogens in stool samples and determine their antimicrobial susceptibility. **Methodology:** In total, 3,322 stool samples collected during 1998-2002 were examined. Stool samples were cultured for bacterial isolation and identification. Serotyping and antimicrobial susceptibility testing were carried out at the Institute for Medical Research. **Results:** Pathogens were isolated from 3% (105/3,322) of stool samples examined; 53% were from children and 31.5% from adults. *Salmonella* sp. were the most commonly-isolated bacteria (75%) in children. Other pathogens isolated were enteropathogenic *Escherichia coli* (7.1%), *Shigella flexneri* (5.4%), *S. sonnei* (3.6%), *Vibrio cholerae* (3.5%), *Aeromonas hydrophila* (1.8%), *Salmonella typhi* (1.8%), and *Campylobacter* sp. (1.8%). The prevalence of various serotypes of the pathogens were *S. paratyphi* B (12), *S. weltevreden* (8), *S. stanley* (6), *S. tshiongwe* (4), *S. enteritidis* (4), *S. bovismorficans* (2), *S. agona* (1), *Salmonella* untypeable group (2), *V. cholerae* O1 Ogawa (2), *S. sonnei* type 0 (1), *S. sonnei* type 12 (1), *S. flexneri* 2a (3), *E. coli* 0127:K63 (1), *E. coli* 055:K59 (2), and *E. coli* 0142:K86 (1). The antibiograms of 45 strains of *Salmonella* and *Shigella* showed that the average resistance rates to various antibiotics, such as ampicillin, chloramphenicol, co-trimozaxole, streptomycin, kanamycin, and tetracycline, were 21.5%. **Conclusion:** The present analysis showed that *Salmonella* was the main aetiologic agent causing diarrhoea in children, and *S. paratyphi* B was the serotype most frequently encountered. It is necessary to establish information on the aetiologic agents and their resistance patterns as there are few therapeutic options available for infants and children. **Acknowledgements:** The authors thank the Institute for Medical Research, Kuala Lumpur, Malaysia, for providing the data, to all staff in bacteriology diagnostic laboratory, and also to the University.

P113 (059)

Health Status in Some Primitive Tribes of Orissa, India, with Special Reference to Diarrhoeal Disorders, Including Cholera

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Background: Orissa, an eastern state of India occupies a unique place in the tribal map of India (having 62 tribal communities, including 13 primitive tribes). There is a consensus agreement that the health status of tribal population in India is worst and still worst in the primitive tribes because of their isolation, remoteness, and being highly unaffected by the developmental process. **Objective:** Assess the health status and morbidity pattern in respect of diarrhoeal disorders, including cholera. **Methodology:** A community-based study has been undertaken in 4 such primitive tribes, Bondo, Didayi, Kondha, and Juanga, located at 4 different geographical regions of the state. The situation analysis, baseline data collection, clinical examination, and laboratory investigations were performed. A randomly-selected sample population of 1,849 (Bondo–526, Didayi–683, Juanga–361, and Kutia Kondha–279) from 36 villages were clinically examined during February 2000-September 2002, and 679 rectal swabs (Bondo–212, Didayi–201, Juanga–60, Kutia Kondha–201) were collected from both community and hospitalized diarrhoeal cases for bacteriological analysis and molecular characterization of *Vibrio cholerae*. **Results:** The situation analysis revealed that there was a continuous occurrence of diarrhoea throughout the year in all these tribal populations attaining its peak in rainy season from July to October. Analysis of stool samples revealed *V. cholerae* in 3.2%, *V. cholerae* O1 in 3.1% and O139 in 0.9%, *Escherichia coli* in 37.3%, *Salmonella* spp. 0.3%, and *Shigella* spp. 2.2% of cases among the culture-positive enteropathogens. Molecular analysis of *V. cholerae* isolates by multiplex PCR revealed the presence of *ctxA* and *tcpA* toxic genes. **Conclusion:** With the administration of suitable intervention during diarrhoeal outbreaks (ORS and antibiotics) and continuous information, education, and communication activities in the study area, it was observed that there is a progressive decrease in the incidence of diarrhoeal diseases, including cholera, along with the increase of health awareness. **Acknowledgements:** The financial support of the Ministry of Health and Family Welfare, Government of India and of the Indian Council of Medical Research is thankfully acknowledged.

P114 (090)

Status of Giardiasis among School Children of Bhaktapur City of Kathmandu Valley

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Background: There was a frequent report of diarrhoea among school-going children in Bhakatapur, an adjacent city of Kathmandu Metropolitan. **Objective:** Identify stool parasites as a cause of diarrhoea and disseminate the findings to school teachers to reduce the incidence and to improve child health through the school health-education talk programme on the importance of sanitation, personal hygiene, and hand-washing practices with soap and use of boiled and filtered water. **Methodology:** Stool samples were collected from children studying in Nursery to Class X using a clean, dried and disposable wide mouth plastic container. The specimens soon after its collection were processed by wet mount using normal saline and iodine separate for direct microscopy and were examined systematically for parasites scanning entire cover glass with low power objective and high power to make sure of correct identification. **Results:** Of 4,931 specimens examined in 11 months, 470 (9.53%) were positive for *Giardia lamblia* either in trophozoite or cyst form. The incidence of giardiasis was the highest (11.79%) (179/1,517) among the students of Play Group (Nursery and Kinder Garten Group) and the lowest (3.52%) (19/539) among the students of higher classes (VII-X). The rate of incidence was observed to decrease as the classes upgraded. **Conclusion:** *G. lamblia* was the primary cause of diarrhoea among school children. After the treatment by metronidazole, use of boiled and filtered water and hand-washing practices before food and after toilet were recommended.

P115 (113)

Clinico-epidemiological Profile of Diarrhoea Cases: Evidence from Hospital-based Surveillance System at Kolkata

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Background: Infectious Diseases Hospital (IDH), Kolkata, is a referral hospital for all diarrhoea cases in West Bengal where 40-60% of beds remain occupied by diarrhoea patients. Therefore, to understand the magnitude of the problem, a hospital-based surveillance system was set up at IDH, Kolkata, since 1995. **Objective:** Monitor clinical profile of diarrhoeal illness and the changing pattern of aetiological agents. **Methodology:** In this study, a systematic sample (i.e. every 5th patient on two randomly-selected days per week) of all diarrhoea patients attending the casualty of the hospital was enrolled. Clinico-epidemiological information was collected on admission, and faecal samples or rectal swabs were examined for pathogens associated with diarrhoea. **Results:** During 1996-2002, 8,363 diarrhoea patients were enrolled in the surveillance system. Of these patients, 95% had watery diarrhoea, 3% had bloody diarrhoea, and 2% had mucoid diarrhoea. Vomiting was a predominant feature in 62% of the cases. On admission, dehydration was present in 91% of the cases, of which 49% were severely dehydrated. Prior to hospital admission, 44.4% of the patients received oral rehydration salt (ORS) solutions, 27.6% received home-available fluids, and 8.6% received intravenous fluids. The isolation rate of *Vibrio cholerae* varied from 9% to 26% over different years. The seasonality of *V. cholerae* was observed similar to overall diarrhoea with peak during July-September. Isolation rate of *Shigella* and *Salmonella* was low (1-2%), whereas isolation rate of rotavirus varied from 5% to 16% in different years. Rotavirus was observed particularly in winter. **Conclusion:** This study reveals the necessity of a surveillance system in an endemic region like Kolkata for detecting various enteropathogens from diarrhoea patients and their clinical profile, including drug-susceptibility patterns, which is needed to formulate a treatment strategy from time to time to study the trend of diseases and predict impending epidemic outbreaks.

Diarrhoea Trends in DTU-CHK

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Objective: Assess morbidity and mortality of diarrhoea patients attending DTU from 1998 to June 2003. **Methodology:** Children aged 0 month-15 years, who attended DTU with complaints of loose motions, were weighed, and then grading of malnutrition was done according to modified Gomez classification. The patients were categorized according to WHO case management for their dehydration status and treated per Plan A, B, and C. The recording of each case was done in a register regularly. When required, additional treatment was also instituted. Data to be presented also include co-morbid conditions. **Results:** The total paediatric OPD in 5 years (1998-June 2003) were 5,84,499, and DTU attendance in the same period was 52,689 (9.01%). The hydration status of patients as assessed by the WHO protocol was: no dehydration-32,439 (61.56%), some dehydration-12,588 (23.8%), and severe dehydration-6,331 (12.1%), whereas the nutritional status was: normal-33,783 (64.1%), PCM-1-7,441 (14.12%), PCM-11-5,139 (9.7%) and PCM-111-6,326 (12%). The different co-morbid conditions encountered were dysentery, persistent diarrhoea, pneumonia, severe pneumonia, very severe disease, rickets, and seizures. **Conclusion:** The total number of visits to DTU over the year has remained constant, but the proportion of severe dehydration cases has risen by 60% possibly due to the increased use of public hospital and 24 hours availability of trained staff. Yearly mortality rates have remained constant at 7%. The case-management strategy should be adhered to and be continuously taught.

P117 (289)

Changing Patterns of Socioeconomic Characteristics of Patients Attending Dhaka Hospital of ICDDR,B, 1997-2003

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Background: Socioeconomic characteristics influence healthcare-seeking behaviour of patient population in many developing countries, including Bangladesh. Since March 2000, ICDDR,B decided to franchise its out-patient services of Dhaka Hospital to Progoti Samaj Kallyan Protisthan (PSKP), a Johns Snow International-supported NGO. The franchising aimed to reduce patient load, financial pressure, improve healthcare delivery, and develop a new infrastructure for operations research and surveillance at ICDDR,B. A registration fee of Tk 20.00 per patient per visit for services was also introduced. **Objective:** Investigate changes in the socioeconomic status (SES) of children, aged less than 5 years, attending the Dhaka Hospital by comparing SES of study children treated between two time periods, i.e. before franchising (March 1997-February 2000; n=4,282) and after franchising (March 2000-February 2003; n=3,517) the Dhaka Hospital out-patient services of ICDDR,B. **Methodology:** For this analysis, hospital surveillance system database (collecting information using a systematic sampling procedure) was used. SES of study subjects' family was evaluated by constructing a wealth index using asset ownership (fan, radio, TV, almirah, luxury cot, ordinary cot), dwelling materials, such as wall, floor, or roof, toilet facility, sources of drinking-water, use of gas for cooking, and availability of electricity. Only the poorest wealth quintile of the 5 wealth quintiles (poorest, second, middle, fourth, and richest) was used for comparison of lack of maternal formal schooling, presenting dehydration status, short- or long-stay hospitalization, possession of less than 4 household items, and nutritional status of children between the two time periods, i.e. before and after franchising. **Results:** Significant reductions were observed in reporting to the health facility of young children coming from poorest socioeconomic strata following franchising as evaluated by lack of formal schooling of mothers (77% vs 70%; $p<0.001$), no dehydration on presentation (23% vs 15%; $p<0.001$), some severe dehydration (29% vs 21%; $p<0.001$), short-stay hospitalization (23% vs 16%; $p<0.001$), long-stay hospitalization (34% vs 23%; $p<0.001$), and possession of <4 household articles (39% vs 31%; $p<0.001$). However, the nutritional status—stunting (44% vs 40%; $p=0.198$), under-weight (58% vs 61%; $p=0.324$), and wasting (28% vs 28%; $p=0.966$)—remained similar in the two time periods. **Conclusion:** Franchising may have influenced poorest children with different clinical and epidemiological characteristics in less reporting to the health facility. Further quantitative and qualitative research is needed to draw more conclusive inferences. **Acknowledgements:** The financial support of the Department for International Development (DFID), UK, and the United States Agency for International Development (USAID) is acknowledged.

Diarrhoea in the Elderly: Aetiology, Clinical and Epidemiological Characteristics

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Background: Diarrhoea is a leading cause of morbidity among children and young adults, and there is lack of information on diarrhoeal morbidity among the elderly. **Objective:** Examine the aetiology, clinical and epidemiological characteristics of elderly people attending a diarrhoeal disease hospital in urban Bangladesh. **Methodology:** 2% systematic sample of patients (n=13,782) attending the Dhaka Hospital of ICDDR,B, during 1996-2001 was studied. Patients aged 60 years or older (4% of 13,782, i.e. 478) were included in analysis. **Results:** *Vibrio cholerae* O1 was the most common enteric pathogen (20%), followed by enterotoxigenic *Escherichia coli* (ETEC) (13%), *Shigella* (11%), *V. cholerae* O139 (10%), *Campylobacter jejuni* (5%), *Salmonella* (3%), EPEC (2%), rotavirus (4%), and *Entamoeba histolytica* (2%). Compared to children aged less than 5 years, the isolation rates of *V. cholerae* O1 (20% vs 12%), *V. cholerae* O139 (10% vs <1%), *Shigella* (11% vs 6%), and *E. histolytica* (2% vs <1%) were significantly ($p<0.05$) higher among the elderly. Significantly ($p<0.05$) higher proportions of the elderly had shorter duration (0-72 hour(s)) of illness (89% vs 65%), higher (>6 per 24 hours) stool frequency (25% vs 12%), severe dehydration (38% vs 5%), and visible blood in stool (8% vs 1%). Compared to younger adults (15-59 years), the elderly had significantly higher rates of isolation of *V. cholerae* O139 and *Shigella* (10% vs 6%, and 11% vs 7% respectively; $p<0.05$ for both the comparisons), had visible blood in stool more often (8% vs 5%), had higher rates of hospitalization for >24 hours (29% vs 18%), and had referral (1% vs <1%) to other health facilities. **Conclusion:** Early initiation of rehydration (oral or intravenous), referral, and efficient clinical assessment and appropriate antimicrobial therapy might be helpful in the management of diarrhoea in the elderly. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged.

P119 (268)

A Recent Outbreak of Cholera Due to *Vibrio cholerae* O1 Ogawa in and Around Chandigarh, North India

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Background: Epidemics of cholera have been reported from various parts of India. Resurgence of *V. cholerae* O139 in certain areas has also been reported. Cholera has been quiescent in the past few years in Chandigarh. Very few cases have occurred (4 confirmed cases) in the last two years. A limited outbreak due to *Vibrio cholerae* O139 (36 cases) occurred in July 1994. Since then, *V. cholerae* O139 has not been isolated, and a few cases that occurred have been due to *V. cholerae* O1 Ogawa. **Objective:** Monitor and report on an outbreak of cholera due to *V. cholerae* O1 biotype ElTor serotype Ogawa in July 2002. **Methodology:** Stool samples were collected from patients suspected to have cholera and admitted to two local hospitals (Government Hospital, Sector 16 and Postgraduate Institute of Medical Education and Research, Chandigarh). They were examined by standard bacteriological techniques. Isolates of *V. cholerae* were biotyped and serotyped, and antimicrobial susceptibility testing was done. A team surveyed the affected areas and collected 88 water samples (10 wells, 45 taps, 20 hand-pumps, and 13 stored water). Water samples were tested by multiple-tube method for faecal coliforms and *Escherichia coli,* and membrane-filter technique for *V. cholerae*. **Results:** In total, 303 patients suspected to have cholera were admitted to two hospitals. The maximum numbers of patients were from two rehabilitation colonies located at periphery of Chandigarh. Stool samples could be collected from 148 patients, and 82 stool samples were positive for *V. cholerae* O1. Forty-eight patients (58.5%) were children, and 16 were aged less than 5 years. Males and females were almost equally affected (42 males, 40 females). All patients presented with acute watery diarrhoea. Seventy-eight percent had vomiting, 56% developed mild-to-moderate dehydration, and 10% developed severe dehydration. Pain in abdomen occurred in 18% of patients. The patients were treated with oral rehydration solution, intravenous fluids, and antibiotics. One patient died, and all others recovered. Case-fatality was <0.01%. All isolates were biotyped as ElTor and were susceptible to amoxicillin, ciprofloxacin, cephalexin, gentamicin, tetracycline, and cefotaxime. The resistance to furazolidone, co-trimoxazole, and chloramphenicol was 37%, 25.8%, and 8.9% respectively. Although stool samples were collected from hospitalized patients only, survey of the affected areas revealed a large number of people having symptoms suggestive of cholera in the two rehabilitation colonies (more than a thousand in area A and 113 cases in area B). This was due to mixing of drinking-water with sewage in these areas due to broken pipelines. Of the 88 water samples tested, 31 (35.2%) were positive for confirmed coliforms, and 13 (14.7%) were positive for confirmed *E. coli*. Water samples collected from tubewells were free of contamination, while water samples collected from hand-pumps (12/20), taps (11/45), and stored water (8/13) showed evidence of faecal pollution. *V. cholerae* could not be grown from any of the water samples. The outbreak was controlled by providing safe drinking-water to the residents from mobile water-tanks, correcting the defects in the sewage and water pipelines, treating the patients, and providing health education to the residents about personal and domestic hygiene practices. **Conclusion:** Regular surveillance of rehabilitation colonies for occurrence of cholera cases and bacteriological testing of drinking-water supplies may prevent the occurrence of outbreaks of enteric diseases.

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Multinomial Logistic Regression Model to Explore Inferential Risk Age Groups of *Vibrio cholerae* O1, O139, and Non-O1 Non-O139 Infection during 1996-2000 in Calcutta, India

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Background: For modelling categorical outcomes, multinomial logistic regression model (MLR) is an effective technique than discriminant function analysis and log-linear models for profiling individual category of dependent variable at the greatest risk of predictor. **Objective:** Explore the year-wise changes of inferential age groups with *Vibrio cholerae* O1, O139, and non-O1 non-O139 infection during 1996-2000 by MLR using the hospital-based surveillance system. **Methodology:** Systematic sampling of every 5th hospitalized case was made in two randomly-selected days in a week. Clinical information and stool samples were collected from the Infectious Diseases Hospital, Calcutta. Data of 1,566 *V. cholerae* O1, O139, and non-O1 non-O139-positive cases during 1996-2000 were analyzed. The MLR model involves one categorical dependent variable Y in which log of odds Y to the explanatory variable X in a linear form. The predictor X can be categorical or continuous. It was assumed that the number of cases had multinomial distribution. The relationship between the dependent variable age and the predictor variables *V. cholerae* was compared to identify the changes. **Results:** Of the 1,566 *V. cholerae*-infected cases, the predominant age category was <5 years (n=478, 30.5%). The independent variables *V. cholerae* O1 ($p<0.001$), O139 ($p<0.008$), and non-O1 non-O139 ($p<0.001$) were significantly associated with children aged less than 5 years. *V. cholerae* O1 showed significantly increasing trend (Exp(B)) year-wise ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$) when compared with respective years since 1996 to 2000. Increasing trend of *V. cholerae* O139 was significant in 2000 ($p=0.007$), and *V. cholerae* non-O1 non-O139 trend was increasing at the inception (1997) and was low at the end (2000) with significance of $p=0.004$ and $p<0.001$ respectively with children aged 5 years. **Conclusion:** The MLR model is a procedure which estimates the net effects of a set of predictor variables on the dependent variable. The MLR model shows that children aged less than 5 years are more vulnerable to infection caused by *V. cholerae* O1.

Ongoing Shigellosis Surveillance in Nha Trang, Vietnam: Preliminary Findings

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Background: Shigellosis is highly endemic in Vietnam. However, little population-based data on the incidence of shigellosis are available in Vietnam. **Objective:** Describe the incidence of shigellosis in Vietnam. **Methodology:** A catchment population for the study included 201,978 individuals living in Nha Trang, central Vietnam. Diarrhoea patients of all ages were enrolled at the project heath facilities (1 provincial hospital, 4 polyclinics, and 16 commune health centres). Rectal swabs were collected and transported to the project laboratory (Pasteur Institute, Nha Trang). **Results:** Of 19,582 patients enrolled from September 2000 to August 2003, *Shigella* spp. were isolated from 659 patients (3%). About 46% of the patients were children aged less than 5 years. Of these children, 15% had mucoid diarrhoea, 19% had watery diarrhoea, and 12% had bloody diarrhoea. 77% of *Shigella* isolates were *Shigella flexneri,* 1% *S. boydii*, 22% *S. sonnei,* and 0.2% *S. dysenteriae*. Serotyping pattern of *S. flexneri* showed the predominance of sub-type 2b (37%), followed by 2a (26%) and 3a (20%). Only 0.5% of *Shigella* isolates were resistant to ciprofloxacin. Resistance to co-trimoxazole ranged from 40% to 92%, ampicillin from 20% to 78%, nalidixic acid from 0% to 14%, and amoxacillin-clavulinic acid from 0% to 7% depending on the species. **Conclusion:** Increasing resistance of *Shigella* strains to various first-line antimicrobials was observed, adding urgency to the introduction of a protective vaccine. *S. flexneri* is the most common species in this area of Vietnam, and 2a, 2b, and 3a are the main subtypes. The heterogeneity of *Shigella* species, serotypes, and subtypes has to be considered in the development of a polyvalent vaccine to protect against shigellosis in Vietnam. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

A Shigellosis Study in Saraburi, Thailand: Preliminary Findings

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Background: The disease burden due to shigellosis in Thailand is poorly understood. **Objective:** Report the preliminary findings of a shigellosis study conducted in Saraburi, Thailand. **Methodology:** The burden of shigellosis was studied during May 2002-May 2003 in a population of 78,744 residing in a recently-industrialized area, Kangkoi district, Saraburi province, Thailand. Health officers in 22 study healthcare centres were asked to document all cases of acute diarrhoea. Demographic and clinical information was recorded. Rectal swabs were collected from eligible volunteers. Specimens were cultured by conventional methods at the Saraburi Regional Hospital Laboratory. *Shigella* isolates were confirmed and classified into serotypes at the Faculty of Allied Health Sciences, Thammasat University. All specimens were also tested by a novel serogroup-specific monoclonal antibody-based dot-ELISA. **Results:** In total, 2,950 diarrhoeal specimens were collected during the study period. Only 38 *Shigella* strains were isolated and confirmed, but 958 samples were positive for *Shigella* by dot-ELISA. The incident rate of shigellosis, caused by the two predominant strains, *S. sonnei* and *S. flexneri,* was 0.34 and 0.11/1,000 persons per year respectively. Most *S. flexneri* and *S. sonnei* isolates were susceptible to norfloxacin (100%), nalidixic acid (100%), kanamycin (90.9%), gentamicin (100%), and ciprofloxacin (100%). A healthcare-use survey indicated that passive surveillance in healthcare centres which participate in shigellosis surveillance might miss one-fourth of dysentery cases in children and more than half of dysentery cases in adults. **Conclusion:** *S. sonnei* is the predominant *Shigella* species in Saraburi province, Thailand, in contrast to other Asian regions. This finding should be considered in the development of a shigellosis vaccine. **Acknowledgements:** The study was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

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Emergence of *Shigella* spp. as an Important Cause of Diarrhoea in North Jakarta, Indonesia

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Background: Previous studies in Indonesia have shown that 2-4% of diarrhoea cases are due to *Shigella* spp., although the actual prevalence might be higher. After an absence of two decades, the re-emergence of *Shigella dysenteriae* in Indonesia has been reported. **Objective:** Estimate morbidity caused by cholera and shigellosis in North Jakarta, Indonesia. **Methodology:** During August 2001-July 2003, a surveillance for cholera and shigellosis was conducted in 2 sub-districts of North Jakarta. Approximately, 140,000 individuals are residing in the catchment area. **Results:** In total, 16,872 stool samples were collected, of which 1,246 (7%) were *Shigella* spp. The most commonly-isolated type was *S. flexneri* (n=904; 73%), followed by *S. sonnei* (n=282; 23%), *S. boydii* (n=34; 3%), *S. dysenteriae* (n=21; 2%), and non-typeable *Shigella* (n=5; 0.4%). Patients with *S. flexneri* were mostly in the 15-59-year age group, in contrast to patients with *S. sonnei* who were mostly in the 1-4-year age group. Only 23% presented with dysentery, and 21% had fever. 60-95% of *Shigella* isolates were resistant to first-line antibiotics (ampicillin, co-trimoxazole), but less than 1% were resistant to cephalosporins and ciprofloxacin. 439 (3%) stool/rectal swab samples grew vibrios, including 155 *Vibrio cholerae* O1, 198 *V. cholerae* non-O1, and 141 *V. parahaemolyticus*. *V. cholerae* isolates were sensitive to commonly-prescribed antibiotics. **Conclusion:** *Shigella* spp. were more frequently detected than expected. *Shigella* spp., in contrast to *V. cholerae,* are now frequently resistant to commonly-used antibiotics. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

Shigellosis in an Urban Area of Dhaka, Bangladesh

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Background: Shigellosis is highly endemic in Bangladesh. Little population-based data on shigellosis in urban areas are available. **Objective:** Define disease burden due to *Shigella* in an urban slum of Dhaka and examine the antimicrobial resistance pattern of strains isolated. **Methodology:** Diarrhoea patients of all ages were enrolled at the Kamlapur urban surveillance clinic. Stool or rectal swabs were collected and processed to culture enteric pathogens. Antimicrobial susceptibility of *Shigella* spp. was determined. Serotyping and sub-serotyping of *Shigella* were performed using monoclonal antibody where indicated. **Results:** Of 2,744 patients enrolled from December 2001 to August 2003, *Shigella* spp. were isolated from 13% of the patients. About 73% of the patients were aged less than 5 years, and 49% of these patients had mucoid diarrhoea, 43% watery diarrhoea, and 8% bloody diarrhoea. Of the *Shigella* isolates, 50% were *Shigella flexneri,* 24% *S. boydii,* 10% *S. sonnei,* 9% *S. dysenteriae,* and 7% non-typable *Shigella*. Serotyping pattern of *S. flexneri* showed the predominance of sub-type 2a (28%), followed by 3a (27%), that of *S. boydii* serotype 14 (23%) and 12 (17%); *S. dysenteriae* serotype 9 (30%), 2 and 14 (20%); *S. sonnei* phase II (73%). No *Shigella* isolates were resistant to ciprofloxacin and mecillinam. Resistance to co-trimoxazole ranged from 39% to 92% depending on *Shigella* species, ampicillin from 22% to 57%, nalidixic acid from 25% to 83%, and amoxicillin-clavulanic acid from 12% to 30%. **Conclusion:** Increasing resistance of *Shigella* strains to various antimicrobials causes frequent treatment failures adding urgency to the introduction of a protective vaccine. The heterogeneity of *Shigella* species and subtypes has to be considered in the development of polyvalent vaccines. **Acknowledgements:** The study was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

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The DOMI Existing Data Website

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Background: Recent interviews with policy-makers highlighted the need for disease-burden data on cholera, shigellosis, and typhoid fever. DOMI has developed a new activity to systematically collect epidemiological data from published and unpublished sources in 6 participating countries (Pakistan, India, Thailand, Vietnam, China, and Indonesia). As a part of this study, the DOMI team has also developed the EDC website. This website allows investigators to overview materials relating to the EDC study, such as background, method, data, and preliminary results. **Objective:** Determine the incidence of treated episodes of cholera, shigellosis, and typhoid fever; determine mortality rate due to shigellosis, cholera, and typhoid fever; determine age-specific incidence and mortality of treated episodes of cholera, shigellosis, and typhoid fever; determine the number or fraction of cases requiring admission and number or fraction of cases treated as outpatients; determine antibiotic resistance profiles of the causative organisms; and determine distribution of *Vibrio cholerae* by biotype and serotype and *Shigella* by species and serotype. **Methodology:** Systematic review and abstraction of data collected between 1991 and 2000 from 3 sources were conducted: government statistics (cholera, shigellosis, diarrhoea, dysentery, typhoid fever, and enteric fever), representative hospital and laboratory data, and published literature (local and international). **Results:** Through this activity, DOMI will provide more comprehensive estimates of the incidence and mortality of the 3 diseases in 6 partner countries. These routine estimates will be augmented later by estimates from the Programme's prospective studies. **Conclusion:** The activity has already produced valuable data which are used by the collaborators of the Programme. The database allows scientists to compare the disease burden for the three target diseases across countries over a decade. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

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Shigellosis in Zhengding, Hebei Province, China in 2002

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Background: Few population-based studies have estimated the disease burden of shigellosis in rural China. **Objective:** Estimate the disease burden caused by specific *Shigella* species and serotypes in Hebei province in 2002. **Methodology:** A prospective, population-based, passive surveillance study was conducted in Zhengding from January 2002 to January 2003. The catchment area included 75,630 individuals, of which 2,997 (4%) were aged less than 60 months. The surveillance system was composed of all 4 township hospitals and 101 village clinics in the catchment area. Laboratory-confirmed shigellosis patients were visited on day 3, 7, 14, and 90. **Results:** The average incidence rate of shigellosis was 4.38/1,000 per year. The highest incidence rate of diarrhoea and *Shigella* was observed in children aged less than 5 years, and the incidence of *Shigella* rose in the people aged less than 50 years. No death and one unrelated hospitalization were detected during 90-day follow-up. Of 331 *Shigella* strains isolated during the surveillance period, 306 (93%) were *Shigella flexneri*, and the remaining 25 isolates were *S. sonnei*. No *S. dysenteriae* and *S. boydii* were detected. The 3 most frequently-encountered *S. flexneri* serotypes were 1a (34%), type X (34%), and 2a (28%). The high percentage (>90%) of *Shigella* strains was resistant to nalidixic acid. **Conclusion:** The incidence of shigellosis is high in children and the elderly in this part of China. *S. flexneri* serotype X is more frequently isolated than previously expected. The emergence of resistant *Shigella* strains adds urgency to the introduction of protective vaccines. **Acknowledgements:** The work was supported by the Diseases of the Most Impoverished (DOMI) programme, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute, South Korea.

ENVIRONMENTAL HEALTH

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Association of Culturable and Viable but Non-culturable *Vibrio cholerae* O1 and O139 with Copepods in Microcosms

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Background: *Vibrio cholerae* O1 and O139 have been implicated as causative agents of epidemic cholera. It is not known how long *V. cholerae* can persist in the viable but non-culturable state in association with copepods. Moreover, the survival pattern of the new epidemic strain of *V. cholerae* O139 and their specific association with copepods in microcosms have not yet been studied. **Objective:** Determine the survival potential of toxigenic *V. cholerae* O1 and O139 attached to copepods in microcosms in the culturable and VBNC states. **Methodology:** Survival of *V. cholerae* O1 and O139 was monitored using culture and fluorescent antibody (FA) techniques. Microcosms were prepared with pond-water, copepods, unicellular alga, and *V. cholerae* O1 or O139 strains and kept in the laboratory. **Results:** *V. cholerae* O1 and O139 survived up to 10 and 12 days respectively attached to copepods in culturable state but the culturability of the bacteria in water where copepods were living was only 5 days for both the strains. VBNC *V. cholerae* O1 and O139 were detected at least up to 62 and 72 days respectively in association with copepods. In water around copepods, VBNC *V. cholerae* O1 and O139 were detected up to 34 and 38 days respectively. *V. cholerae* O1 and O139 were attached to the posterior cephalothoracic region, oral region, and caudal setae of copepod. **Conclusion:** The study demonstrated that both *V. cholerae* O1 and O139 acquire survival advantages in association with copepods compared to water in which copepods were living. **Acknowledgements:** The research was funded by the National Institutes of Health (NIH) (grant no. 1R01A139129-01), USA and by the ICDDR,B: Centre for Health and Population Research. ICDDR,B is supported by countries and agencies which share its concern for the health problems of developing countries.

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Long-term Persistence of *Vibrio cholerae* O1 and O139 in Association with Copepods in Microcosm

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Background: Microcosm studies have demonstrated that *Vibrio cholerae* O1 and O139 can independently survive longer in association with copepod than water in which copepods were living. However, no microcosm study was undertaken in which co-existence of both *V. cholerae* O1 and O139 in association with copepods has been monitored. **Objective:** Find out the co-existence of *V. cholerae* O1 and O139 in association with copepods in microcosms. **Methodology:** The persistence of *V. cholerae* was monitored, in association with copepods, in water in which copepods were living and in control water without copepods using culture, fluorescent antibody, multiplex PCR, and dot-blot hybridization techniques. **Results:** Both *V. cholerae* O1 and O139 survived in culturable state and viable but non-culturable state longer in association with copepod than water in which the copepods were living. The lower numbers of both *V. cholerae* O1 and O139 were detected by multiplex PCR and dot-blot hybridization. *V. cholerae* O1 survived 8 days in culturable state but detected up to 65 days in viable but non-culturable state in association with copepods. Similarly, *V. cholerae* O139 survived 6 days in culturable state but 65 days in viable but non-culturable state. Preferential attachment of *V. cholerae* in the caudal setae and oral region of copepods was observed by the fluorescent antibody technique. **Conclusion:** The results demonstrated the long-term persistence of *V. cholerae* O1 and O139 in association with copepods. The findings again emphasized the role of copepods as a reservoir of *V. cholerae* in the aquatic environment. The co-existence of both the serotypes may be more harmful due to synergistic effect and can cause more devastating epidemics in future. **Acknowledgements:** The research was funded by the National Institutes of Health (NIH) (grant no. 1R01A139129-01), USA and by the ICDDR,B: Centre for Health and Population Research.

P129 (312)

Field Trial of a Flocculent-Disinfectant Treatment of Pond-water for Drinking in Bangladesh

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Background: The continued prevalence of diarrhoeal disease in Bangladesh, along with arsenic contamination of tubewells, emphasizes the urgent need for innovative, practical strategies to provide safe drinking-water. **Objective:** Evaluate the efficacy of a combined flocculent-disinfectant point-of-use water treatment for improving the quality and microbial safety of surface pond-water in rural Bangladesh. **Methodology:** Water from 35 surface ponds in Matlab, Bangladesh, was collected and treated with the flocculent-disinfectant 3 times, at one month interval. Samples collected before and after treatment were analyzed for faecal coliforms by the membrane-filtration technique using filter coliform agar. Water samples were classified as potable if they met the WHO bacterial guideline of <1 CFU/100 mL faecal coliforms. Free chlorine levels were measured 30 minutes after treatment. Turbidity was measured with a portable turbidity meter. Since the samples were from surface water, arsenic levels were not evaluated. **Results:** Prior to treatment, all samples (n=101) were non-potable and were contaminated with a mean of 2.9×10^4 CFU/100 mL faecal coliforms. Turbidity ranged from 6 to 92 NTU, with a mean of 32 NTU. Following treatment, 97 (96%) samples met the potability guideline. Treatment resulted in a mean 87% reduction in turbidity (mean 4 NTU) with 93% of the samples meeting the WHO turbidity guideline of <5 NTU. Free chlorine was detected in 83% of the samples. **Conclusion:** Following treatment with the flocculent-disinfectant, 96% of the surface pond-water samples met the WHO bacterial potability guideline. Samples showed markedly improved clarity. This strategy may be useful in programmes for improving the quality and safety of drinking-water. **Acknowledgements:** The study was funded by the Procter and Gamble Health Sciences Institute, USA.

P130 (040)

Improvement of Sanitation Condition of Slum Areas of Sylhet City: An Evaluation

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Background: Sylhet is lagging behind in providing utility facilities, especially in water supply and sanitation sectors. In slum areas, open defecation is a normal practice. Moreover, slum dwellers have limited access to safe drinking-water. Consequently, various types of diseases are spreading out. The deteriorated water supply and sanitation scenario causes severe environmental degradation, which affects the total environment of Sylhet city. **Objective:** Assess the sanitation conditions of low-income areas of Sylhet city, motivate the community for practising appropriate defecation systems, and monitor the behavioural changes after providing some sanitary facilities. **Methodology:** Necessary information and data were collected using a questionnaire and visual inspection of 5 selected slums. Several motivational programmes were conducted for creating their awareness among slum people about health and sanitation. Some sanitary units were installed with the active participation of the inhabitants in those areas. Regular monitoring was performed to observe the behavioural and other changes of the communities. **Results:** Open defecation, even by children, was remarkably reduced after intensive motivational works and providing sanitation facilities, which reduced the spreading of various diseases. Before undertaking the research work, only 14.5% of targeted dwellers were observed using sanitary latrines. But it has now sharply increased to almost 78%. Besides, active participation of the beneficiaries at different stages of the programme made them responsible for using and maintaining toilets. **Conclusion:** The overall health and sanitation conditions of the community were significantly improved. **Acknowledgements:** The financial support of the International Training Network (Danida-World Bank), BUET, Dhaka, Bangladesh, is acknowledged.

P131 (075)

Environmental Health Assessment in Urban Wards of Indore, Madhya Pradesh

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Background: Diarrhoea is the second most serious killer of children aged less than 5 years, accounting for an estimated 1.5 million deaths annually. Improved hygiene can lead to a 30-50% reduction in diarrhoea morbidity. **Objective:** Assess environmental health in slums of Indore to study water, sanitation and drainage services and household hygiene practices, and identify programmatic options for diarrhoea prevention through hygiene improvement. **Methodology:** Interviews with Indore municipal officials and community representatives were conducted to understand provision of water and sanitation services and to identify prospective programme partners and resources. Focus-group discussions were held, and a quantitative survey among 150 households in two slums was conducted. **Results:** Indore (population 1.6 million) is divided into 69 wards. Only 11% of slum dwellers have piped water supply. Half (50%) of the municipal lines were functional. The sewerage system covers less than half of the population. 1,400 public toilet blocks with an average of 10 seats cover 60% of slums. Only 10% were functional. 88% adults and 59% children resorted to open defecation. The main reasons for not using available public toilets were inadequate water (75%), poor maintenance (40%), and foul smell (35%). Hand-washing after defecation was the least priority behaviour due of lack of resources, such as water. The community did not recognize the relationship among sanitary disposal of faeces, hygiene behaviour, and diarrhoea. **Conclusion:** A programmatic approach is to link interests of municipality and slums through support for rehabilitation and expansion of public latrines, capacity-building of municipal water/sanitation sector for service-delivery in slums, and capacity-building of community representatives to maintain public toilets and promote improved hygiene behaviours. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) is acknowledged. Active contribution of stakeholders, viz. the community, CBOs, NGOs, and Indore Municipal Corporation in carrying out the study needs special mention.

P132 (121)

Impact of Conflict Conditions on Hygiene Behaviour in the Occupied Palestinian Territories

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Background: The Palestinian Territories are experiencing chronic conflict conditions since long time. Impacts of the conflict on social, economic and physical environment have been tackled, but impact on hygiene behaviour and practices has never been dealt with. **Objective:** Provide baseline data on the indoor and outdoor environmental hygiene and sanitation conditions; evaluate hygiene behaviour of Palestinian caretakers; investigate the incidence of diarrhoea and trends in high-risk communities in the Palestinian Territories; identify the risk variables relating to water shortage and hygiene behaviour associated with the occurrence of diarrhoea; and identify the impact of the conflict conditions on hygiene behaviour in Palestine; and determine the value of incorporating environmental health with primary healthcare services (to be national policy recommendation). **Methodology:** The community-based study included random selection of 40 rural localities, classified localities according to political intensity, conducted public environmental survey in each locality, also conducted household survey using semi-structured interviews, also included an observation checklist to be implemented by community health workers of Ministry of Health, Palestine, and investigated diarrhoeal incidence by follow-up study of cohort of 1,200 children aged less than 5 years for 12 weeks. **Results:** The study is ongoing. Initial results included: the incidence of diarrhoea in the rural areas in Palestine was about 20%. Public environmental conditions were worse in the localities with political intensity. 80% of the localities experienced water shortage. Hygiene behaviour was high among caretakers, regardless of educational level. The incidence of diarrhoea was higher in localities with political intensity. **Conclusion:** Poor hygiene behaviour and diarrhoeal incidence are more common among caretakers in localities with more political intensity. Political conditions result in stress and frustration that result in poor hygiene behaviour, regardless of caretaker's awareness of the possible risks implied by these practices. **Acknowledgments:** The research was supported by generous fund from the Karim Rida Said Foundation, London, UK.

P133 (220)

Organization of CTX Prophage in Environmental Isolates of *Vibrio mimicus*

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Background: *Vibrio mimicus* has been implicated as the causative agent of gastroenteritis and for various other infections. The occurrence of *ctx* gene in environmental *V. mimicus* has been reported earlier but yet to date the organization of CTX prophage in the genome of environmental *V. mimicus* isolates has not been determined. **Objective:** Search for *ctx*-containing *V. mimicus* strains from the aquatic environment of Bangladesh and determine the organization of CTX prophage within the genome of *V. mimicus.* **Methodology:** In total, 1,600 non-sucrose fermenting vibrios were examined for the presence of *ctx* gene using the patch technique by DIG-labelled colony blot hybridization. The organization of CTX prophage was determined by RFLP using DIG-labelled *ctxA* probe. **Results:** Of 1,600 isolates, 6 contained *ctxA* gene and were identified as *V. mimicus*. The presence of *ctxA*, *ctxB*, and *tcpA* was examined by PCR in 6 *V. mimicus* strains. The CTX prophage was present as a single copy flanked by at least a single RS element. **Conclusion:** Ribotype pattern revealed that strains of *V. mimicus* that acquired CTXO in the aquatic environment belonged to a single clone. **Acknowledgements:** The research was funded by the National Institutes of Health (grant no. 1R01A139129-01), USA and by the ICDDR,B: Centre for Health and Population Research.

Pandemic Strains of O3:K6 *Vibrio parahaemolyticus* in the Aquatic Environment of Bangladesh: Implications for Disease Transmission

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Background: *Vibrio parahaemolyticus* is the leading cause of seafood-borne gastroenteritis and travellers' diarrhoea, especially in countries where seafood consumption is high. The pandemic serotypes of *V. parahaemolyticus* in patients have been detected in Bangladesh. However, it is not known whether the pandemic strains are also present in the aquatic environment of Bangladesh. **Objective:** Determine if the pandemic serotypes prevailed in the aquatic environment of Bangladesh as a possible source of human disease. **Methodology:** In total, 1,500 environmental strains of *V. parahaemolyticus*, isolated from the aquatic environment of Bangladesh, were screened for the presence of the *tdh* gene by the colony blot hybridization method using a digoxygenin-labelled *tdh* gene probe. **Results:** Of the 1,500 strains, 5 carried the *tdh* sequence, which was further confirmed by polymerase chain reaction (PCR) using primers specific for the *tdh* gene. Examination by PCR confirmed that the 5 strains were *V. parahamolyticus* and lacked the *trh* gene. All the 5 strains gave positive Kanagawa phenomenon reaction with characteristic □-haemolysis on Wagatsuma agar medium. Southern blot analysis of the *Hin*dIII-digested chromosomal DNA demonstrated presence of two *tdh* genes common to Kanagawa phenomenon-positive strains in all the 5 strains. However, the 5 strains belonged to 3 different serotypes (O3:K29, O4:K37, and O3:K6). Of these, two were pandemic serotype O3:K6 and gave positive results in group-specific (GS) PCR and ORF8 PCR assays, characteristics unique to the pandemic clone. Clonal variations among the 5 isolates were analyzed by comparing random amplified polymorphic DNA (RAPD) and ribotyping patterns. **Conclusion:** Different patterns for 3 serotypes were found, but the pattern was identical among the O3:K6 strains. This is the first report on the isolation of pandemic O3:K6 strains of *V. parahaemolyticus* from the aquatic environment of Bangladesh. **Acknowledgements:** The research was funded by the National Institutes of Health (NIH) (grant no. 1R01A139129-01), USA and by the ICDDR,B: Centre for Health and Population Research.

Villagers' Perception about Arsenic-contaminated Drinking-water in Matlab, Bangladesh

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Background: There has been a remarkable change in the source of drinking-water from surface to underground during the last couple of decades in rural Bangladesh. While underground water has been relatively safer biologically than surface water, the recent discovery of arsenic in ground-water has posed a serious public-health threat to rural population. The tackling of emerging crisis may necessitate a modification of people's behaviour in relation to water use. **Objective:** Understand villagers' perception of drinking-water quality and taste in relation to arsenic contamination and health. **Methodology:** An ethnographic study, including 8 focus-group discussions, 9 in-depth interviews, timeline, and observational techniques were applied to collect information during August 2001-May 2002 in two villages of Matlab, Bangladesh. **Results:** Villagers perceived that tubewell water possesses iron and is tasteful; canal- or river-water is iron- and arsenic-free but germ-contaminated; pond-water is muddy, greenish, and unpleasant to drink. Canal- or pond-water is mainly used for cooking and washing. The young and literate villagers reported that tubewell water now possesses arsenic and causes blackening of skin and hardening of palms and soles. Some villagers perceived that boiling or filtering or using water-purifying tablet removes arsenic from water. Most key informants reported that they would not believe the harmful consequence of drinking arsenic-contaminated water without seeing any arsenic patient. **Conclusion:** Awareness among villagers about arsenic contamination and its consequences should be raised on an urgent basis. **Acknowledgements:** The study was conducted with the support of SIDA, WHO, USAID, and ICDDR,B.

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Response to Edrophonium in Patients with Neurotoxic Envenoming by Snakes

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Background: Snake bite envenomation is an important and potentially fatal occupational health hazard in countries of South-East Asia, including Bangladesh. Definitive treatment of snake bite is antivenom. Anticholinesterases have an important role in the management of neuroparalytic effect of snake venom. **Objective:** Study the ability of anticholinesterase drugs to reverse the paralytic effects of snake venom. **Methodology:** A placebo-controlled, double-blind, cross-over trial of intravenous edrophonium (tensilon) was conducted among 17 adults admitted with neurotoxic envenoming in the snake bite clinic of Chittagong Medical College Hospital, Chittagong, Bangladesh. The patients were randomly selected to receive intravenous edrophonium/placebo first and then acted as their own case/control by receiving other drugs. They were examined at baseline (before injection) and at an interval of 0 (after injection), 5, 10, 15, and 20 minute(s) after receiving medication. Five parameters noted were: ptosis (percentage of iris uncovered), duration of upward gaze, peak flow rate, interdental cleft distance, and maximum blow pressure. The study was attempted in 21 patients but had to be abandoned in 4 due to development of respiratory arrest during the trial. **Results:** There was significantly more improvement in ptosis and endurance of upward gaze after administration of edrophonium than after administration of placebo. The improvement was more marked at the 5th minute—the mean difference (±SD) in the percentage of iris that was uncovered was 15 (50±27.95 vs 35±21.9), and the mean difference in the number of seconds of upward gaze was 10 (22 vs 12). The forced vital capacity and ability to open the mouth (inter-dental cleft) also improved after administration of edrophonium. Marked improvement was observed among few patients in their ability to talk, swallow, and cough. **Conclusion:** Anticholinesterases are beneficial in the management of neurotoxic envenoming by snakes of this part of Bangladesh, and use of anticholinesterase routinely in patients with neurotoxic envenoming is recommended. **Acknowledgements:** The support of the staff of Medicine Units of Chittagong Medical College Hospital and of the Wellcome Trust of the UK is acknowledged.

Anti-snakevenom Use and Adverse Reaction in a Snake Bite Study Clinic

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Background: Snake bite is an important health hazard in rural areas of Bangladesh which may lead to fatality. The incidence of snake bite is 4.3 per 100,000 population with morbidity of 20%. Venomous snake bite can be presented with local or systemic envenoming with neurotoxicity as the prime cause of death. The mainstay of management is antivenom which is highly effective but liable to cause adverse reaction, including anaphylaxis. **Objective:** See the use and types of adverse reaction of anti-snakevenom. **Methodology:** In total, 537 patients were admitted to the Chittagong Medical College Hospital from May 1999 to June 2001. In this prospective study, 35 neurotoxic snake-bite patients receiving anti-snakevenom were included. **Results:** The neurotoxic snake-bite cases were 10% (54) with 51 cobra bites. Seventy percent victims were aged less than 30 years. The average latent period of neurotoxicity from the time of bite was 4.5 hours. The common neurotoxic features were ptosis (100%), external ophthalmoplegia (94.2%), dysphagia (77.1%), dysphonia (68.5%), and broken neck sign (80%). Ventilatory support was needed in 14.2% of the cases. All the cases were treated with polyvalent antivenom, with 8.62% needing the second dose. Hundred percent cases were also treated with anticholinesterases. Antivenom reaction was 88.55%, and pyrogenic reaction was 80.64%, followed by anaphylaxis (64%). The common features of anaphylactic reaction were urticaria (80%), with vomiting and bronchospasm (40%), and angioedema in 10% of the cases. The average time of onset of anaphylaxis was 28 minutes, with 55 mL of antivenom having passed. The antivenom reaction was treated mainly with injection adrenaline for anaphylaxis and paracetamol suppository in pyrogenic reaction. The recovery time was 4 and half hours with 100% recovery with no residual deficit. **Conclusion:** Anti-snakevenom were withheld by physicians despite indication of possible danger which is easy to manage with proper approach. A national guideline for management of snake bite should be practised by every medical professional. **Acknowledgements:** The support of the Snake Bite Study Clinic is acknowledged.

P138 (276)

Improper Poultry Dressing: A Threat for Keeping Good Health

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Background: The consumption of dressed chicken meat is increasing in Bangladesh. The conditions of most poultry-dressing units are unhygienic and do not follow the proper steps of processing. Thus, dressed meat is being contaminated with bacteria that pose serious health risks. **Objective:** Find out the knowledge and awareness on proper dressing of poultry, the quality and safety of meat, and the ways of transmitting bacteria into poultry meat. **Methodology:** The study was carried out among 125 poultry dressers and 85 poultry dressing units in Dhaka metropolitan area, Savar, Gazipur, and Dhamrai area of Bangladesh during August-September 2003. Simple random sampling technique was used. A pre-tested questionnaire was used as a research instrument. Data were collected through interviews. Analysis was done with the help of scientific calculation. **Results:** More than 86% (108/125) of poultry dressers were not enough educated (<SSC). Over 97% (122/125) had no institutional training on dressing of poultry, and 87.2% (109/125) did not use PPE. 92% (115/125) of poultry dressers had no knowledge about proper dressing, while 92% (115/125) did not follow all steps of dressing. Over 97% (122/125) did not follow the step of short-time deeping in cold water. 93.6% (117/125) of dressing area were not hygienic, and 98.4% (123/125) did not change water for scalding. **Conclusion:** It is important to educate and train them about the guidelines to follow the proper steps of dressing and to maintain hygienic environment which will, no doubt, help minimize development of microbial contamination and transmission of meatborne diseases. **Acknowledgements:** The financial support of the Animal Health Division of Autodesk Ltd. is acknowledged.

P139 (325)

An Innovative Low-cost Solar Device for Destruction of Diarrhoeal Pathogens in Arsenic-free Surface Water

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Background: Surface water, which is free of arsenic, should be the main source of drinking-water through destruction of diarrhoeal pathogens. However, the challenge remains to develop a suitable technique for the rural poor. **Objective:** Develop a very simple and low-cost device to destroy diarrhoeal pathogens in water using free solar energy. **Methodology:** Exposure for half an hour at 60 °C is necessary to destroy diarrhoeal pathogens in water, and the required time drops drastically at higher temperatures (pasteurization). An innovative device made of hay, a bamboo-tray, and transparent polythene sheets has been developed by the author to attain temperatures higher than the above in relatively clear sun which works according to the 'Green House Effect'. The polythene sheets used in this device may be used for collecting rainwater by tying them to sticks stuck to the ground to make a conical funnel shape. **Results:** The device developed, costing about US$ 2, has successfully raised the temperature of about 5 litre of water to about 70 °C in winter (December) and about 80 °C in summer (July) in about 2 hours destroying diarrhoeal germs effectively. Two harvests in a day with clear sun are possible to make 10 litre per day which may be adequate for a family. Another group has reported additional contribution of UV in sunshine to accelerate the destruction at raised temperatures. **Conclusion:** This device has the potential of providing a major solution to the drinking-water problem in the developing world. This device may be set up even on a raft or on a boat during flood to make flood-water drinkable. Water treated in polythene bags can be supplied to people in distress in the aftermath of natural or manmade calamities. **Acknowledgements:** The author is grateful to Prof. K.M. Nazrul Islam (Retired) of IPGMR (now BSMMU), Dhaka, who first suggested trying solar energy giving the information that 60 °C is adequate. The author also acknowledges the contributions of many students at different phases of the work.

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Quantification of Oral Streptococci in Saliva of Bangladeshi School Children to Evaluate Caries Risk

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Background: The most prevalent oral infectious diseases are dental caries and periodontal diseases. *Streptococcus mutans* has been strongly implicated as the principal aetiological agent in dental caries that also constructs the foundation of many infectious diseases in the body. However, quantitative status of bifilm-forming streptococci in the oral cavity of Bangladeshi school children is unknown. **Objective:** Evaluate the prevalence of caries risk by quantification of pathogenic streptococci in the stimulated saliva of Bangladeshi school children. **Methodology:** Decayed, missing and filled teeth (DMFT) and paraffin-stimulated saliva were collected form 100 children (average age 11.22 years) of a school in Dhaka city. The saliva samples were brought to Japan in freezing condition. DNA was isolated from the saliva and real-time PCR performed by the ABI PRISM 7700 sequence detection system. **Results:** Average DMFT for permanent teeth was 1.16±1.54. About 96% of the children had more than 10 DNA copies of *S. mutans,* and 55% had *S. mutans* DNA copies ranging from 10^3 to 10^6 in 1 mL of saliva. About 20% of the children having DMFT >1 had more than 10^2 DNA copies of *S. sobrinus.* 27% of the children, with DMFT=0, did not deliver any *S. sobrinus*. **Conclusion:** Caries experience was low at age below 12 years. However, there is prevalence of caries risk in children having high quantity of both *S. mutans* and *S. sobrinus*. Apparently, *S. sobrinus* rather than *S. mutans* played as the principal aetiological agent to induce caries in Bangladeshi children. **Acknowledgements:** The research was supported by grants for JSPS for foreign postdoctoral fellows and COE Program, FRMDRTB at TMDU, Japan.

P141 (061)

Skin Infections among Hospitalized Children in a Tertiary-care Hospital of Bangladesh

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Background: Nutritional deficiencies lead to subnormal growth and development. Malnutrition is a widespread problem among children in developing countries. Growing children, especially those of pre-school age, are most severely affected with malnutrition. Skin infections manifesting as primary and secondary cutaneous complaints constitute a considerable number of outpatient visits to paediatricians. **Objective:** Compare the pattern of sociodemographic features, nutritional profile, and clinical features of skin-infected and non-infected children with malnutrition. **Methodology:** All children aged 1 month–5 years were examined soon after admission in the paediatric ward of Sylhet MAG Osmani Medical College Hospital during August-September 1998. Weight of children was measured to the nearest 0.1 kg using the Salter Scale and was recorded. Skin was examined for clinical evidence of infections, vitamin, and trace-element deficiencies. Diagnoses were made based on clinical findings and were recorded. A pre-designed questionnaire was used for recording all findings. Gomez classification was used for assessing the nutritional status of children. **Results:** Of 154 children studied, 47.4% were aged 1 month-1 year, and 52.6% were aged more than 1 year to 5 years. Reasons for admission into the hospital were pneumonia (n=5, 29.2%), diarrhoea (n=39, 25.3%), protein-energy malnutrition (n=24, 15.6%), enteric fever (n=5, 3.2%), acute bronchial asthma (n=3, 1.9%), and miscellaneous causes (n=38, 24.6%). None of the children were admitted for skin diseases, although skin infections were present in 43 (27.9%) patients, of which 38 (24.6%) were bacterial infections, including 5 (3.2%) with acute glumerulo-nephritis, and 6 (3.8%) were fungal infections. According to Gomez classification, 34.4%, 29.9%, and 27.3% of the children were having severe, moderate, and mild-degree malnutrition respectively. **Conclusion:** The high rate of infection could be due to poor personal hygiene, malnutrition, and low standard of living. Especially the climatic condition of Sylhet, which is hot and humid during the study period (August-September), predisposes the skin to infection. **Acknowledgements:** The permission of Director of Sylhet MAG Osmani Medical College Hospital for undertaking the study is thankfully acknowledged.

P142 (091)

Rapid Diagnosis of HHV-6 Infection by a Novel DNA Amplification Method—LAMP Method

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Background: A novel nucleic acid amplification method, termed loop-mediated isothermal amplification (LAMP), which amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions, may be a valuable tool for the rapid detection of infectious agents. **Objective:** Evaluate the reliability of LAMP developed for human herpesvirus 6 (HHV-6). **Methodology:** The LAMP reaction was conducted according to the original reports described by Notomi *et al*. Primers for HHV-6 LAMP were designed against the HHV-6 B U31 gene. **Results:** Although LAMP products were detected in HHV-6 B and HHV-6 A DNA, they were not detected in HHV-7 and human cytomegalovirus DNAs. The sensitivity of the original HHV-6 LAMP protocol was 50 copies/tube. HHV-6 LAMP was modified by increasing the primer concentration to increase the sensitivity (25 copies/tube). Following these initial validation studies, 13 patients with fever were tested for HHV-6 by viral isolation, serological analysis, and HHV-6 LAMP. In 3 of 8 patients with primary HHV-6 infection, HHV-6 DNA was detected in whole blood by the original HHV-6 LAMP protocol in not only the acute phase but also the convalescent phase. HHV-6 DNA was detected by modified HHV-6 LAMP in all 8 plasma samples collected in the acute phase; however, no HHV-6 DNA was detected in plasma samples collected in the convalescent phase. Although HHV-6 DNA was detected in both acute and convalescent phases in whole blood samples collected from patients with past HHV-6 infection, it was not detected in any plasma samples. **Conclusion:** Thus, detection of HHV-6 DNA in plasma using modified HHV-6 LAMP protocol is appropriate for diagnosis of active HHV-6 infection. **Acknowledgements:** The technical support of Eiken Chemical, Japan, is acknowledged.

Risk Factors of Severe and Complicated Malaria in an Endemic Area of Bangladesh

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Background: Descriptive epidemiology and locally prevalent risk factors for severe and complicated malaria (SCM) could be useful for planners to direct resources in a cost-effective way in the national malaria-control programme. **Objective:** Assess the operational risk factors for SCM cases in Bangladesh perspective by a cross-sectional comparative design. **Methodology:** Study cases were two primary-care hospitals and one district hospital. The study was conducted from February 1999 to April 2000. All consecutive patients admitted with SCM (presence of one or more severe symptom(s) of malaria) having asexual *Plasmodium falciparum* parasitaemia were enrolled. Treatment was given using the national guidelines. The outcome was recorded as complete recovery, death, or other outcomes. **Results:** In total, 829 cases (60% males) were recorded. Predominant severe manifestations included involvement of the central nervous system (CNS) in 44% of the cases. The overall mortality was 4.8%, with the highest case-fatality rate (CFR, 27%) in unrousable coma. Severe prostration (27.5%) and hyper-parasitaemia (20.5%) were common SCM manifestations. About two-thirds of the patients sought treatment in the community, three-fourths of whom received no antimalarial treatment. All age groups and occupations were recorded. The median duration of severe symptoms and travel time was longer, and travel cost was higher among the fatal cases. Of females aged 15-50 years, 37 (27%) were pregnant. CFR in pregnancy (22%) was significantly higher ($p=0.03$) compared to non-pregnant cases (7%). **Conclusion:** Community treatment of malaria in the study area is ineffective, and first contact treatment providers should be trained for improvement of diagnosis, presumptive treatment, and referral of cases. Interventions (prophylaxis) could be considered for pregnant females in the locality. **Acknowledgements:** The financial support of the Tropical Disease Research, World Health Organization, Switzerland, is acknowledged.

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Hepatic Involvement in Severe Malaria

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Background: Severe falciparum malaria causes abnormalities in liver function. The presence of jaundice in falciparum malaria indicates severe illness with poor prognosis. The frequency of hepatic involvement is not known among Bangladeshi patients. **Objective:** Describe the frequency of hepatic involvement, associated characteristics, and case-fatality rate in severe malaria. **Methodology:** A study in adults admitted to the Chittagong Medical College Hospital, fulfilling the inclusion criteria, was conducted. Patient data were recorded on a pre-designed case record form. Treatment was given according to the national guidelines using quinine. Outcomes were recorded as death, recovery, or otherwise. Data were analyzed using SPSS (10). **Results:** Sixty-eight parasitologically-confirmed cases (68% males) were enrolled. Thirty (44%) had serum bilirubin more than 3 gm/dL with predominantly-conjugated fraction. Fifty-five (79.4%) patients recovered, and 13 (20.6%) died. CNS involvement was the commonest (59%) manifestation. Clinical associations were jaundice (68%), severe prostration (67.2%), multi-organ failure (22.4%), severe anaemia (10.4%), organomegaly (70.6%), and renal failure (20.9%). Renal failure was associated with increased morbidity ($p<0.01$). The majority (62%) of patients was either from or had history of travel to a malaria endemic zone. The duration of present illness (6.96±3.24 days) before hospitalization and the duration of severe illness (2.31±1.96 days) were longer among those who died than among survivors. There were no stigmata of chronic liver disease in these patients. Platelet count was significantly lower in hyperbilirubinaemic patients ($p<0.01$) and also in patients with fatal outcome ($p<0.01$). Liver functions were (mean±SD) AST 139.16 (131.47) U/L, ALT 60.46 (51.18) U/L, alkaline phosphatase 243.79 (200.39) U/L, total protein 6.54 (0.63) gm/dL, and albumin-globulin ratio 0.75 (0.21). Prothrombin time was prolonged (4-15 seconds) in 9. Liver enzymes and serum creatinine were significantly raised in hyperbilirubinaemic patients ($p<0.01$) influencing outcome. Histopathological findings among 7 biopsies were congestion of sinusoids 2, pigments 3, inflammatory cells 2, fatty change 2, and portal fibrosis 1. **Conclusion:** Hepatic involvement in severe falciparum malaria is a poor prognostic factor. Identification and addressing such involvement is required during management of severe malaria. **Acknowledgements:** The support of the staff of Medicine Units of Chittagong Medical College Hospital is acknowledged.

P145 (291)

Inadequacy of a Single Antibody Test to Rule Out Dengue in Patients with Clinically Compatible Illness

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Background: Diagnostic testing for dengue is generally done early in the course of illness, often as soon as the patient presents to hospital. Since most tests involve antibody detection, early testing has the potential for reduced sensitivity. **Objective:** Determine whether a single antibody test is adequate to rule out dengue in patients with a clinically compatible illness. **Methodology:** Epidemiological and clinical information were obtained from all suspected dengue cases identified through surveillance at the Dhaka Medical College and Holy Family Red Crescent Hospitals from July through December 2002. Serum specimens obtained during acute illness were assessed for dengue IgG and IgM antibodies by capture ELISA; 40 antibody units were considered to be positive for dengue. IgM/IgG ratio of $\geq$1.78 was defined as primary (first time) infection, and IgM/IgG ratio <1.78 was defined as secondary infection (previous exposure). **Results:** Of 833 suspected cases, 624 (75%) had serologic evidence of dengue. Of those with laboratory confirmation, 461 (73.9%) had secondary infection, and 163 (26.1%) had primary infection. Death occurred in 3 (0.48%) confirmed cases. More than one serum specimen was available for 112 suspected cases. Of 233 suspected cases whose initial serum specimen was negative for dengue antibodies, 43 had a second specimen available; 24 (55.8%) were positive for dengue. For the 24 cases, the mean duration from onset of fever to first serum collection was 4.7 (SD±1.2) days (median 5 days; inter-quartile range 4-6 days; range 2-6 days) and from onset of fever to second serum collection the mean duration was 8.9 (SD±1.5) days (median 8 days; inter-quartile range 8-10 days, range 7-12 days) (p=<0.001). For 190 patients with a single negative result, the mean duration of onset of fever to first serum collection was 5.8 (SD±1.9) days (median 6 days; inter-quartile range 4-7 days; range 2-12 days). It is possible that more than half of the 190 suspected cases with negative tests of initial specimens would have been diagnosed with dengue if a second specimen had been tested. **Conclusion:** Negative antibody tests from specimens collected early in the course of illness should be followed by a repeat specimen at least 4 days later before ruling out dengue as the cause of clinically consistent illness. Because most cases of suspected dengue were evaluated during surveillance with a single serologic specimen, it is likely that the number of dengue cases was substantially underestimated during the surveillance. **Acknowledgements:** The financial support of the United States Agency for International Development (USAID) and the Canadian International Development Agency (CIDA) is acknowledged.

P146 (328)

Clinical Signs and Epidemiology of Ascariasis in Patients Who Were Referred to Hamadan Faculty of Medicine, Iran

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Background: *Ascaris lumbricoides,* one of the most common intestinal parasites in the world, causes 8-15% clinical signs in patients. It has recently been demonstrated that the parasite causes malnutrition in children. **Objective:** Determine different kinds of clinical signs in patients who were referred to the Hamadan Faculty of Medicine in 2001. **Methodology:** Faecal samples of 274 patients of Hamadan hospitals and clinical centres who were referred to Hamadan Faculty of Medicine were examined using the Formol-Eter method. Clinical signs of patients were also noted. Finally, data were analyzed. **Results:** 16.4% of the patients were infected by *Ascaris*. *Ascaris* was the most common parasite observed in 6-10-year old patients. Abdominal pain (14.4%) and nausea (35.5%) were the most common clinical signs respectively. These signs were also more common among 1-5-year old children. Nausea was the most common sign among 11-15-year old patients. **Conclusion:** In Hamadan, the prevalence of *Ascaris* compared to other parts of Iran was relatively high. Abdominal pain was the most common sign. It was also most common among 6-10-year old patients. Nausea, diarrhoea, abdominal pain, and vomiting signs in this study were similar to findings of a number of previous studies.

P147 (203)

Burden of Disease of Typhoid Fever, a Passive Surveillance Study in Slum Areas of 2 Sub-districts of North Jakarta, Indonesia

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Background: During the 1980s, Indonesia reported one of the highest typhoid fever incidence rates in the world. It has been estimated that, in Indonesia, there are 600,000-1,300,000 typhoid cases with at least 20,000 deaths each year. **Objective:** Estimate the disease burden of typhoid fever in North Jakarta, Indonesia. **Methodology:** During August 2001-July 2003, a surveillance study for typhoid fever was conducted in 2 sub-districts of North Jakarta. Blood samples were inoculated into BACTEC media or 10% Oxgall solution bottles. **Results:** 5,775 blood samples were collected. The isolation rate for *Salmonella* Typhi was lower than expected. The highest number of typhoid fever cases was found among the 10-20-year age group. Microbiological examination of rectal swab/stool samples showed that there were also other *Salmonellae*, with the amount of 43, 37, 29, 42, and 1 for *Salmonella* groups B, C, D, and E, and *Salmonella* spp. *S.* Typhi and *S.* Paratyphi A remained susceptible to commonly-prescribed antibiotics. In contrast, non-typhoidal *Samonella* had increasing resistance to first-line antibiotics. **Conclusion:** The observed incidence of typhoid fever in North Jakarta may be lower than previously reported. The isolated organisms of enteric fevers are still sensitive to commonly-prescribed antibiotics, although the isolated non-typhoidal *Salmonella* no longer follow a similar pattern in Indonesia. **Acknowledgements:** The support of the Diseases of the Most Impoverished (DOMI) programme, Bill and Melinda Gates Foundation, coordinated by the International Vaccine Institute, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, and U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia, is acknowledged.

NUTRITION 3

P148 (022)

Effect of Salt Iodization on Prevalence of Iodine Deficiency Disorders among Children of District Swat, NWFP, Pakistan

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Background: Salt iodization has been one of the most cost-effective methods of controlling iodine deficiency. In Pakistan, Swat district has been declared as a model district where over 95% of edible salt is iodized. **Objective:** Assess the effect of salt iodization on the prevalence of iodine deficiency disorders among children of Swat district, NWFP, Pakistan. **Methodology:** A community-based survey was carried out in Swat district to assess the prevalence of sub-clincal and clinical iodine deficiencies among children aged 8-11 years and to compare the results with previous studies. In total, 900 children were randomly selected and were clinically examined for goitre. A urine sample from a child of every third household was collected for determining urinary iodine concentration. The children were characterized as normal (iodine ≥10 µg/dL), mild iodine-deficient (iodine 5-9.9 µg/dL), moderate iodine-deficient (iodine 2.0-4.9 µg/dL), and severe iodine-deficient (iodine <2.0 µg/dL). Both univariate and mutivariate analyses of data were performed to check the significant difference in the mean iodine contents and goitre prevalence between different groups. **Results:** The results of clinical assessment of goitre of 8-11-year old children showed that only 4% of children had grade I goitre, and 17% were sub-clinically iodine-deficient. The results obtained were much lower than those of previous studies conducted on a similar age group of children, suggesting a positive impact of salt iodization on the prevalence of iodine deficiency disorders. **Conclusion:** Salt iodization has been effective in reducing iodine deficiency disorders in Swat district, Pakistan. **Acknowledgements:** The financial support of UNICEF is gratefully acknowledged.

P149 (339)

Presentation and Sociodemographic Context of Severe Protein-energy Malnutrition among Clinic-attending Weaning-age Children in Urban Bangladesh

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Background: From 1 March 2001 to 31 March 2003, ICDDR,B and 3 pilot NGO clinics serving the urban poor in the Dhaka metropolitan area enrolled 465 severely under-weight weaning-age children for protocolized nutrition rehabilitation on an outpatient basis. Clinic interventions were subsidized from ICDDR,B grant funds. Household interviews with children's caretakers were conducted for a sub-set of study children to obtain information on children's home environment and other background characteristics. **Objective:** Obtain a better understanding of the households and other background characteristics of enrolled children. **Methodology:** Study subjects were all (n=184) weaning-age children (6 to 23 months), severely underweight newlyidentified by the pilot clinics from 1 October 2001 to 30 June 2002. Child anthropometry at enrollment was performed by specially-trained clinic personnel. Trained ICDDR,B interviewers conducted semi-structured household-level interviews with children's mothers or other main caretakers. **Results:** Study children, who were enrolled on grounds of severe underweight (weight-for-age z-score <-3), had a mean age of 13.6 months; their sharply-skewed weight-for-age z-score distribution had a median of -3.74. The children were, on average, also severely stunted (mean length-for-age z-score -3.45), although not severely wasted (mean weight-for-length z-score -2.17). Severe stunting and severe wasting were found among 59.8% and 11.4% of the children respectively. The sociodemographic background of the children was indicative of very severe deprivation. Three-fourths of the children came from urban slums, more than half from households that had lived at their current address for less than a year. Eighty-four percent of the children's households occupied just a single room, and 90% used shared latrine facilities. Families were typically nuclear and male-headed, with a median household size of 5, with about two earning members per household. The majority (55%) of fathers did casual work paid on a daily basis, whereas two-thirds of the children's mothers were housewives. The median monthly earned income per household member was only 536 taka, corresponding to 18 taka (US$ 0.31) per person per day—far below the generally-accepted poverty line of US$ 1 per capita per day. **Conclusion:** Despite their young mean age, the majority of severely underweight weaning-age children identified by the pilot clinics and enrolled for protocolized management were already severely stunted. Management of already prevalent underweight should, therefore, be complemented by earlier interventions to prevent growth-faltering. Pilot interventions in response to already prevalent severe child malnutrition in this urban poor population were, on the other hand, very sharply poverty-targeted and were, therefore, appropriate from a pro-poor policy perspective. **Acknowledgements:** The study was conducted with financial support from the World Bank grant to the ICDDR,B Nutrition Centre of Excellence, BINP Operations Research contract funds, Government of Bangladesh–Gates Award Counterpart funds, and ICDDR,B core funds.

Sociodemographic Determinants of Very Severe Protein-energy Malnutrition among Clinic-attending Weaning-age Children in Dhaka, Bangladesh

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Background: From 1 March 2001 to 31 December 2002, ICDDR,B and 3 pilot NGO clinics serving the urban poor in the Dhaka metropolitan area enrolled 465 severely underweight weaning-age children for protocolized nutrition rehabilitation on an outpatient basis. Pilot project experience suggests the need for active community-level prevention, which should be guided by an understanding of the determinants of more severe degrees of malnutrition found among clinic-attending children. **Objective:** Identify determinants of very severe underweight and wasting, defined respectively as a weight-for-age of less than -4 z-scores and a weight-for-length of less than -2.18 z-scores, which were the mean and median weight-for-length among the study children. **Methodology:** Study subjects were all (n=184) weaning-age (6 to 23 months) severely underweight children newly identified by the pilot clinics from 1 October 2001 to 30 June 2002. Child anthropometry at enrollment was performed by specially trained clinic personnel. Trained ICDDR,B interviewers conducted semi-structured household-level interviews with children's mothers or other main caretakers. Multivariate analyses were performed by logistic regression. **Results:** Variables examined as determinants of more severe malnutrition included household demographic and physical characteristics, household composition, including presence and financial contribution of fathers, socioeconomic status of the parents of study children, mothers' work outside home and child-caring arrangements and mothers' reproductive history, and study children's current breast-feeding status. Among these, mother's management of household finances, mothers' work outside home, and subsequent birth of a younger sibling were identified as independently significant predictors of very severe underweight. An independently significant effect on wasting was only found for children where 3 or more household members were supported by one earning member. **Conclusion:** Among the urban poor, mothers' work outside home and mothers' responsibility for the management of household finances—both likely to reflect a necessity, rather than a choice, short birth intervals, resulting in competition from a younger sibling, and high dependency ratios within the household severely compromised the nutritional status of weaning-age children. Active outreach for identification and prevention of very severe child malnutrition among the urban poor should, therefore, focus on young children of mothers working outside home, mothers who themselves shoulder household financial responsibilities, mothers with two children aged less than two years, and young children from households with high dependency ratios. **Acknowledgements:** The study was conducted with financial support from the World Bank grant to the ICDDR,B Nutrition Centre of Excellence, BINP Operations Research contract funds, Government of Bangladesh–Gates Award Counterpart funds, and ICDDR,B core funds.

P151 (346)

Knowledge, Attitude, and Practice on Iodized Salt, Prevalence of Goitre, and Urinary Iodine Levels in Adolescent Girls of Three Schools

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Background: In 1993's first national IDD survey and 1999's follow-up, no specific data on adolescent girls were collected. A study on pregnant women, carried out in the Dhaka Medical College Hospital and MCHTI during 2001, concluded that the prevalence of goitre was the highest (35.4%) among adolescent pregnant women. A pilot project titled 'Urinary iodine in adolescent college girls' was conducted in 3 colleges in Dhaka city but there were still limitations. The study was carried out on only girls aged 16-18 years and did not cover the whole period of adolescence. Moreover, the study did not include a knowledge, attitude, and practice (KAP) study which was necessary to know the level of awareness among adolescents. The need for conducting a study, including KAP, on adolescent non-pregnant women was greatly urged. **Objective:** Assess the iodine nutriture and prevalence of goitre among adolescent school girls and compare it with 3 different socioeconomic groups. **Methodology:** This was a cross-sectional study. Students were selected randomly, and 308 students were interviewed from the Viqurunnisa Noon School (VNS), Azimpur Girls School (AGS), and the Nilkhet High School (NHS). A pre-tested structured questionnaire was used for obtaining relevant information on their KAP on iodized salt and iodine deficiency disorders (IDD). Clinical examination of goitre was done following the WHO/UNICEF/ICCIDD criteria for every respondent, and casual urine sample was collected from each of them on the spot and examined at the IDD laboratory of Institute of Nutrition and Food Science (INFS), University of Dhaka. Salt iodine content was measured by the titration method in this laboratory. **Results:** 74.7% of the students could answer what iodine was. 95.8% successfully answered the definition of goitre (93% of NHS, 96% of AGS, and 98% of VNS), and 98% could also tell the location of goitre. The observed goitre rate (30%) was the highest among students of NHS. It was 24% and 20.4% among the AGS and VNS students respectively. Urinary iodine excretion showed the highest deficiency (13%) among the students of AGS. It was 8% among the NHS and 7% among the VNS students. Distribution of goitre and urinary iodine deficiency was the highest among students aged 12 years. **Conclusion:** Iodine nutriture did not seem to correlate well with socioeconomic status; as salt, used as the cheapest vehicle for iodine, is used more or less equally by everyone, irrespective of economic background.

Dietary Intake and Nutritional Status in a Group of Pre- and On-maintenance Dialysis Diabetic Subjects

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Background: Patients with chronic renal failure often suffer from malnutrition, and this is more frequent among diabetics. Morbidity and mortality in chronic diseases largely depend on nutritional status. **Objective:** Identify and compare calorie intake and nutritional parameters between predialysis diabetics to those under on-maintenance haemodialysis. **Methodology:** Fifty-five diabetic subjects—35 of predialysis (Gr 1) and 20 from haemodialysis (Gr 2)—and 25 healthy controls (Gr 3) were selected. A 3-day dietary diary was advised to fill up by study subjects to calculate calorie intake. Anthropometric measurements and related clinical and laboratory parameters were assessed. **Results:** Controls were relatively younger among the 3 groups. For Gr 1, 2, and 3, the age was 59±9, 55±10, and 39±14 years respectively ($p<0.001$), but they were matched for body mass index of 24.7±3, 24.5±3, and 23.7±3 kg/m^2 (p=NS). Serum creatinine was 3.13-±1, 9.1±2.5, and 1.02±0.24 mg/dL ($p<0.001$) respectively. Total calculated calorie intake in both the diabetic groups was lower than that in the control—1,404±404, 1,344±404, and 1,872±600 kcal/day ($p<0.001$), and this was 78%, 53%, and 101% of required (1831±232, 2216±389, and 1897±306 kcal/day) calorie ($p<0.001$) respectively. As a whole, protein intake of high biologic value (hbv) was low in diabetics. This was lower in predialysis subjects than those on dialysis—18±9 and 25±16 g/day ($p<0.05$)–and was 30% and 42% of required hbv protein ($p<0.03$) respectively. C-reactive protein increased in diabetics with a higher tendency among dialysis patients—9.9 (2-11) and 15.5 (3-12) mg/L (p=NS). Serum albumin was lower in both the diabetic groups (3.1±0.3 and 3.1±0.4) (p=NS). **Conclusion:** From this study, it may be shown that diabetic renal-failure subjects are protein-calorie deficient.

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Is Female Illiteracy a Determinant for Child Malnutrition: An Analysis of Developing Countries

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Background: Along with poverty, socioeconomic backwardness is the main underlying factor leading to malnutrition among children. In developing countries, inadequate unhygienic food, lack of medical facilities for children, and poor care by uneducated mothers result in unhealthy growth, suggesting a strong relationship between poverty and malnutrition. Increased income usually enables poor families to get better access to health services, however, at the same time, there also lies a potential incongruity among them. The hypothesis is whether improvement, provision, or access to services, such as education, improve malnutrition among children in real terms. If so, then to what extent and which particular area of the education sector demands attention and investment? **Objective:** Establish the relationships between malnutrition and economic development and secondly between malnutrition and social development by analyzing data from several countries across the world which comes in the lower income bracket, i.e. developing countries. **Methodology:** Multiple regression technique was used with regard to socioeconomic variables. Data were drawn from human development reports (various editions). **Results:** From the exercise, it has been established that both economic factors (e.g. family income) and social factors (e.g. education of mother) play an important role in malnutrition of a child. For instance, in Sri Lanka, the adult female literacy rate is 89.3, and GDP per capita is US$ 849, but 29% of children aged less than 5 years are underweight. On the other hand, countries like Bangladesh, Nepal, and Pakistan which have very low adult female literacy rates and also have very low GDP per capita have quite high percentage of children aged less than 5 years who are underweight, i.e. 48%, 48%, and 38% respectively. **Conclusion:** The policy relevance of the analyses is that, since the economic factors are playing a major role, the government resources should be directed in such a way that it should enhance the income levels of households. On the other hand, the impact of social factors is more than of economic factors. The government should invest more on social sectors, such as female education, on a priority basis.

Effect of Lalshak on Nutritional Value of Rice-based Bangladeshi Diet

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Background: There is a growing focus on improving the nutritional quality of rice-based diet through increasing the quantity of vegetables, especially leafy vegetables. However, the effects of especially leafy vegetables on the availability of nutrients are not clearly elucidated. **Objective:** Compare the nutritional value of rice-based diets, specifically to determine the effects of inclusion of lalshak on the digestibility of protein, absorption, and retention of calcium, phosphorus, magnesium, and zinc in young growing rats. **Methodology:** Diets were prepared from rice (*Oryza sativa*), lentil (*Lens esculenta*), leafy vegetables, lalshak (*Amaranthus gangeticus*), and small mola fish (*Amblypharyn godon mola*) to simulate the habitual Bangladeshi rice-based diet, the recommended diet with lalshak, and recommended diet excluding lalshak. Five groups of 8 young male rats each were fed ad libitum with diets. The study was conducted for 28 days, and faeces and urine were collected in two balance periods: days 8-14, and days 22-28. Digestibility and utilization of protein of diets, weight gain in rats, femur bone weight and mineral composition of femur bone, and blood alkaline phosphatase were determined. **Results:** The study showed that inclusion of lalshak in diets increased the intake of protein (283.8 mg from diet with lalshak vs 261.6 mg from diet excluding lalshak), calcium (48.8 mg from diet with lalshak vs 28.7 mg from diet excluding lalshak), phosphorus (35.4 mg from diet with lalshak vs 31.2 mg from diet excluding lalshak), and magnesium (17.0 mg from diet with lalshak vs 5.4 mg from diet excluding lalshak) but reduced the utilization of protein (45.1% from diet with lalshak vs 48.5% from diet excluding lalshak) and retention of calcium (39.4 % from diet with lalshak vs 92.1% from diet excluding lalshak), phosphorus (45.3% from diet with lalshak vs 63.6% from diet excluding lalshak), and magnesium (7.9% from diet with lalshak vs 29.0% from diet excluding lalshak). **Conclusion:** Findings of the study suggest that lalshak contains several inhibitory agents responsible for reduced utilization of protein and important micronutrients in rice-based diet. More attention should be paid to these inhibitory agents of lalshak before recommending further increases in the intake. **Acknowledgements:** The financial support from the Bilateral Programme for the Enhancement of Research Capacity in Developing Countries, Ministry of Foreign Affairs, Denmark, is gratefully acknowledged.

P155 (350)

Antioxidant Status in Children with Oedematous Malnutrition

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Background: Oedematous malnutrition, or kwashiorkor, is one of the most severe forms of malnutrition with a high risk of mortality and morbidity. The 'free radical theory' claims that generation of toxic-free radicals inside the body during infections may causes oedematous malnutrition, suggesting a possible role of antioxidants for its management. **Objective:** Assess the antioxidant status of severely-malnourished children (with or without oedema) and healthy controls by measuring total antioxidant capacity (TAC) of plasma. **Methodology:** Twenty-two children aged 6 months-3 years were selected for the pilot study when they presented for the treatment of diarrhoea or for follow-up visits after recovery from diarrhoea and severe malnutrition. Nineteen children were finally evaluated for the assessment of TAC. TAC was determined by measuring chemiluminescence after adding the sample to a mixture of 2'2-azobis(2-amidinopropane) dihydrochloride and luminol. TAC value was calculated from the lag time before an increase in chemiluminescence was observed. **Results:** The mean (±SD) TAC value in control (n=7), marasmus (n=6), and oedematous (n=6) subjects was 298.0±79.54, 273.7±71.58, and 218.7±82.01 micromole trolox equivalents respectively (p for ANOVA=0.2). TAC values, being the lowest in the oedematous group and the highest in healthy controls, showed a trend (p=0.08) in harmony with the 'free radical theory'. **Conclusion:** Children with oedematous malnutrition had a low antioxidant status compared to healthy controls and severely-malnourished children without oedema. Further studies with a larger sample size would be required to assess the prevalence of oxidative stress in this population and to determine the need for antioxidant supplementation. **Acknowledgements:** The study was supported by NIH-Fogarty training grant awarded to K.M. Jamil for postdoctoral research at the University of California-Davis, USA.

P156 (352)

Anthropometric Status of School-age Children in Bangladesh

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Background: As morbidity and mortality among children aged less thn 5 years decline in Bangladesh due to improved health, more children are getting an opportunity to attend school. Very little is known about the nutritional status of school-age children in Bangladesh. **Objective:** Assess the anthropometric status of school children in rural Bangladesh who are taking part in a school nutrition programme. **Methodology:** A survey was done in March-April 2002 in 20 schools in 7 sub-districts of Jamalpur district and in 19 schools in 3 sub-districts of Sherpur district in northern Bangladesh. Data on sex and weight of a sample of children in all grades were collected. A school nutrition programme was then implemented in all schools in 4 sub-districts of Jamalpur in which all children were dewormed using a single dose of 400 mg of albendazole, and the children were also given each school day milk and fortified biscuits, which provided about 300 kcal of energy. The survey was repeated in all 39 schools in October-November 2003 when data were collected on date of birth (recorded to the nearest month), weight, and height, and a short socioeconomic questionnaire was administered to a parent of each child. **Results:** Data are presented on the anthropometric status of a sample of school children in a school nutrition programme in comparison with children in neighbouring sub-districts who were not. **Conclusion:** The anthropometric status is being discussed in the context of international growth references and differences between the sexes. **Acknowledgements:** The support of the United States Department of Agriculture, Tetra Pak, and the programme partners is acknowledged.

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Dietary Pattern of Newly-diagnosed Type 2 Diabetic Subjects with Hypercholesterolemia

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Background: Dietary measures comprise the first-line intervention for control of dyslipidemia in diabetic subjects. Understanding dietary pattern of diabetic subjects with hypercholesterolemia at presentation may help develop specific intervention for this group of subjects. **Objective:** Assess the contribution of dietary practices in the development of hypercholesterolemia in newly-diagnosed type 2 diabetic subjects. **Methodology:** Fifty newly-diagnosed type 2 diabetic subjects with hypercholesterolemia (fasting plasma total cholesterol >200 mg/dL) were recruited from the Out-patient Department of BIRDEM. The daily intake of macro- and micronutrients was assessed by 24-hour recall method. Seven-day food frequency questionnaire was used for identifying the dietary sources. **Results:** The daily energy intake (median [range]) of the subjects was 1,748 (1,059-2,903) kcal. In 20% of the subjects, this intake was 300 kcal more than the recommended calories. The intake of carbohydrate, protein, and fat was 269 (170-400), 63 (27-171), and 46 (17-86) g/day respectively. Daily carbohydrate intake was higher than the recommended value (up to 60% of total daily energy intake) in 58% of the subjects. Compared to recommended proportion (20-30% of total daily energy intake), daily fat intake was higher in 16% of the subjects and lower in 26% of the subjects. Daily protein intake was adequate (1g/kg of body weight) in only 40% of the subjects. The intake of fiber was only 5 (1-19) g/day; none of the subjects consumed the recommended amount of dietary fiber (15 g/1,000 kcal). The intake of iron and calcium was 26 (13-60) and 341 (103-1,290) mg/day respectively. The daily intakes of vitamins were: retinol 2,331 (67-25,529) mg/day, vitamin B_1 1.4 (0.7-2.5) mg/day, vitamin B_2 0.9 (0.5-2.2) mg/day, and vitamin C 58 (0-386) mg/day. Intakes meeting the recommended dietary allowance were found for iron in 40%, calcium in 44%, retinol in 54%, vitamin B_1 in 66%, vitamin B_2 in 4%, and vitamin C in 58% of the subjects. **Conclusion:** The data suggest that most newly-diagnosed hypercholesterolemic type 2 diabetic subjects consume excessive carbohydrate, moderate fat, and very low amount of protein. A large proportion of subjects also consume an inadequate amount of fiber, vitamins, and minerals. In contrast to usual notion, high fat intake does not seem to be the major cause of dyslipidemia in this population. **Acknowledgements:** The financial support of ENRECA under DANIDA, Denmark and IPICS, Uppsala University, Sweden, is gratefully acknowledged.

Glucose, Insulin, and Non-esterified Fatty Acid Responses to Ladies Finger and Parwar in Type 2 Diabetes Mellitus

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Background: Glycaemic index (GI), insulin, and non-esterified fatty acid (NEFA) responses are useful measures for the biological effects of a carbohydrate diet in relation to diabetes and its complications. **Objective:** Rank ladies finger (LF) and parwar (PA) in terms of their GI and NEFA and insulin (as measured by C-peptide) responses which are useful to create healthy dietary guidelines. **Methodology:** Seven diabetic subjects, aged 32-48 years, having body mass index (BMI) 21-27 and HbA_{1c} 5.20-8.20% participated in the study under a cross-over design. Three test meals (white bread as reference, and LF and PA as test foods), all containing 25 g of total carbohydrate (nitrogen-free extracts + fiber), were given to the participants for ingestion within 10 minutes with 200 mL of water. The serum levels of glucose were estimated at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minute(s) respectively. Serum NEFA and C-peptide levels were estimated at 0 and 180 minute(s) only. Serum glucose was measured by the glucose-oxidase method, serum C-peptide by chemiluminescent ELISA, and NEFA by the colorimetric method, and HbA_{1c}-was measured by the HPLC method. **Results:** Both LF and PA showed significantly lower serum glucose value than that of bread at 60 and 90 minutes ($p<0.01$). LF had significantly lower value compared to PA in 45 minutes ($p<0.01$). There was no significant difference in area under the curve between any 2 of the 3 groups (AUC 1,506±301 in bread, 1377±436 in LF, and 1,296±172 in PA). There was also no significant difference regarding their GI values (LF 91 and PA 88). Both LF and PA showed significantly lower serum C-peptide and serum NEFA response compared to bread at 180 minutes ($p<0.01$). **Conclusion**: In contrast to the general belief that vegetables are rich in dietary fiber and thus necessarily have low GI, the present data showed relatively high of ladies finger and parwar. The presence of bioactive natural agents in these vegetables, resulting in the suppression of insulin secretion/action or having stimulatory effect on insulin antagonists, needs to be investigated. **Acknowledgements:** The study was supported by grants from the ENRECA programme of DANIDA, Denmark and International programme in the Chemical Science, Uppsala University, Sweden. The contribution of the Ministry of Science and Information and Communication Technology, Government of Bangladesh, is also acknowledged.

Glycaemic Response of Variously Processed BRRI 28 Rice in Type 2 Diabetic Subjects

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Background: Glycaemic response to carbohydrate diets is a useful indicator of biological effects of the diet in relation to health. Glyacemic Index (GI) (a useful measure of glucose to a dietary component) of food greatly varies from place to place of origin, type of processing, and also in population to population. **Objective:** Investigate as part of a systematic attempt to determine GI of commonly-consumed rice in Bangladesh, a newly-developed rice variety, named as BRRI 28, under different techniques of processing [non-parboiled (NP), traditional parboiled (TP), and pressure parboiled (PP)]. **Methodology:** Sixteen type 2 diabetic subjects (8 males and 8 females) were studied after ingestion of 50 g of available carbohydrate in 3 types of rice (58 g) and equicarbohydrate amount (65 g) of white bread (reference food) under a cross-over design. The mean (±SD) age of the subjects was 41.8 (±7.9) years, body mass index (BMI) was 25.1 (±3.6), and plasma HbA_{1C} (%) was 7.0 (±0.8). The serum levels of glucose were estimated at fasting (-15 minutes) state, 15 minutes after starting meals, i.e. just at the end of the meal (0 minute) and at 15, 30, 45, 60, 90, 120, 150, and 180 minutes after 0 minute. GI was calculated by standard formula. **Results:** The PP rice showed a higher blood glucose value during 0-120 minute(s) compared to bread ($p<0.01$) and also compared to TP in 0 minute ($p<0.01$) and compared to NP in 30 minutes ($p<0.01$). The area under glucose curve (AUC) of PP (1995 [2778-1375]) was significantly higher than that of bread (1798 [1962-1218]) ($p<0.01$), and there was a rising tendency in NP (1816 [2778-1037]) and TP (1775 [2590-1245]). These glycaemic responses were also reflected in their GI values (NP 108.2±17.5, TP 109.3±15.8, and PP 118.5±16.1). GI of TP and PP of BR 28 rice were significantly higher than that of bread ($p<0.05$ and $p<0.01$). **Conclusion:** Compared to GI of 2 previously-tested varieties (BR25 and BR32), BRRI 28 produces lower GI. The data also suggest that equicarbohydrate amount of 3 types of BRRI 28 rice produces comparatively higher glycaemic responses compared to bread, and PP rice produces higher glycaemic response compared to 2 other types (NP and TP) of BRRI28. **Acknowledgements:** The study was supported by grants from the ENRECA programme of DANIDA, Denmark and International Program in the Chemical Science, Uppsala University, Sweden. The contribution of the Ministry of Science and Information and Communication Technology, Government of Bangladesh, is also acknowledged.

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Clinical, Biochemical and Functional Characterization of Young Adult Males with Lower Degrees of Body Mass Index

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Background: Low body mass index (BMI) is used for defining and grading chronic energy deficiency (CED) in adults but its clinical and biochemical consequences are not still fully understood. These consequences need to be investigated in the context of various racial and environmental backgrounds. **Objective:** Explore certain clinical, biochemical and functional parameters in low BMI adults. **Methodology:** In total, 215 young adult subjects aged 19-39 year(s), with low-to-normal BMI (range 14.0-25.0 kg/m^2) were collected from different areas of Dhaka city. Based on BMI criteria, as suggested by the WHO/FAO Expert Committee, the subjects were divided into various CED groups: BMI >18.5 (normal), BMI 17.00-18.49 (CED 1), BMI: 16.00-16.99 (CED 2), and BMI <16.00 (CED 3). The general clinical conditions of subjects were assessed using a pre-structured questionnaire and through physical examination by a qualified physician. The biochemical parameters included ESR, Hb%, glucose, total protein, lipid profiles, SGPT, and creatinine. The functional characterization was done by working days per week and by estimating consumption of oxygen using a treadmill. **Results:** The BMI groups were found not to differ in any clinical or biochemical criteria, except that the 3 CED groups had significantly (p=0.001) lower blood glucose levels compared to the normal BMI group. A substantial proportion of the CED subjects had blood glucose levels lower than the lowest value (3.0 mmol/L) of the normal BMI subjects (38% of CED1, 29% of CED 2, and 21% of CED 3). The number of working days did not show any significant association with degrees of BMI. Consumption of oxygen (l/min median-range) was 0.46 (0.33-0.71), 0.41 (0.29-0.60), 0.33 (0.28-0.37), and 0.22 (0.19-0.27) respectively in normal, CED 1, CED 2 and CED 3 groups. Consumption of normal BMI and CED 1 had a higher significant value (p=0.01) compared to the CED 2 and CED 3 groups. But no significant difference was found between the normal group and the CED1 group. **Conclusion:** A compromise in blood glucose values may be the earliest biochemical change in the case of lower BMI. The work capacity is affected only in subjects with BMI below 17.0. **Acknowledgements:** The financial support of ENRECA Project of DANIDA, Denmark and the International Program in the Chemical Sciences, Uppsala University, Sweden, is acknowledged.

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Knowledge, Attitude, and Practice of Hypercholesterolemic Type 2 Diabetic Subjects on Dyslipidemia

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Background: Dyslipidemia is a common problem in type 2 diabetic subjects. Studies relating to knowledge, attitude, and practice (KAP) of diabetic subjects on dyslipidemia are relatively rare in developing countries. The present study is the first one in Bangladesh on this issue. **Objective:** Assess KAP of newly-diagnosed hypercholesterolemic type 2 diabetic subjects on dyslipidemia and analyze the influence of some demographic and socioeconomic factors on the level of KAP. **Methodology:** Fifty newly-diagnosed type 2 diabetic subjects (male:female ratio 7:3, age 45±10 years, mean±SD) with hypercholesterolemia (fasting plasma total cholesterol >200 mg/dL) were selected from the Out-patient Department of BIRDEM. Data were collected using a pre-designed, pre-tested, interviewer-administered questionnaire. The subjects were graded as low, medium, and high as follows: knowledge-score <50%, 50-60%, and >60%; attitude-score <60%, 60-80%, and >80%; and practice-score <50%, 50-70%, and >70% respectively. **Results:** The levels of knowledge were low in 30%, medium in 26%, and high in 44% of the study subjects. The corresponding attitude levels were low in 10%, medium in 68%, and high in 22%, and the levels of practice were low in 54%, medium in 36%, and high in 10% of the subjects. The subjects of urban, semi-urban and rural areas did not significantly differ on knowledge. Attitude-score of the subjects of semi-urban area was higher than those of urban area (81.1□5.1% vs 71.8□9.6, $p<0.05$), and practice-score of urban subjects was higher than rural subjects (50.8□14.2% vs 35.8□15.8%, $p<0.05$). Knowledge-score was high in the secondary (58.6□7.4%, $p<0.05$) and graduate (60.3□9.1%, $p<0.05$) groups compared to the illiterate primary group (49.4□10.6%). Practice-score of the illiterate primary group (40□15.8%) was lower than the secondary group (52.5□15.0%, $p<0.05$), but they did not differ on attitude. Knowledge-score of the high-income group (58□9.7%) was better than the low-income group (40.3□6.3%, $p<0.05$). Attitude-score of the high-income group (75.1□8.6%) was better than the low-income group (57□1.8%, $p<0.05$). Practice-score was low in the medium-income group (34.2□13.3%, $p<0.05$) compared to the high-income group (52.5□13.3%). **Conclusion:** Knowledge, attitude, and practice, the 3 important components of awareness, do not follow a one-to-one relation in the newly-diagnosed hypercholesterolemic type 2 diabetic subjects. The variation is largely explained by demographic, educational and socioeconomic factors which determine the outcome in a complex fashion. Thus, a coordinated development policy is required to promote knowledge and attitude on healthy diet and to translate those into practice. **Acknowledgements:** The financial support of ENRECA under DANIDA, Denmark and IPICS, Uppsala University, Sweden, is acknowledged.

PATHOGENESIS

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Inflammatory Changes in Cholera—A Histopathologic Study in Rabbit Ileal Loop Model

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Background: Cholera is still a major cause of morbidity and mortality, especially in children, in developing countries. *Vibrio cholerae*, a gram-negative organism, produces toxin(s), mediates diarrhoea, and causes cholera. Among the large number of recognized serogroups, 01 and 0139 serogroups are associated with epidemics of cholera, whereas non-O1 non-O139 strain has been associated with sporadic cases of diarrhoea. The actual pathogenic process after colonization of the pathologic *V. cholerae* strain in the intestine is yet to be determined. **Objective:** Know the location of the lesion and the pathomorphological changes. **Methodology:** A study was carried out using 3 strains from culture collection in rabbit ilea loop model. The strains were: (a) VC20 (serogroup 01), (b) SG24 *(V. cholerae* O139 Bengal), and (c) PL-21, a non-O1 non-O139 strain devoid of *ctx* genetic element. A protein was purified from the culture supernatant of the strain PL-21. It was a metalloprotease with 34-kDa size and had haemagglutinating activity. This study was carried out in rabbit ileal loop model, and each strain was used separately in individual rabbit along with a control. After 18 hours of inoculation, the rabbit was sacrificed, and tissues were collected in 10% buffered formalin and 3% chilled cacodylate-buffered glutaraldehyde, processed and stained separately for light and electron microscopy. The study design was approved by the Ethical Committee of the National Institute of Cholera and Enteric Diseases. **Results:** The pathomorphological changes observed in 3 different strains of cholera had some specific features. Distorted micro-villous border, hypertrophic endoplasmic reticulum, swollen mitochondria, inflammatory cells, such as neutrophils and mononuclear cells, in increased number, were observed. The purified protein showed fluid accumulation at a minimum of 40-microgram inoculation dose, and inflammatory changes were evident by light microscopy from 20-microgram dose onwards. **Conclusion:** Although a number of factors are thought to be involved in the pathogenesis of cholera, inflammatory changes are important events in disease manifestation, and besides cholera toxin, the role of other proteins is also very significant.

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Role of Enteric Neuropeptides in Immunopathogenesis of Shigellosis

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Background: Physiological and pathological responses of the intestine require an integrated neuroimmune network. Both nerve fibers and inflammatory immune cells are able to release neuropeptides that modulate physiological and pathophysiological effects, including cell growth and differentiation, immunity, inflammation, and tissue repair. Research during the past decade has highlighted the importance of neuroimmuno mechanisms in the pathogenesis of diarrhoea. Shiga toxin and endotoxin from *Shigella* species have been shown to cause nerve damage and limb paralysis in animals and humans. Neurologic manifestations have been documented in children as complications of shigellosis. **Objective:** Understand the involvement of the enteric neuropeptides, neurotransmitters, and mast cells in the immunopathogenesis of shigellosis. **Methodology:** Rectal biopsy and stool samples were obtained from adult patients with shigellosis (*Shigella dysenteriae* type 1, n=3, *S. flexneri*, n=14, *S. boydii*, n=3) and healthy controls (n=20). Tissue expression of neuropeptides, mast cells, and argentaffin cells was detected by immunohistochemical technique, while ultrastructural features of nerves were studied by transmission electron microscopy. Concentrations of neuropeptides in faecal extracts were measured by enzyme-linked immunosorbent assay. **Results:** Increased expression of nerve growth factor (NGF) produced predominantly by argentaffin and mast cells in the rectal mucosa in the acute stage (3-5 days after onset of diarrhoea) suggested regeneration of enteric nerves. Increased expression of substance P and vasoactive intestinal peptide (VIP) in nerves was evident 14-16 days after onset in patients compared to healthy controls ($p<0.03$). Frequency of argentaffin cells in the crypt epithelium was maximum 14-18 days after onset, while serotonin expression was the highest at late convalescence (30-35 days after onset). Accumulation of histamine-containing mast cells at the acute stage was associated with increased severity of inflammation ($p=0.04$). Secretion of substance and serotonin in stool was parallel to their expression in rectal mucosa. Immunohistochemical staining revealed apoptotic neuronal death at the acute stage. Ultrastructural analyses showed axon swelling and degeneration and degranulating mast cells localized in the vicinity of nerve fibres at the acute stage. Extensive regeneration of nerves was prominent at the early convalescence. **Conclusion:** In acute shigellosis, apoptotic and degenerative neuronal axons were evident in the inflamed rectal mucosa in parallel to increased numbers of degranulating mast cells. Regeneration of axons, increased production of neuropeptides, and reduction in mast cell numbers were seen during early convalescence with restitution and healing of the mucosa. Thus, neuropeptides, neurotransmitters, and mediators of mast cells may participate in a complex, interdependent network that modulates inflammation, wound healing, and immune responses in shigellosis.

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A Novel Electrical Impedance Technology Developed Locally with Application in Gastric Function Studies

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Background: Electrical impedance tomography (EIT), a computerized imaging system developed in the last decades, can provide non-invasive gastric emptying and acid-secretion studies which are useful in predicting diarrhoeal sensitivity. This was also found to be useful in studying gastric emptying in children with malnutrition when they developed diarrhoea on administration of high-calorie diets. The above studies were carried out in collaboration with ICDDR,B and Children's Hospital, Bangladesh. However, the EIT system uses 17 electrodes that are difficult to manage. The system is also sophisticated and expensive, and cannot be used for large-scale studies and screening tests, particularly in developing countries. **Objective:** Develop a simple and low-cost non-invasive electrical impedance system which can give reasonably acceptable results compared to that obtained using EIT, on the above-mentioned measurements. **Methodology:** The new technique developed has been named Focused Impedance Measurement (FIM). It uses 7 skin surface electrodes, attached to the region around the stomach, the electric impedance being displayed numerically on a digital meter. The instrument, which costs very little, has been developed locally and was used for studying the time variation of gastric emptying after a drink of saline. To use this system for estimation of volumes of gastric acid, some simulation experiments were carried out. **Results:** The time variation of gastric emptying agreed very well to that obtained using EIT. The simulation experiment mentioned above showed a regular variation in impedance with volume of an object with constant electrical resistivity. **Conclusion:** The above results show the potential of using FIM for non-invasive gastric emptying and gastric acid-secretion studies which may have direct relevance to measurements of diarrhoeal sensitivity and in studies on children with malnutrition. **Acknowledgements:** The EIT equipment used for comparison was custom-made by Sheffield University and was a gift from the British ODA.

Role of Intracellular Calcium Store in Mechanism of NAG-ST-Induced Calcium Influx in Rat Intestinal Epithelial Cells

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Background: The mechanism of action of non-agglutinable *Vibrio* ST (NAG-ST) is not known clearly. NAG-ST induces initial rise in free cytosolic calcium occurred with activation of inositol tri-phosphate (IP_3) in rat intestinal epithelial cells. Chelating of intracellular calcium with BAPTA showed that NAG-ST-induced calcium influx across the plasma membrane depends upon initial and transient rise in free cytosolic calcium. **Objective:** Evaluate the mechanism behind the NAG-ST-induced calcium influx. **Methodology**: Calcium was measured by fluorescence ratio imaging using fura-2AM. Nitric oxide and inositol tetra-kis-phosphate (IP_4) were measured fluorimetrically. **Results:** In rat intestinal epithelial cells, NAG-ST, in presence of 1mM $CaCl_2$, showed an immediate increase in intracellular calcium concentration $[Ca^{2+}]i$ compared to control (50±2 vs 155±11.78, p□0.01, n=3), and this was followed by a sustained phase up to 8.5 minutes. The same population of cells when exposed to thapsigargin (TG), a model ligand for capacitative calcium entry, a transient rise in $[Ca^{2+}]i$ (112±8 nM, p□0.01, n=3) was observed, and in presence of 1 mM $CaCl_2$, a sustained rise similar to that observed with NAG-ST (168±14.22 nM, p□0.01, n=3) was found. Adriamycin, an inhibitor of IP_3 kinase, which converts IP_3 to IP_4, had no role in NAG-ST-induced calcium entry. However, NAG-ST raised intracellular nitric oxide level, and calcium influx in rat intestinal epithelial cells by NAG-ST could be blocked by nitric oxide synthase inhibitors. **Conclusion:** NAG-ST increased the $[Ca^{2+}]i$ by capacitative calcium entry with the involvement of nitric oxide in rat intestinal epithelial cells.