



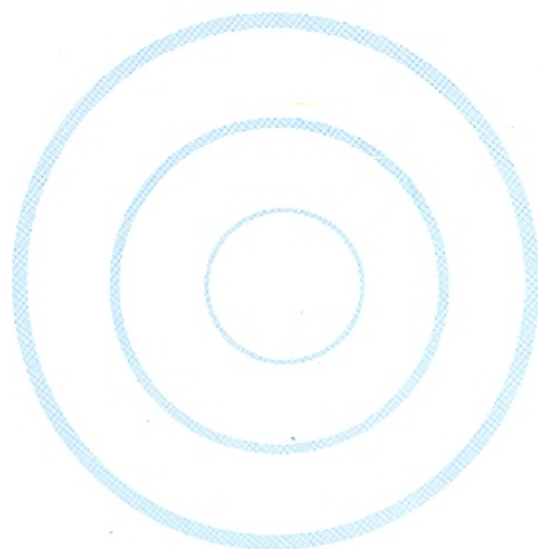
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INTERNATIONAL CENTRE FOR  
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*(See inside of the back cover...)*

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## An Outbreak of Group C Rotavirus Gastroenteritis Among Children Attending a Day-care Centre in Belém, Brazil

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### ABSTRACT

In August 1993, an outbreak of group C rotavirus-associated gastroenteritis occurred among children attending a day-care centre in Belém, Brazil. Of the 64 children, 21 (33%) became ill. Group C rotavirus was identified in faecal specimens from 8 (38%) children with diarrhoea by electron microscopy (EM) and an enzyme immunoassay (EIA), using antibodies specific to the Cowden strain of porcine group C rotavirus. By polyacrylamide gel electrophoresis (PAGE), a pattern similar to that of group C rotavirus was observed in 5 (62.5%) of the 8 EM- and EIA-positive samples. These 5 faecal samples were confirmed to be positive for group C rotavirus by reverse transcriptase-polymerase chain reaction, using specific VP6 and VP7 primers. This is the first report of an outbreak of diarrhoea in North Brazil associated with group C rotavirus. These findings suggest that group C rotavirus may be an important aetiological agent of diarrhoea in this region, which requires further study.

**Key words:** Rotavirus; Diarrhoea, Infantile; Gastroenteritis; Disease Outbreaks; Day-care centres

### INTRODUCTION

Rotaviruses are important causes of diarrhoeal illnesses among infants and young children worldwide (1,2). Rotaviruses belong to the family *Reoviridae*, and are classified into seven antigenically and genetically distinct serogroups (A-G) (3), of which group A, B, and C are known to infect both humans and animals (4). Group A rotaviruses are associated with high morbidity and

mortality, particularly in the developing countries, leading to an estimated 800,000 deaths per year among children aged less than five years (5). Group B rotaviruses were initially detected in cows and pigs, but in 1984, these agents were also associated with large epidemics of severe diarrhoea among children and adults in China (6-9). Group C rotaviruses were first isolated from piglets with diarrhoea in the USA (10), and were subsequently identified by Bridger *et al.* (11), in 1986, to be human pathogens. Since then, workers in several countries have detected group C rotavirus in association with both sporadic diarrhoeal illness (12-16) and outbreaks of diarrhoea (17-20) which appear to have a global distribution (21). A recent report by Riepenhoff-

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Talty *et al.* suggest that group C rotaviruses may be involved in the pathogenesis of extra-hepatic biliary atresia in infants (22).

It is likely that the incidence of group C rotavirus infection in humans is severely underestimated, because appropriate diagnostic methods, PAGE of RNA and electron microscopy, are not used in most clinical laboratories, and are rarely used for routine detection. In addition, more sensitive techniques, such as reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme immunoassays with group C rotavirus-specific reagents are available only in a few reference centres (12,15).

A recent serosurvey, carried out in Sweden, indicates that the prevalence of antibody to group C rotavirus ranges between 35% and 45%, depending on age (23). In the UK, a survey, involving 1,000 human serum samples, obtained from all age-groups and screened by an enzyme-linked immunosorbent assay (ELISA), yielded an overall seroprevalence of 43%, with the highest rate of 66% in the 71-75-year age-group (24). These results strongly suggest a high level of exposure of humans to group C rotaviruses throughout life.

In Brazil, group C rotaviruses were first identified by Pereira *et al.* (25) in a faecal specimen from a child with gastroenteritis in Rio de Janeiro, and have since been detected in other regions of the country, including São Paulo, Santa Catarina, Belém, Brasília, and Valentin Gentil (26-30). The present report documents an outbreak of diarrhoea caused by group C rotavirus, affecting at least 38% of the children who attended a day-care centre (DCC) in Belém, Pará, Brazil.

## MATERIALS AND METHODS

**Patients and faecal specimens:** During July 1993-June 1995, we conducted a regular surveillance of acute diarrhoea among children aged less than five years who attended a DCC in Belém, Pará. Children were visited twice a week to detect outbreaks of gastroenteritis. In addition, the DCC staff telephoned us whenever cases of diarrhoea occurred between visits. The DCC was attended by children from low-income families who were, in general, undernourished at admission.

On 18 August 1993, we were notified of an outbreak of diarrhoea, affecting 21 (33%) of the 64 children aged 10 months to 3 years. This was the second outbreak of a series of nine which occurred in this DCC during the survey period. The clinical symptoms were, in general, mild, and diarrhoea resolved without leading to dehydration. Faecal specimens were collected from all

children with diarrhoea and 4 from staff members who had no symptoms. These specimens were examined for bacterial and parasitic pathogens and stored at -20 °C until processed for viral diagnosis.

**Enzyme immunoassay (EIA):** Stools were diluted to make a 10%-20% suspension in phosphate-buffered saline and centrifuged at 3,000xg for 15 minutes at 4°C. The supernatants were tested for the presence of group A rotavirus and adenovirus antigens, using an EIA (EIARA) provided by Bio-Manguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (31). To detect group C rotavirus antigen, we used a specific EIA kit, as previously described (12,32). This consists of a sandwich assay in which a polyclonal hyperimmune gnotobiotic pig serum (U 340) to the group C Cowden strain acts as a capture antibody and a biotinylated hyperimmune gnotobiotic pig serum (U339) to the Cowden strain as a detector antibody. These samples were also tested for the presence of astroviruses, using an EIA kit prepared at the Centers for Disease Control and Prevention (CDC), as previously described (33).

**Polyacrylamide gel electrophoresis:** Deproteinized rotavirus RNA was electrophoresed in 5% polyacrylamide gel, using a discontinuous buffer system (34), and was stained with silver nitrate (35).

**Direct electron microscopy (DEM):** Stools were examined by DEM after negative staining, using the method reported by Barth (36). Grids were stained with 2% phosphotungstate solution (pH 7.2), and were examined in a Zeiss EM 900 electron microscope at 40,000X magnification.

**Reverse transcriptase-polymerase chain reaction:** Group C rotavirus dsRNA was extracted from stool suspensions, as described by Boom *et al.* (37). The RT-PCR was carried out, using group C rotavirus-specific primers for the VP6 (primers BMJ41 and BMJ42) and VP7 (primers BMJ107 and C7-10) genes, as described previously (28). The mixture was amplified through 40 cycles of denaturation (94 °C for 1 minute), primer annealing (48 °C for 1 minute), and DNA polymerization (72 °C for 2 minutes). The final cycle was followed by an extension period at 72 °C for 10 minutes. The PCR products were analyzed by electrophoresis on 3% agarose gel at 100V, and were stained with ethidium bromide.

**Bacteriological and parasitological procedures:** Samples were tested for the presence of bacteria and parasites, following the techniques described in the WHO Manual for laboratory investigations of acute enteric infections (38).

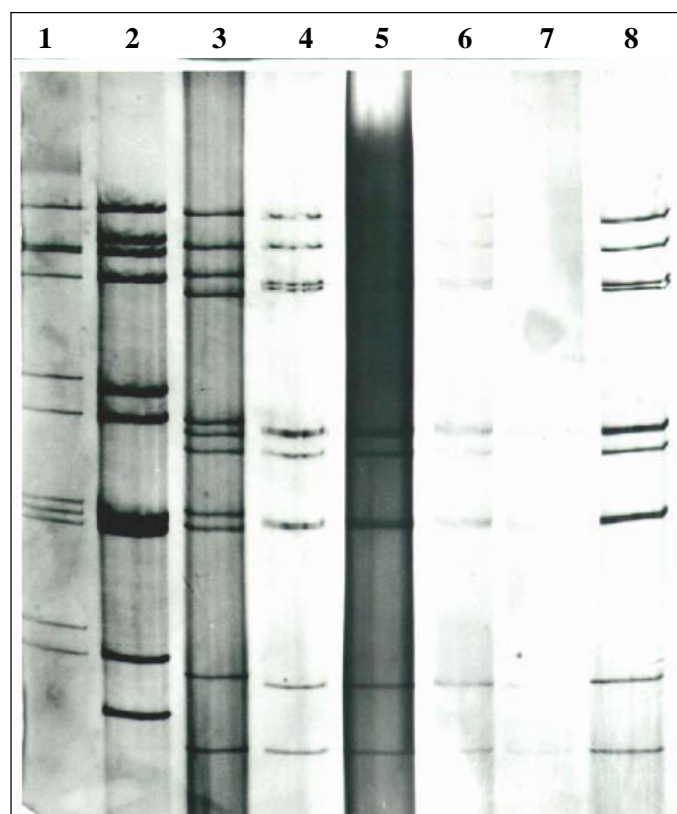
## RESULTS

During this outbreak, 21 (33%) of the 64 children had diarrhoea. Faecal specimens, collected from 21 children with diarrhoea and from 4 staff members who had no symptoms, were negative by EIA for group A rotavirus, adenovirus, and astrovirus. When tested by EIA for group C rotavirus, 9 (36%) were positive, including one sample from an adult who did not have diarrhoea. PAGE of

dsRNA from the 21 samples showed 5 (24%) with the characteristic pattern of group C rotavirus, i.e. segment 5, 6, and 7 displaying a typical triplet pattern (Fig. 1) (39). When the results for group C rotavirus, obtained by PAGE and EIA, were compared, more specimens were positive by EIA (36%, 9/25) than by PAGE (20%, 5/25), and all samples were positive by PAGE were confirmed by EIA (Table).

**Table.** Comparison of results obtained from PAGE and EIA for group C rotavirus in faecal samples from diarrhoeic children and controls

| PAGE         | Enzyme immunoassay    |                        |                       |                       | Total (%)<br>(n=25) |
|--------------|-----------------------|------------------------|-----------------------|-----------------------|---------------------|
|              | Diarrhoeic            |                        | Control               |                       |                     |
|              | Positive (%)<br>(n=8) | Negative (%)<br>(n=13) | Positive (%)<br>(n=1) | Negative (%)<br>(n=3) |                     |
| Positive (%) | 5 (24)                | -                      | -                     | -                     | 5 (24)              |
| Negative (%) | 3 (14)                | 13 (62)                | 1 (25)                | 3 (75)                | 20 (76)             |



**Fig.1.** RNA genomic profiles of group C rotaviruses obtained from faecal specimens of five children with diarrhoea during an outbreak of gastroenteritis in a day-care centre in Belém, Brazil. Lanes: 1: Group A rotavirus, short pattern (control); 2: Group A rotavirus, long pattern (control); 3: Group C rotavirus, Cowden strain (W926); and 4-8- Group C rotaviruses from the outbreak.

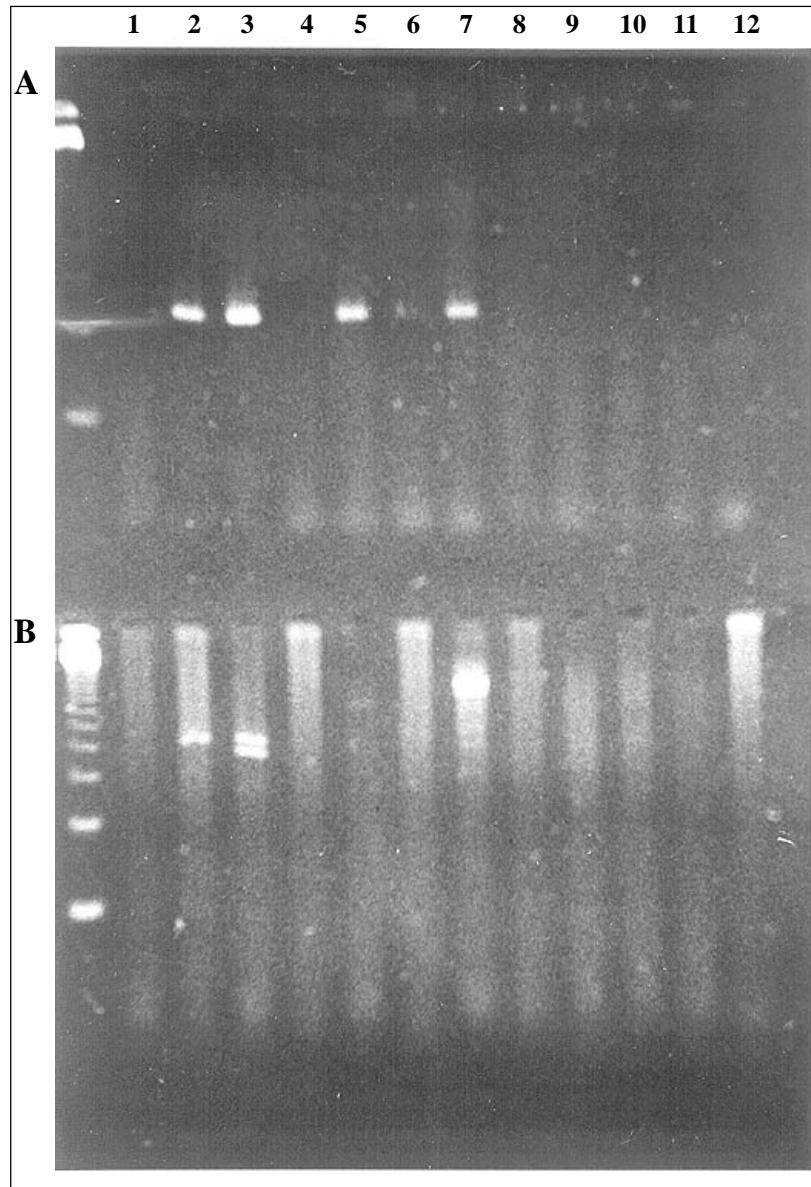
All 9 specimens positive for group C rotavirus by EIA were also tested by RT-PCR, using the group C rotavirus VP6- and VP7-specific primers. PCR products with sizes of 270 bp and 467 bp for VP6 and VP7, respectively, were noted in five (3 VP6/VP7 and 2 VP6) of the diarrhoeic specimens (Fig. 2). Typical rotavirus particles were observed by DEM in all EIA-, PAGE- and RT-PCR-positive samples.

Of the eight diarrhoeic cases tested positive for group C rotavirus, five were detected on 18 August 1993, when most faecal specimens (one per child) were obtained. *Entamoeba histolytica* was detected in two (10%) patients, one of them was also group C rotavirus-positive. Mixed infection, involving group C rotavirus and *Salmonella* group D, was also detected in one child with diarrhoea. All the specimens from adults, including the group C rotavirus-positive one, were collected on 20 August.

## DISCUSSION

This is the first report of an outbreak of diarrhoea associated with group C rotavirus in North Brazil. We had previously detected group C rotavirus in Belém in two children with sporadic episodes of diarrhoea (26). The detection of group C rotavirus in this outbreak was possible, because we were conducting a 2-year survey of diarrhoeal episodes in this DCC to assess the aetiologic roles of different viral enteropathogens. During this period, two of the nine outbreaks of diarrhoea recorded were associated with group A rotaviruses.





**Fig.2.** Characterization of group C rotavirus VP6 (a) and VP7 (b) gene products by RT-PCR. Samples were analyzed in 3% agarose gel, and were stained with ethidium bromide. M-123-bp ladder molecular weight marker. Lanes: 1 - 11- samples from the outbreak and 12 - negative control (water).

Outbreaks of group C rotavirus-associated gastroenteritis have been described throughout the world (15,17-20). Most have involved either children aged one to five years or adults. In this outbreak, most children with group C rotavirus-associated diarrhoea in the DCC were aged about two years. Different age-groups were recorded in two outbreaks occurring in schools: one in London

(England) (18) and the other one in Fukui (Japan) (19). Most patients were aged 4 to 12 years. However, group C rotavirus can be associated with gastroenteritis in infants as reported by Jiang *et al.* (12) in a 5-year survey conducted in the United States, which demonstrated, for the first time, the presence of group C rotavirus among hospitalized diarrhoeal children aged less than one year.

The detection of group C rotavirus in an asymptomatic member of the staff indicates possible transmission of this virus to adults, as reported elsewhere and the possible role of immunity with age (15,20). Souza *et al.* (30) have reported that, during the same period as this outbreak in Belém, an outbreak of group C rotavirus-associated gastroenteritis occurred in Valentin Gentil, a small town of São Paulo State, affecting both adults and children.

The RNA profiles, identified during the outbreak, were identical to those noted in our previous study in Belém (26), and to that from the outbreak in Valentin Gentil, Southeast Brazil. This electropherotype similarity suggests a low degree of genetic variability for group C rotavirus strains in our region. This is in contrast to the findings of a study conducted by Ishimaru *et al.* (40), involving hospitalized children with acute gastroenteritis in Matsuyama, Japan, where two distinct RNA patterns were identified in different years (1985/1986 and 1988). In addition, both patterns, observed in the Japanese study, were different from those obtained in Belém, suggesting that group C rotavirus strains may differ in their genetic composition by region.

The first group C rotavirus isolate in Belém was analyzed by Cooke *et al.* (41) who sequenced PCR products from the VP6 gene. They observed that the Belém VP6 gene shares 98% nucleotide homology with that of the human group C/Bristol VP6 gene and 83% nucleotide homology with the corrected porcine group C/Cowden sequence. Subsequently, Grice *et al.* (42) compared the complete

nucleotide sequence of the Belém VP7 gene to the corresponding gene of the Bristol and Preston strains isolated in the UK, and showed that these genes were identical in size (1.063 bp) and nearly identical in sequence (97.8-99.8%). Recently, Jiang *et al.* (43) analyzed the VP7 gene from 14 human group C strains isolated from the USA and from four other continents, and found that these genes were highly conserved in predicted primary and secondary structures. Sequencing studies are planned with group C rotavirus strains obtained to date in Belém to assess the degree of nucleotide homology between them.

Two diagnostic techniques--the EIA specific for group C rotavirus and the PAGE--were compared in this study. ELISA was found to be more sensitive than PAGE. All EIA- and PAGE-positive samples were further tested by RT-PCR, using a pair of group C-specific primers, and five were confirmed. It is likely that more positive specimens might have been detected by RT-PCR, if all specimens, both ELISA-positive and ELISA-negative, had been examined, including additional (not used in the present study) internal primers (nested PCR).

In the present study, the clinical symptoms of diarrhoea due to group C rotavirus were milder than those reported for other two local outbreaks caused by group A rotavirus. Matsumoto *et al.* (19) also reported that group C infections were associated with mild clinical symptoms, characterized mainly by abdominal pain and vomiting in a large outbreak occurred in seven elementary schools in Fukui city, Japan.

We do not know the source of the group C strains, but interspecies transmission has been suggested previously (4). To establish this linkage, we plan to study group C rotavirus strains from pigs living in close contact with these children and see if the sequences of the VP6 and VP7 genes are closely related to those found in strains from our children.

Further studies are needed to understand the aetiology of group C rotavirus in diarrhoea among children and the epidemiology of group C rotavirus-associated infections in humans. The techniques of EM, EIA, and RT-PCR should be considered for routine use in the detection of group C rotavirus from faecal specimens of children or adults with diarrhoea.

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## Characterization of Virulence Factors of *Aeromonas* isolated from Children with and without Diarrhoea in Tripoli, Libya

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### ABSTRACT

During September 1992-August 1993, stool samples from 157 children with diarrhoea and 157 matched healthy controls were examined for the presence of *Aeromonas* and other enteropathogens. *Aeromonas* strains were tested for haemolytic activity, haemagglutination patterns, and antibiotic susceptibility. In total, 62 *Aeromonas* were isolated, of which 27 (17.2%) were from children with diarrhoea and 35 (22.3%) from healthy controls. Only 23 (14.6%) of the diarrhoeal children and 28 (17.8%) of the healthy controls were positive for *Aeromonas*; of which, 4 (2.5%) of the diarrhoeal children and 6 (3.8%) of the healthy controls showed multiple species. *Aeromonas hydrophila* was isolated from 5 (3.2%) children with diarrhoea and from 9 (6.4%) controls, *A. veronii* by *sobria* from 8 (5.1%) and 7 (4.5%), *A. caviae* from 13 (8.3%) and 17 (10.8%), and *A. schubertii* from 1 (0.6%) and 2 (1.3%) respectively. No significant difference in the haemolytic activity of *Aeromonas* was found between diarrhoeal and healthy children. However, a significant difference ( $p < 0.002$ ) was observed in mannose-resistant haemagglutination (MRHA) by diarrhoeal isolates of *Aeromonas* (7/27, 26%) compared to the healthy controls (1/35, 3%). *Aeromonas* strains were uniformly sensitive to ciprofloxacin, gentamicin, and nalidixic acid. The results of this study suggest that *A. caviae* strains may be associated with diarrhoea in children and MRHA may be used as one of the virulence markers for distinguishing between *Aeromonas* isolated from diarrhoeal children and healthy controls or environmental isolates.

**Key words:** *Aeromonas*; Diarrhoea, Infantile; Haemolysin; Haemagglutination; Microbial sensitivity tests

### INTRODUCTION

*Aeromonas* species are oxidase-positive, Gram-negative, facultative anaerobic, rod-shaped bacteria, and are associated with acute diarrhoea in children (1-3).

*Aeromonas hydrophila* and *A. sobria* (4, 5) and, recently, *A. caviae* (6,7) have been described as causative agents of gastroenteritis. It is reported that there is no significant difference in isolation rates of *Aeromonas* species from children with diarrhoea compared to controls (8,9).

*Aeromonas* species have been reported to elaborate a number of virulence factors, such as exotoxins (haemolysins, cytotoxins, enterotoxins), haemagglutinins, and invade tissue in culture (10-15). Burke *et al.* (16) found that when haemolysin production alone

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**Table 1.** Distribution of *Aeromonas* species in diarrhoeal children and healthy controls

| Subjects            | No. tested | No. of strains (%) belonging to the species |                                    |                  |                      | Total     |
|---------------------|------------|---|------------------------------------|------------------|----------------------|-----------|
|                     |            | <i>A. hydrophila</i>                        | <i>A. veronii</i> bv <i>sobria</i> | <i>A. caviae</i> | <i>A. schubertii</i> |           |
| Diarrhoeal children | 157        | 5 (3.2)                                     | 8 (5.1)                            | 13 (8.3)         | 1 (0.6)              | 27 (17.2) |
| Healthy controls    | 157        | 9 (6.4)                                     | 7 (4.5)                            | 17 (10.8)        | 2 (1.3)              | 35 (22.3) |

was used for characterizing *Aeromonas* species, 97% of the strains concurrently produced enterotoxins. Other workers also reported a correlation between haemolysin and cytotoxin production (9). However, enterotoxigenic isolates of *A. hydrophila* showed haemagglutination (HA) which was not sensitive to mannose and fucose, but *Aeromonas* strains that were HA-sensitive to mannose or no haemagglutination (NHA) were non-toxic strains of *A. caviae* commonly isolated from non-diarrhoeal infection or the environment (10).

To understand the prevalence of *Aeromonas* species and their potential to cause gastroenteritis, some investigators (17) suggested to isolate these organisms from well-defined populations. This study was, therefore, carried out to determine the prevalence of *Aeromonas* strains in children with diarrhoea and in age- and sex-matched controls in Tripoli area (population >1,250,000), to identify the species and to investigate their haemolytic activity, haemagglutination patterns, and antibiotic susceptibility.

#### MATERIALS AND METHODS

During September 1992-August 1993, stool samples from 157 children (85 males) with acute diarrhoea (three or more liquid stools per day), admitted to the Aljalla Children Hospital in Tripoli, Libya, were examined. During the same period, stool samples from 157 age- and sex-matched children without diarrhoea (controls) were obtained. Control samples were from patients who had not had diarrhoea or received antibiotics within 2 weeks of specimen collection. The age of the study children ranged from few days to 3 years, but most (84.7%) were aged less than one year.

Clinical data obtained included: age, sex, date of onset of diarrhoea, presence of frank mucus or blood in stools, presence of fever or vomiting, and antibiotics taken before stool collection. Whenever possible, information on the source of water (treated or untreated) used for drinking and recent travel abroad was also obtained.

Stool samples were also examined for *Salmonella*, *Shigella*, and *Yersinia enterocolitica*.

To isolate *Aeromonas*, faecal specimens were sub-cultured on blood agar, containing 15 mg/L ampicillin after enrichment in alkaline peptone water (pH 8.6)

overnight at 37°C. Plates were incubated at 37°C for 18-24 hours. Suspected colonies were tested for the presence of oxidase growth in nutrient broth with 0% and 6% NaCl, resistance to the compound O/129 (2, 4-diamino-6, 7-diisopropylpteridine), and were further characterized by the API 20E System (bioMerieux, France). Isolates were further characterized to the species level, using a set of biochemical tests in the Aerokey II as described by Carnahan *et al.* (18). Based on the seven biochemical tests, such as aesculin hydrolysis, gas from glucose, acid from arabinose, indole production, acid from sucrose, Voges-Proskauer reaction, and resistance to cephalothin (30 µg), *Aeromonas* were identified as *A. hydrophila*, *A. veronii* bv *sobria*, *A. caviae*, and *A. schubertii*.

Haemolysin production test was performed as described by Burke *et al.* (11), using human erythrocytes. Haemagglutination tests were carried out, using human erythrocytes in absence of mannose [mannose-sensitive haemagglutination (MSHA)] and in presence of mannose [mannose-resistant haemagglutination (MRHA)] as described by Evans *et al.* (25).

Antibiotic susceptibility tests were performed by the disc-diffusion method (20). Antibiotics (Oxoid, UK) tested included: amoxicillin-clavulanic acid combination (30 µg), carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), tetracycline (30 µg), and trimethoprim-sulphamethoxazole (25 µg).

#### RESULTS

During the 12-month, *Aeromonas* species were isolated from 23 (14.6%) children with diarrhoea and from 28 (17.8%) healthy controls. *A. hydrophila* was isolated from 5 (3.2%) children with diarrhoea and 9 (6.4%) controls, *A. veronii* bv *sobria* from 8 (5.1%) and 7 (4.5%), *A. caviae* from 13 (8.3%) and 17 (10.8%), and *A. schubertii* from 1 (0.6%) and 2 (1.3%) respectively. Multiple *Aeromonas* were isolated from 4 (2.5%) diarrhoeal children and 6 (3.8%) controls (Table 1). Of the 23 diarrhoeic children with *Aeromonas* in their stools, 4 had *Salmonella*, and 3 had *Shigella*. No significant difference was found in the frequency of isolation of *Aeromonas* between diarrhoeal children and matched healthy controls.

**Table 2.** Information about children with diarrhoea and their *Aeromonas* isolates

| Patient | Age (months) | Sex | Month of occurrence | Faeces with mucus | Blood | Fever | Vomiting | Duration of diarrhoea (days) | Episodes per day | Antibiotic taken          | Other pathogens detected | Species of <i>Aeromonas</i>                               |
|---------|--------------|-----|---------------------|-------------------|-------|-------|----------|------------------------------|------------------|---------------------------|--------------------------|---|
| 1.      | 11           | F   | Sep                 | -                 | -     | +     | +        | 2                            | 10               | No                        | -                        | <i>A. caviae</i> , <i>A. veronii</i> bv <i>sobria</i>     |
| 2.      | 8            | M   | Sep                 | -                 | -     | -     | -        | 3                            | 3-4              | No                        | -                        | <i>A. caviae</i>  |
| 3.      | 4            | F   | Sep                 | -                 | -     | -     | -        | 7                            | 7-8              | ampicillin                | -                        | <i>A. caviae</i>  |
| 4.      | 7            | F   | Sep                 | -                 | -     | +     | +        | 4                            | 7-8              | ampicillin                | -                        | <i>A. hydrophila</i>                                      |
| 5.      | 8            | F   | Sep                 | +                 | -     | -     | -        | 7                            | 9                | ampicillin, metronidazole | -                        | <i>A. caviae</i>  |
| 6.      | 36           | F   | Oct                 | +                 | +     | -     | -        | 2                            | 5-7              | No                        | Shigella sp.             | <i>A. hydrophila</i>                                      |
| 7.      | 2            | M   | Oct                 | -                 | -     | -     | -        | 1                            | 5                | ampicillin                | Salmonella sp.           | <i>A. hydrophila</i>                                      |
| 8.      | 5            | M   | Nov                 | +                 | -     | -     | -        | 4                            | 6-7              | ampicillin, gentamicin    | -                        | <i>A. caviae</i>  |
| 9.      | 3            | F   | Nov                 | -                 | +     | -     | -        | 4                            | 7                | No                        | -                        | <i>A. caviae</i>  |
| 10.     | 4            | F   | Nov                 | -                 | -     | -     | -        | 5                            | 10               | No                        | -                        | <i>A. caviae</i>  |
| 11.     | 5            | F   | Dec                 | +                 | -     | +     | +        | 7                            | 7-8              | ampicillin                | -                        | <i>A. veronii</i> bv <i>sobria</i>                        |
| 12.     | 8            | F   | Jan                 | -                 | +     | +     | +        | 1                            | 10               | No                        | -                        | <i>A. caviae</i>  |
| 13.     | 3            | M   | Jan                 | +                 | -     | +     | +        | 5                            | 5                | ampicillin                | Salmonella sp.           | <i>A. veronii</i> bv <i>sobria</i>                        |
| 14.     | 7            | M   | Jan                 | -                 | -     | -     | +        | 4                            | 6                | metronidazole             | -                        | <i>A. caviae</i>  |
| 15.     | 14           | F   | Feb                 | +                 | -     | +     | -        | 2                            | 9                | ampicillin                | -                        | <i>A. caviae</i> , <i>A. veronii</i> bv <i>sobria</i>     |
| 16.     | 25           | F   | Mar                 | +                 | -     | -     | -        | 3                            | 3                | ampicillin                | -                        | <i>A. hydrophila</i>                                      |
| 17.     | 7            | M   | Apr                 | +                 | +     | +     | +        | 1                            | 10               | ampicillin                | Shigella sp.             | <i>A. veronii</i> bv <i>sobria</i>                        |
| 18.     | 10           | M   | Apr                 | +                 | -     | +     | +        | 5                            | 7                | metronidazole             | Salmonella sp.           | <i>A. veronii</i> bv <i>sobria</i> , <i>A. schubertii</i> |
| 19.     | 8            | F   | May                 | -                 | -     | -     | -        | 2                            | 4                | No                        | -                        | <i>A. veronii</i> bv <i>sobria</i>                        |
| 20.     | 15           | F   | Jun                 | -                 | -     | -     | +        | 2                            | 4                | No                        | -                        | <i>A. caviae</i>  |
| 21.     | 13           | M   | Jun                 | +                 | +     | -     | +        | 10                           | 6-7              | metronidazole             | Shigella sp.             | <i>A. hydrophila</i> , <i>A. veronii</i> bv <i>sobria</i> |
| 22.     | 5            | M   | Jun                 | -                 | -     | -     | -        | 7                            | 4-5              | No                        | -                        | <i>A. caviae</i>  |
| 23.     | 9            | M   | Aug                 | -                 | +     | -     | -        | 6                            | 5                | ampicillin                | Salmonella sp.           | <i>A. caviae</i>  |

*Aeromonas* were isolated from 9.6% of the 83 children aged less than 6 months, 20% of the 50 diarrhoeal children aged between 6 months and one year, and 20.8% of the children with diarrhoea aged over one year. *Aeromonas* were detected in 20.8%, 11.9%, 11.4%, and 12.5% of the specimens collected during autumn, winter, spring, and summer seasons respectively. Information on the source of water used for drinking was available for 12 diarrhoeal children and 10 controls, of whom 83% and 90% respectively used untreated drinking water supplies. The organisms were found in 18.1% and 11.8% of the female and male children with diarrhoea and in 23.6% and 12.9% of the female and male controls respectively. None of the children with diarrhoea or controls travelled abroad in the last 30 days prior to stool collection.

Of the 23 diarrhoeal children with faecal *Aeromonas*, 43.5% had mucus, and 26.1% had blood in their stools; 34.8% and 39.1% presenting with fever and vomiting respectively. Clinical data on diarrhoeal children with positive *Aeromonas* are shown in Table 2.

Results of the haemolysin and haemagglutination assays are shown in Table 3. Regardless of whether isolated from children with diarrhoea or controls, no significant differences in the haemolytic activity of *A. hydrophila*, *A. veronii* bv *sobria*, or *A. schubertii* were found. Of the 14 *A. hydrophila*, 15 *A. veronii* bv *sobria*, and 3 *A. schubertii* strains, 71%, 67% and 66% were respectively positive in the haemolysin assay. None of the 30 *A. caviae* strains were positive in this test. Of the 27 *Aeromonas* strains from the diarrhoeal children and 35 strains from the controls, 56% and 43% showed MSHA ( $p > 0.05$ , Fisher's exact test) respectively. Whereas 26% of the strains from the children with diarrhoea and 3% from the controls showed MRHA ( $p < 0.002$ , Fisher's exact test).

Ninety-seven percent of the 62 *Aeromonas* strains, in the present study were resistant to amoxicillin-clavulanic acid combination, 95% to carbenicillin, 64% to cephalothin, 6% to chloramphenicol, 3% to kanamycin, 11% to tetracycline, and 23% to

trimethoprim-sulphamethoxazole. All strains were, however, susceptible to ciprofloxacin, gentamicin, and nalidixic acid.

### DISCUSSION

In this study, *Aeromonas* were isolated with nearly equal frequencies from children with diarrhoea (15%) and children without diarrhoea (18%). This observation is in agreement with earlier workers who reported that there is no difference in isolation rates of *Aeromonas* species between children with diarrhoea and healthy controls (8,9).

Many workers reported *Aeromonas*-associated gastroenteritis to be distinctly seasonal, with a sharp summer peak, and it is suspected to be water-borne (21). Most of our children with diarrhoea (83%) and controls (90%), from whom information on the source of drinking water was available, used untreated drinking water. *Aeromonas* were isolated from 55% of the 600 water samples obtained from untreated drinking water supplies in Tripoli area, and their prevalence, in such supplies, was highest during autumn and winter months (22). This may explain the high isolation rates of *Aeromonas* obtained in our study, especially during autumn.

Some studies (3,9) reported *A. caviae* to be the most prevalent species in faeces from children with and without diarrhoea, while others (23) found *A. hydrophila* to be the most common. *A. caviae* was the most often isolated species in our study from children with and without diarrhoea. This organism is also the second most common species, after *A. hydrophila* from water samples obtained from wells in Tripoli and submitted to our laboratory for bacteriological analysis (K.S. Ghenghesh, unpublished observation). This observation supports the findings of earlier workers who demonstrated that *A. caviae* are diarrhoeagenic suggested by the fact that these

strains were potentially enterotoxigenic (24), and were often found in the environment (6).

Reports on the prevalence of *A. schubertii* in cases of childhood gastroenteritis and in controls are lacking. Studies are, thus, required to determine their role in diarrhoeal diseases. In the population we studied, this organism was not an important enteric pathogen.

It has been reported that children with *A. sobria* (*A. veronii* bv *sobria*)-associated infection were more likely to have associated systemic manifestations (3). In our study, 56% and 67% of the children with diarrhoea due to *A. veronii* bv *sobria* had fever and vomiting respectively. Also mucus and blood were found more in stools (50% and 33% respectively) of cases with *A. sobria* bv *sobria*.

Contrary to reports by other workers (25), we found no significant differences in the haemolytic activity of *Aeromonas* species regardless of whether they were isolated from diarrhoeal children or controls. The results of our study showed that haemolysin production was significantly associated with *A. veronii* bv *sobria*, *A. hydrophila*, and *A. schubertii*, but not with *A. caviae* isolates, regardless of their isolation from diarrhoeal children or controls. These findings are consistent with those of other investigators (8).

Human diarrhoeal isolates and enterotoxigenic strains of *Aeromonas* species are strong haemagglutinators of human blood cells (26). In the present study, we found a significant association between *Aeromonas* isolated from children with diarrhoea and MRHA. Others (10) reported a strong association between diarrhoea and agglutination not inhibited by fucose, galactose, or mannose. We observed that MRHA was markedly species-dependent, 66% (2 of 3) of *A. schubertii*, 50% (4 of 8) of *A. veronii* bv *sobria*, 23% (3 of 13) of *A. caviae*, and none of *A.*

**Table 3.** Haemolytic and haemagglutination activities of *Aeromonas* species isolated from diarrhoeal children and healthy controls

| Species                            | Subjects            | No. studied | No. of strains (%) positive |                   |                    |
|------------------------------------|---------------------|-------------|-----------------------------|-------------------|--------------------|
|                                    |                     |             | Haemolysin                  | Haemagglutination |                    |
|                                    |                     |             |                             | MS                | MR                 |
| <i>A. hydrophila</i>               | Diarrhoeal children | 5           | 4(80)                       | 3(60)             | 0.0                |
|                                    | Healthy controls    | 9           | 6(67)                       | 5(56)             | 0.0                |
| <i>A. veronii</i> bv <i>sobria</i> | Diarrhoeal children | 8           | 6(75)                       | 6(75)             | 4(50)              |
|                                    | Healthy controls    | 7           | 4(57)                       | 3(42)             | 0.0                |
| <i>A. caviae</i>                   | Diarrhoeal children | 13          | 0.0                         | 5(38)             | 3(21)              |
|                                    | Healthy controls    | 17          | 0.0                         | 6(35)             | 0.0                |
| <i>A. schubertii</i>               | Diarrhoeal children | 1           | 1(100)                      | 1(100)            | 0.0                |
|                                    | Healthy controls    | 2           | 1(50)                       | 1(50)             | 1(50)              |
| <i>Aeromonas</i> sp. (Total)       | Diarrhoeal children | 27          | 11(41)                      | 15(56)            | 7(26) <sup>†</sup> |
|                                    | Healthy controls    | 35          | 11(31)                      | 15(43)            | 1(3)               |

MS=mannose-sensitive; MR=mannose-resistant; <sup>†</sup>( $p < 0.002$ , Fisher's exact test)

*hydrophila* strains. Furthermore, our findings are in agreement with the recently published reports (12, 27, 28) which showed a strong association of haemagglutination activity and enteropathogenicity of *Aeromonas* isolated from clinical and environmental sources.

With very few exceptions, *Aeromonas* are known to be uniformly resistant to ampicillin (29). Nearly 50% of our patients took ampicillin prior to stool collection for reasons other than diarrhoea. This may have predisposed them to infection with these organisms. Moyer (30) reached to the same conclusion and reported that antibiotic therapy and drinking of untreated water are two significant risk factors for the susceptible host. Although gastrointestinal infections with *Aeromonas* are generally self-limiting, marked alleviation or resolution of gastrointestinal symptoms of patients taking antimicrobials to which *Aeromonas* strains are susceptible has been reported (31). The results of the antibiotic sensitivity test indicate that ciprofloxacin, gentamicin, and nalidixic acid might be effective agents for the treatment of diarrhoea due to *Aeromonas*.

In conclusion, *A. caviae* strains may have the potential to cause diarrhoea in children, and MRHA may be used as one of the virulence markers to distinguish between diarrhoeal and healthy control or environmental isolates.

The failure of exotoxin-producing strains of *Aeromonas* to cause diarrhoea in human volunteers (32) suggests that exotoxins are not the only determinants of pathogenicity (33). Future studies with human volunteers should include *Aeromonas* that possess multiple virulence factors, particularly mannose-resistant adhesion.

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# Detection of Group- and Subgroup-specific Antigenes of Bovine Rotaviruses in Bangladesh

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## ABSTRACT

**The study was carried out to detect group- and subgroup-specific antigens of bovine rotaviruses. Stool specimens, collected from diarrhoeic calves of the Savar Dairy Farm, Bangladesh, were examined by an enzyme-linked immunosorbent assay, using group- and subgroup-specific monoclonal antibodies. Thirty-three specimens showed specificity for group A rotavirus. While subgrouping, 21 group A-positive specimens showed subgroup I specificity. Twelve specimens did not react with either of the subgroup I- and subgroup II-specific monoclonal antibodies.**

Key words: Rotavirus; Antibodies, Monoclonal; Antigens, Bacterial; ELISA

## INTRODUCTION

Bovine rotavirus, the first group A rotavirus, was isolated from cell culture in 1969 (1). The cell culture-grown virus was characterized and confirmed as a most important cause of gastroenteritis in calves (1,2). Subsequent studies revealed the widespread prevalence of group A- rotavirus associated infections in young animals, including calves and pigs, and their association with the diarrhoeal disease complex in animals aged less than one month (3-6). Although the outbreaks of gastroenteritis due to other groups of rotaviruses have been reported, it is now recognized that group A rotavirus from animals, including humans, shares common epitopes on the inner-capsid protein VP6, the group antigen (7).

In addition to group antigen, subgroup antigens are also located on VP6, and subgroup specificities were initially identified by immune adherence haemagglutination assay (IAHA) and ELISA, using convalescent sera from gnotobiotic calves infected with

different rotavirus strain (8). The rotavirus genome is composed of 11 segments of double-stranded RNA (ds RNA). The inner capsid of rotavirus particles contains group and subgroup antigens that reside in the RNA segment 6. Most group A rotaviruses can be classified into either subgroup I or subgroup II, and the presence of a third subgroup has been proposed (9-11).

Most animal rotaviruses belong to the subgroup I, but some strains, isolated from chicken, turkey and horse and murine species, remain to be subgrouped (12-15). Subsequently, subgroup-specific monoclonal antibodies were developed and used in epidemiological studies of animal and human rotaviruses (11,13). Serotype specificity, defined by neutralization test, is associated with an outer-capsid protein (VP7) encoded by RNA segment 8 or 9 (14,15,16,17,18).

This report describes the detection of group- and subgroup-specific antigens of bovine group A rotaviruses by ELISA, using group- and subgroup-specific monoclonal antibodies collected from faecal specimens of calves in Bangladesh.

## MATERIALS AND METHODS

Faecal specimens (n=150), collected from calves during monthly visits to the Savar Dairy Farm, Savar, Bangladesh, were kept in sterile tube and labelled, and

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| <b>Table.</b> Characterization of bovine rotaviruses-isolated from faecal specimens of calves |                                      |    |                               |       |                                |   |                                  |
|---|--------------------------------------|----|-------------------------------|-------|--------------------------------|---|----------------------------------|
| Total no. faecal samples tested   | Group A rotavirus antigen determined |    | Subgroup antigens             |       |                                |   |                                  |
|   |                                      |    | Subgroup I antigen determined |       | Subgroup II antigen determined |   | Subgroup I and II not determined |
|   | Total no. positive                   | %  | Total no. positive            | %     | Total no. positive             | % |                                  |
| 150   | 33                                   | 22 | 21                            | 63.63 | -                              | - | 12                               |

were kept in -20 °C. Each specimen was prepared as a 10% suspension in phosphate buffered saline (PBS) (pH 7.4) mixed and centrifuged at 3000 rpm for 30 minutes. The supernatants were separated and examined for the presence of group and subgroup antigens by ELISA. For detecting group antigen, group A-specific Y0-156 monoclonal antibody (Mab) was used. For subgrouping, subgroup I-specific S2-37 and subgroup II-specific Y0-5 Mabs were employed (11). The Y0-156 Mab recognizes common group A rotavirus antigenic site on inner-capsid protein VP6 (11) was employed to detect group A rotavirus antigen from faecal specimens.

Briefly, polyvinyl microtitre plates were coated with 1:10,000 dilution of mouse ascetic fluid containing Mabs and incubated overnight at 4 °C. After washing the wells three times with PBS (pH 7.4), wells were incubated with 1% bovine serum albumin overnight at 4 °C and subsequently washed. A mixture of 10% faecal suspension (37 µL) and 10% skim milk (125 µL) was allowed to react in the wells overnight at 4 °C. After being washed, 50 µL of rabbit anti-human rotavirus serum (1:10,000 dilution) was added and incubated for one hour at 37 °C. After washing, 50 µL of peroxidase-conjugated goat anti-rabbit immunoglobulin G was added. The plates were incubated for one hour at 37 °C and washed. The reaction with the substrate o-phenylene diamine (OPD) was allowed to develop for 30 minutes at room temperature and then stabilized by adding 25 µL of 3N sulphuric acid. The reaction was evaluated by visual observation by comparing the intensity of the colour visible in the group and subgroups-positive wells and group and subgroups-negative wells.

### RESULTS

In total, 150 faecal specimens, collected from calves with acute gastroenteritis, were examined by ELISA for rotavirus. Of these, 33 (22%) were positive for group A rotavirus by ELISA, using group-specific monoclonal antibody. Group A rotavirus-positive specimens were further examined for the presence of subgroup I- and subgroup II-specific antigen by ELISA, using subgroup I- and subgroup II-specific monoclonal antibodies. While subgrouping, 21 specimens reacted with subgroup I-specific monoclonal antibody, whereas 12 specimens did

not show any reactivity with either of the subgroup I- and subgroup II-specific monoclonal antibodies (Table).

### DISCUSSION

The faecal specimens, used in this study, were collected from calves with acute gastroenteritis from the Savar Dairy Farm. The calves were presumed to be sporadic cases of gastroenteritis, since no epidemic of gastroenteritis was reported by the dairy farm authorities during the study period. The data obtained from this study, therefore, may be useful to determine the prevalence of rotavirus infection in calves. Studies, using reassortant rotaviruses, have established that a 42,000-dalton major inner-capsid protein is responsible for group and subgroup specificity of rotaviruses and that the protein is encoded by the 6th gene segment (13).

Rotavirus has two antigenic specificities: subgroups and serotypes (15,16) The recent development of monoclonal antibodies against the 42,000-dalton protein of rotaviruses and their successful application to subgrouping by ELISA have facilitated epidemiological studies of subgroups of rotaviruses (11,17). Although subgroup specificity does not necessarily correlate with neutralization specificity, it can be used as one of the means of examining the antigenic diversity of rotaviruses.

Group A rotavirus has been detected in faecal specimens by ELISA, using group A-specific monoclonal antibody. Subgrouping of specimens positive for group A rotavirus was examined by ELISA, using subgroup-specific monoclonal antibodies. In our studies, most bovine rotavirus strains reacted with subgroup I-specific monoclonal antibody. This finding correlated with the earlier finding that bovine rotavirus has subgroup I specificity, using subgroup-specific monoclonal antibodies (5,19).

Group A rotaviruses contain a common antigen that cross-reacts between viruses isolated from different mammalian hosts (20). The serological study showed that group antigen was located on the inner capsid, and was, thus, masked intact double-shelled particles. Subgroup antigen is associated with a major component of the inner capsid which is encoded by RNA segment 6 (8).

The presence of at least two antigenic determinants on PV6 has been confirmed with monoclonal antibodies. One determinant is common to all rotavirus strains, and the second determinant reacts with viruses having one of the two well-characterized subgroup specificities (17). All bovine specimens that reacted in this assay are subgroup I-specific, while specimens from humans have been found to be subgroup I- and subgroup II-specific. However, the finding VP6 monoclonal antibodies with subgroup I or subgroup II specificity reacted with the trimeric and not with the monomeric forms of VP6 suggests that subgroup specificity may be determined by conformational epitopes produced by the folding of VP6 or the interaction between VP6 monomers (21,22,23). In contrast, immunoblot analyses of both monomeric and trimeric forms of VP6 with commonly reactive Mabs suggest that common epitopes on VP6 have continuous determinants (22,23).

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SHORT REPORT

## Enteropathogenicity and Antimicrobial Susceptibility of New *Escherichia* Spp.

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### ABSTRACT

To determine the mechanism of enteropathogenicity of the newly described *Escherichia* species, a total of 50 clinical isolates of *Escherichia* spp. from diarrhoeal stools were studied. Twelve isolates (24%) were found to be *E. vulneris*, 6 (12%) *E. fergusonii*, 2 (4%) *E. hermannii*, and the rest 30 (60%) were *E. coli*. Most isolates of the new species were resistant to ampicillin, tetracycline, and co-trimoxazole, but were susceptible to cephalosporins and aminoglycosides. The representative strains of all the new species produced significant fluid accumulation in the rat ileal loops both by live cells and their culture filtrates. *E. vulneris*, isolated from stools, showed maximum fluid accumulation. Thus, it can be inferred that these species are diarrhoeagenic, but their roles on extra-intestinal infections remain to be determined.

Key words: *Escherichia coli*; Enteropathogenicity; Aminoglycosides; Microbial sensitivity tests

### INTRODUCTION

The genus *Escherichia* had been represented for a long time by the single species *Escherichia coli*. However, the last decade has witnessed a new taxonomic reorganization of the genus into 4 species apart from *E. coli*. The new species have been named *E. hermannii*, *E. vulneris*, *E. fergusonii*, and *E. blattae* (1-5); the last one having been isolated from non-human sources. These new species have been described based on a variety of biochemical tests, intra-species DNA homology, genome size, and guanine cytosine content. Besides *E. vulneris* which has been isolated exclusively from wound infections (2,6), the other two species have been isolated from faeces along with other clinical samples (1,3). Although the clinical significance of the

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new species has been suggested, intra-peritoneal injection of *E. vulneris* and *E. hermannii* in mice have failed to show any pathogenicity (6).

### MATERIALS AND METHODS

**Bacterial strains:** In total, 50 strains of *Escherichia* spp. were isolated from an equal number of adult patients with diarrhoea. All the stool specimens yielded either pure or predominant growth of *Escherichia* spp. with the absence of any other diarrhoeagenic bacteria, protozoa, helminths, or fungi. Stock cultures were initially subcultured into nutrient agar and MacConkey agar to obtain fresh colonies.

**Biochemical characterization:** Isolated colonies were picked up from the subcultured plates and grown in brain-heart infusion broth (BHIB) at 37 °C for 4 hours, and biochemical and related tests were performed, using the broth culture. For identification, the schemes described previously (5,7) were followed and performed by conventional methods (7,8). The tests used were:

indole production; lysine and ornithine decarboxylase; fermentation of lactose, sorbitol, mannitol, adonitol, cellobiose, and melibiose; malonate use; and production of yellow-pigmented colonies.

**Enteropathogenicity.** Ten representative strains of the 3 new species characterized by the above scheme were tested for diarrhoeagenic potential in the ileal loops of Charles-Foster strain of albino rats, using whole live cells and culture filtrates (10,11).

To test the whole cells, organisms were grown in BHIB (pH 7.2) for 4-5 hours to give a turbidity equivalent to  $10^5$ - $10^6$  colony-forming units (CFU)/mL, and were used for ileal inoculation. Culture filtrates of the organisms were prepared, following the method of Saha and Sanyal (12). Briefly, for each strain, 50 mL of BHIB contained in a 250-mL conical flask was seeded with 5 smooth colonies from overnight growth of the strain on nutrient agar. The flasks were incubated at 37 °C for 18 hours with constant shaking at 80-120 oscillations/minute. Growth was measured at 640 nm wavelength. The culture was then centrifuged at 22,000 g for 30 minutes at 4 °C. The supernatant was filtered, using a millipore filter of 0.22 µm pore diameter and stored at 4 °C.

0.5 mL of the broth culture/culture filtrate was injected into the ileal loops measuring 5 cm. In all experiments, toxigenic *Vibrio cholerae* 569B, grown in BHIB, and unseeded BHIB served as positive and negative controls respectively. The experiments were carried out in duplicate, and the amount of fluid accumulation was expressed as mL/cm of gut.

**Antimicrobial susceptibility:** Antibiograms were determined by Stoke's disk-diffusion technique (9) on Mueller-Hinton agar, and *E. coli* NCTC10418 strain was used as sensitive control.

## RESULTS

Thirty of the 50 strains tested were found to be *E. coli*. Of the remaining, 12 (24%) were *E. vulneris*, 6 (12%) *E. fergusonii*, and 2 (4%) *E. hermannii*.

Ten representative strains, comprising 4 of *E. vulneris*, 4 of *E. fergusonii*, and 2 *E. hermannii*, were used for enteropathogenicity test. All the strains tested showed significant fluid accumulation ranging from 0.2 to 0.4 mL/cm for *E. vulneris*, 0.15 to 0.2 mL/cm for *E. fergusonii*, and 0.15 to 0.18 mL/cm for *E. hermannii*. When the culture filtrates of the above strains were tested, they produced fluid accumulation in the same range. The positive control strain of *V. cholerae* 569B gave fluid accumulation ranging from 0.18 to 0.30 mL/cm, using both whole cell and culture filtrates.

All the 12 *E. vulneris* strains were resistant to one or more antimicrobial agent(s) (Table). Cefotaxime was effective against all the strains. Moderate-to-good activity was shown by cephalixin, cefoxitin, and netilimicin. Ampicillin, tetracycline, and co-trimoxazole, were the least-active agents. In contrast, all the strains of *E. fergusonii* and *E. hermannii* were uniformly susceptible to cephalosporins and netilimicin, but had a higher prevalence of resistance to ampicillin, tetracycline, and co-trimoxazole.

## DISCUSSION

The new species of genus *Escherichia* have been isolated from a variety of clinical specimens, such as stool, blood, urine, pus, etc. *E. fergusonii*, first described in 1985 (3), has been isolated more frequently from stools apart from gall bladder, blood, and urine (3,4,13,14), but its clinical significance has not been established. Six of the 50 *Escherichia* strains in our study belonged to this species. Ampicillin, tetracycline, and co-trimoxazole were the

**Table.** Antimicrobial resistance of new *Escherichia* spp.

| Antimicrobial agent | No. of resistant strains       |                                 |                                |
|---------------------|--------------------------------|---------------------------------|--------------------------------|
|                     | <i>E. vulneris</i><br>(n = 12) | <i>E. fergusonii</i><br>(n = 6) | <i>E. hermannii</i><br>(n = 2) |
| Ampicillin          | 10                             | 5                               | 2                              |
| Cephalexin          | 4                              | 0                               | 0                              |
| Cefoxitin           | 2                              | 0                               | 0                              |
| Cefotaxime          | 0                              | 0                               | 0                              |
| Chloramphenicol     | 9                              | 3                               | 1                              |
| Ciprofloxacin       | 5                              | 1                               | 0                              |
| Co-trimoxazole      | 11                             | 5                               | 1                              |
| Erythromycin        | 3                              | 0                               | 0                              |
| Gentamicin          | 2                              | 0                               | 0                              |
| Netilimicin         | 1                              | 0                               | 0                              |
| Nalidixic acid      | 8                              | 2                               | 1                              |
| Tetracycline        | 11                             | 6                               | 1                              |

least-active agents against them unlike the previous study (4) where 77% and 62% of the strains were susceptible to ampicillin and tetracycline respectively.

*E. hermannii* was first reported in 1982 (1), and has been isolated predominantly from wounds, sputum, and faeces (1,6), although it is not considered pathogenic (6). We could isolate only 2 strains of this species, and both were resistant to ampicillin.

The third new species *E. vulneris* which constituted the majority of the isolates in the study (24%) was first described in 1982 (2). It has been recovered most frequently from wound infections (2,6) and from one case of urosepsis (15). Interestingly, there is no report of its isolation from stool specimens unlike our study where it is the most frequent isolate among the new species. Antimicrobial susceptibility of the isolates was similar to earlier observations (2,6).

In previous studies, except for *E. vulneris*, the other two new species have been found to be associated with diarrhoea. However, no enteropathogenicity tests, using an experimental model, have been performed. In our study, using the rat ileal loops, we found that all the species were potentially diarrhoeagenic, causing significant fluid accumulation in the ileal loops. Both whole live cells and culture filtrates gave similar results, indicating that enteropathogenicity is most probably mediated through enterotoxin(s), although the role of other virulence factors, such as adherence, invasiveness, or adhesiveness, cannot be ruled out. Furthermore, one particular strain of *E. vulneris*, a species which has not been recovered from stool earlier, showed maximum fluid accumulation of 0.4 mL/cm.

The clinical significance of these new species of *Escherichia* is still not clear, and doubt exists regarding their pathogenic potential. In our study, we investigated the enteropathogenic aspect of the organism, and it has been ascertained. However, further studies and clinical reports are needed to evaluate their significance in causing infections in both immuno-compromised and immuno-competent subjects.

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LETTER TO THE EDITOR

## WHO-ORS in Treatment of Shigellosis

Sir,

The role of oral rehydration solution (WHO-ORS) as an adjunct in the treatment of shigellosis needs to be reviewed because of the prevalence of different types of *Shigella* species causing varied clinical presentations, especially with regard to severity of dehydration. Use of an appropriate antimicrobial agent, along with a rehydration fluid (WHO-ORS), remains the cornerstone of therapy in management of symptomatic *Shigella* infection (1).

To our knowledge, very few clinical studies were conducted to assess the therapeutic need of WHO-ORS in *Shigella* infection, particularly in children. According to a study by Rabbani *et al.* (2), hydration was achieved with normal fluid and plain water intake in children and adults with shigellosis having frequent small evacuation of bloody mucoid stool. A community study in Bangladesh showed that only 25% of all patients suffering from dysenteric illness received ORS, in addition to antimicrobial agents and other supportive treatments (3). In Thailand, a recent study demonstrated that only a limited number of patients (n=21) in a large group with shigellosis having mild-to-moderate dehydration required treatment with WHO-ORS, and patients with moderate dehydration were benefited most (4).

In Bangladesh, a small study has recently been carried out in children suffering from shigellosis without obvious dehydration. The study was conducted based on the hypothesis that there may be subclinical deficit of electrolytes, including sodium and potassium, in patients with shigellosis in absence of clinically detectable dehydration. Such deficit may cause anorexia and impairment of general well-being, in addition to *Shigella* infection. The use of WHO-ORS alone may, thus, expedite the improvement of loss of appetite and sense of general well-being in these patients. Accordingly, the children were randomized, given either WHO-ORS (35 mL/kg.day, n=16) along with an antimicrobial agent or the antimicrobial agent alone (n=14). The study could not demonstrate any additional benefit with the use of WHO-ORS, in addition to the appropriate antimicrobial therapy, and untoward effects were not observed in either group. (AM Khan and ASG Faruque of ICDDR,B., unpublished observations).

Clinical spectrum of dehydration in shigellosis may vary; in most cases, dehydration is either absent or mild. Stoll *et al.*, in a study from surveillance at the International Centre for Diarrhoeal Disease Research, Bangladesh, found that 44% of 412 patients had no dehydration, 43% had mild dehydration, 12% had moderate dehydration, and only 1% had severe dehydration (5). Thus, in 87% of the patients with shigellosis, dehydration was not clinically significant.

Pathophysiology of dehydration in *Shigella* infection varies according to type of *Shigella* species. Therefore, identification of the type of *Shigella* species is important to understand the clinical situation where the use of WHO-ORS will be appropriate and beneficial. *Shigella flexneri* and *S. dysenteriae* type 1 infections are usually characterized by frequent evacuation of small amounts of faecal material mixed with mucus or blood. Other clinical features commonly include fever, marked anorexia, abdominal cramp, tenesmus, etc. Sometimes *S. dysenteriae* type 1 may present with watery stool initially followed by typical dysenteric stool where moderate dehydration is common; severe dehydration has even been found to be rarely associated with *Shigella* infections. Similarly, *S. sonnei* and *S. boydii* infections usually present with mild clinical symptoms, and the stool is usually watery, but little mucus or blood may occasionally be present. In *S. flexneri* and in most cases of *S. dysenteriae* type 1 infections, dehydration develops as a result of multiple factors, e.g. diminished intake of fluid due to anorexia, insensible water loss due to fever, and development of severe bacterial colitis associated with passage of mucus and blood. The use of WHO-ORS in every case may not, therefore, be appropriate, because WHO-ORS was originally formulated to treat cholera and other secretory diarrhoea with dehydration.

We have reviewed recent surveillance data of the ICDDR,B which showed a high prevalence of *S. flexneri*, followed by *S. dysenteriae* type 1; other types were occasionally observed, showing a similar pattern of dehydration indicated earlier. Thus, in *Shigella* infection in Bangladesh, passage of bloody mucoid or mucoid stool is a predominant clinical feature, and watery diarrhoea is infrequently seen. On the other hand, in industrialized countries, *S. sonnei* and *S. boydii* infections are common which usually present as asymptomatic or mild clinical disease.

In conclusion, we suggest that careful clinical assessment of *Shigella* cases be made with regard to providing or withholding treatment with the WHO-ORS. Further evaluations of other ORS formulations with low-electrolyte contents to treat dehydration in shigellosis may provide important therapeutic guidelines.

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LETTER TO-THE-EDITOR

## Virulence Factors in *Aeromonas veronii* bv *veronii*

Sir,

*Aeromonas* spp. have been implicated in extra-intestinal infections and diarrhoea in man (1,2), the strains often originating from water. Virulence markers, such as production of haemolysin, haemagglutinin (HA), chitinase and enterotoxin, and serum sensitivity, have been used for determining its pathogenicity (3-5). *Aeromonas veronii* bv *veronii* is commonly isolated from aquatic environment, and its isolation has been reported from intestinal and extra-intestinal infections in humans. We report here the occurrence of possible virulence markers in this species.

Three strains of *A. veronii* bv *veronii*, two from water source and one from human blood infection, provided by Dr. JM Janda, Berkeley, USA, identified earlier by standard bacteriological techniques, were included in the study.

*A. veronii* bv *veronii* strains, grown in brain-heart infusion broth (BHIB, Difco) for 3 hours, were streaked onto the 5% sheep blood agar plates. After overnight incubation at 37 °C, the strains were examined for haemolysis.

A haemagglutination (HA) test was performed, following the method of Atkinson and Trust (6), at room temperature by mixing 20 µL of erythrocytes suspension with 20 µL of bacterial suspension (ca 10<sup>9</sup> bacteria per mL) on a slide, alongside a control suspension of erythrocytes and phosphate buffered saline (PBS), and gently rocking by hand. Strains were considered HA-negative, if agglutination did not occur within 5 minutes.

The *A. veronii* bv *veronii* strains were challenged against 65% normal human serum (NHS, blood group O) in the tube assay, according to the method of Carruthers and Kabat (7). Plate counts were done at an interval of 30, 60, and 120 minutes. The strains that exhibited a 10-fold (one log) decrease in a viable count after 120 minutes (when compared with t=0) were considered serum-sensitive.

The chitinase activity in culture supernatant (CS) was determined, following the method of Jeuniaux (8). In brief, each CS in 0.5 mL amount in two tubes, mixed with 0.1 mL of 0.8 M potassium tetraborate (Sigma), was boiled in a water bath for (exactly) 3 minutes and brought to room temperature. One percent dimethyl amino benzaldehyde (p-DMAB, Sigma, prepared in

glacial acetic acid, containing 1.25% 10 N HCl) in 3.0 mL amount was added to each tube, mixed well, and allowed to stand for 20 minutes at 37 °C. The samples were brought to room temperature, and absorbency was noted at 540 nm in a colorimeter within 10 minutes. A calibration curve was established with 10 µ, 20 µ, 40 µ, 80µ and 160 µg per mL concentration of N-acetyl glucosamine (Sigma; USA). The enzyme activity was expressed in terms of µg of N-acetyl glucosamine (N-AG) per mL.

The culture filtrates, prepared in BHIB with each strain of *A. veronii* bv *veronii* (only when the live cells of a particular strain gave positive ileal loop reaction), were tested for accumulation of fluid in the rabbit ileal loop (RIL), following the method of De and Chatterji (9).

All *A. veronii* bv *veronii* strains showed haemolysis on 5% sheep blood agar. Only two of them caused fluid accumulation in the initial rabbit ileal loop tests, and the other isolate from blood infection did so after a single passage through the RIL (Table).

None of the *A. veronii* bv *veronii* strains showed HA. However, all of them showed resistance to 65% NHS (Table). Moreover, all isolates were enterotoxic, and none of these isolates underwent any change in HA property after serial passage through the rabbit gut.

All strains of *A. veronii* bv *veronii* produced constitutive chitinase. However, it was found that the two strains from water source elaborated inducible chitinase in their CS, and also caused fluid accumulation in the initial test (Table). This finding suggests that there is a correlation between production of inducible chitinase and enterotoxin.

The observation that one isolate requires passage to cause fluid accumulation suggests a repression-derepression phenomenon influencing the toxin gene. However, the non-haemagglutinating strains did not show HA after ileal loop passage, although they became enterotoxic. This suggests that repression-derepression phenomenon may not apply to the haemagglutinin gene.

The above finding that these isolates are serum-resistant suggests their potential virulence in extra-intestinal infections. This finding may explain the recent report that extra-intestinal infections, such as wound

| <b>Table.</b> Haemolysis, haemagglutination, serum sensitivity, chitinase production, and enterotoxicity of <i>Aeromonas veronii</i> bv <i>veronii</i> strains |             |            |     |                   |  |
|--|-------------|------------|-----|-------------------|--|
| Strain number  | Source      | Haemolysis | HA  | Serum sensitivity | Fluid accumulation (mL per cm in RIL) <sup>a</sup> |
| *4995-91A  | Water       | +          | NHA | R                 | 0.60-0.80  |
| *4996-91A  | Water       | +          | NHA | R                 | 0.66-0.84  |
| 7534-93A   | Human blood | +          | NHA | R                 | 0.00-0.00  |
| Positive control <sup>+</sup>  |             |            |     |                   | 0.90-1.20  |
| Negative control <sup>++</sup>   |             |            |     |                   | 0.00-0.00  |

<sup>a</sup> Range of fluid accumulation in ileal loops of two rabbits before passage.  
\* Strains produce inducible chitinase.  
<sup>+</sup> BHIB culture of toxigenic *V. cholerae* strain 569B; <sup>++</sup> unseeded culture of BHIB.  
HA: haemagglutination; NHA: no haemagglutination; R: serum resistant; RIL: rabbit ileal loop

infection by *Aeromonas* spp., are water-related (10). However, the findings of our study, except one isolate from blood infection that failed to show any secretory response in the initial test but became positive after one passage, suggest a possible correlation between these two properties and which may indicate versatility in the role of this species in various diseases.

These observations, thus, suggest that strains of *A. veronii* bv *veronii*, isolated from water and blood infection, possess the potential virulence factors, such as enterotoxin production, including haemolysin, serum resistance, and inducible chitinase production, but not haemagglutination, which may play some role in enhancing the virulence of this organism.

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Abstracts of Papers Presented at the  
8th Annual Scientific Conference (ASCON)  
held on 13-14 February 1998 at ICDDR,B

# Vaccine Research and Environmental Health

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## **Introduction**

The Eighth Annual Scientific Conference (ASCON) has adopted two themes—Vaccine Research and Environmental Health. Both of these themes are the keys to addressing child health and survival, maternal health, and prevention strategies.

Vaccine research had its beginning in the Matlab field site in 1963. It was there where the Cholera Research Laboratory, the forerunner of ICDDR,B, conducted cholera vaccine trials that proved the vaccine developed in the mid-sixties and early seventies to be ineffective. The results from these trials led to the discontinuation of recommending the vaccine, saving the world from misuse of millions of dollars. Since that time, cholera vaccine research has continued, and new trials for cholera vaccines may be on the horizon. Other diarrhoeal disease vaccines, such as for rotavirus, shigella, and salmonella also show promise. Once again, Matlab sub-station of the Centre is poised to serve, in many respects, as an ideal field test site due to its thirty-year history of maintaining demographic surveillance on its 210,000 population. The availability of these longitudinal data for such a long and uninterrupted period on this rural Bangladeshi population will continue to make Matlab an attractive field site for vaccine trials and other community-based research. In vaccine research, we must bear in mind that immunogenicity and efficacy of vaccines must be tested in the appropriate vulnerable groups. Otherwise, the results will not be valid, and financial resources will be wasted.

There are numerous other questions surrounding vaccines. They include concerns, such as the proper distribution of vaccines, immunogenicity trials, the purpose and outcomes of community-based efficacy and effectiveness trials, appropriate methods of vaccine research, ethical issues raised in vaccine trials, assessment of the disease burden; and prioritization of vaccines to be incorporated into the national EPI and operations research on implementing effective immunization campaigns. Additionally, given the Centre's history, many will be interested in our research efforts, our track record in vaccine trials, and global implications of the work in vaccine research conducted at ICDDR,B. There is no doubt that some of these questions will be raised and addressed over the course of the next two days.

The Centre has been intimately involved in environmental health research both in the laboratory and the community. The results have been significant. Hundred years after discovery of the cholera germ, scientists of this Centre, in collaboration with scientists from abroad, have discovered the reservoirs of cholera. The results of basic research conducted here as well as the methodology for cholera epidemic control preparedness have been disseminated throughout the world from the Centre in Bangladesh. These achievements of the Centre are also claimed as major achievements for Bangladesh. Through their basic research work, the Centre's scientists continue to find practical solutions to prevent diarrhoeal disease outbreaks. The environmental health research in Bangladesh will have its impact on other communities worldwide that face similar environmental problems resulting in outbreaks of diarrhoeal diseases.

The importance of the public health concerns of environmental health cannot be underestimated. The promotion of public health messages and prevention strategies based on an understanding of the nature and extent of the growing environmental problems in our

community are essential for developing appropriate solutions that will benefit people of all age groups and at every level of society. It is the work done at the field level by concerned communities, government agencies, and NGOs working together that is required to yield sustainable solutions. Education is critical for advancing environmental health concerns. Thus dissemination of information cannot be restricted to the community health workers and NGOs operating in the field, and messages must reach every household at every level. At the 8<sup>th</sup> ASCON, we will have the opportunity to review some challenges of prevention of infectious diseases through clean water supply and of controlling the levels of toxins in the atmosphere and water sources. The effectiveness of the public health messages and prevention strategies employed through urban and rural community-based activities can be examined, and many of the issues explored five years ago in the 3<sup>rd</sup> ASCON can be re-examined, along with progress being made in this area.

Research by scientists at the Centre and others has been significant in both of the topics presented in the 8<sup>th</sup> ASCON, with collaborative research being done at the international level. This, in part, reflects the unique research environment of ICDDR,B.

We are pleased to welcome our colleagues from the Government of Bangladesh, academic research community, and from the NGO research sector to the ASCON. This 8<sup>th</sup> ASCON is open to outside presenters as we have been continuing the practice from our two previous ASCONs. This underscores the important work done throughout the research community in Bangladesh in both vaccine research and environmental health. It also provides an excellent opportunity and a forum for us to interact with our colleagues and collaborators and learn of the research conducted in Bangladesh in these two important fields. Undoubtedly, some of the results and outcomes presented here will ultimately be shown to be important with implications for health policy and programmes in Bangladesh and in the global community.

We invite you to fully participate in the conference proceedings, by interacting with the presenters. The Conference should serve as an occasion to further explore some of the findings presented here and create an impetus for new vaccine research and strategies to address environmental health concerns. Once again, we welcome all of you. Enjoy the 8<sup>th</sup> ASCON.

George Fuchs, MD  
Chairperson, Organizing Committee  
8th Annual Scientific Conference (ASCON)  
and Interim Director, ICDDR,B

## Morbidity and Vaccine Research

### Response to Tuberculin Skin Testing at Six Months of Age and Relation with Birth Characteristics in Infants of Dhaka City Slums

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**Objective:** Investigate whether the response to tuberculin skin testing at 6 months of age is related to infants' birth characteristics.

**Methodology:** A total of 345 infants from Dhaka city slums, whose mothers had received either zinc or placebo supplementation during pregnancy, received the standard Bacille Calmette Guerin (BCG) vaccination within 72 hours after birth. The infants were followed for weekly morbidity assessment and monthly anthropometric measurements. Cell-mediated immune response was tested at 6 months of age by delayed hypersensitivity skin test to purified protein derivate (PPD) tuberculosis (5 tuberculin units). Size of induration was determined after 72 hours and considered positive when an induration of >5 mm was observed.

**Results:** A total of 207 (60.5%) infants had a negative tuberculin skin reaction at 6 months of age. Birth-weight was significantly related to tuberculin reaction ( $p < 0.001$ ). The percentage of negative skin responses was significantly higher in low-birth-weight compared to the normal-birth-weight infants (71.9% vs. 52.7%;  $p < 0.001$ ). The prevalence of measured fever during the first 6 months of life was also significantly related to tuberculin skin response ( $p < 0.001$ ). No relation was observed between other birth characteristics, sex of infant or zinc treatment of mother and response to tuberculin testing.

**Conclusion:** The results of this study indicate that infants born with low-birth-weight have impaired cell-mediated immunity at 6 months of age compared to normal-birth-weight infants as is shown by a more frequent negative response to tuberculin skin testing. Supplementation with zinc during pregnancy does not affect this relationship.

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### Safety and Immunogenicity of an Oral Inactivated Enterotoxigenic Escherichia coli Vaccine in Bangladesh

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**Objective:** Carry out phase I trial on an oral ETEC vaccine in Bangladeshi adults and children.

**Methodology:** Healthy adult volunteers (18-45 years of age,  $n=38$ ) and children (3-10 years of age,  $n=21$ ) were given two doses of the ETEC vaccine 14 days apart. The vaccine was composed of a mixture of inactivated ETEC bacteria expressing colonization factors (CFs) together with recombinant cholera toxin B-subunit (rCTB). The immune responses were studied by measuring antibody-secreting cells (ASCs), and antibody levels in faeces and plasma.

**Results:** The vaccine was safe with little or no adverse effects. The immune responses to the CFs and the toxoid in both children and adults peaked after a single dose of the vaccine. The majority of the vaccinees (<sup>3</sup>90%) responded with ASCs to CFA/I (GM=80-194 ASC/ $10^7$  MNC) as well as to rCTB (GM=307-1533 ASC/ $10^7$  MNC). Intestinal IgA antibody responses were also observed in intestinal fluids to CFA/I (>67%) and rCTB (>80%). About 81% of the children and 55% of the adults responded with IgA antibodies to CFA/I, and over 90% of both children and adults responded to rCTB in plasma.

**Conclusion:** The results of the phase I clinical trial indicate that the ETEC vaccine is both safe and immunogenic. A single dose of the vaccine acts as a booster in adults as well as children who have been primed by previous ETEC exposure.

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## Maternal Immunization with Meningococcal Vaccine

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**Objective:** Evaluate the safety and immunogenicity of *Neisseria meningitidis* (Nm) vaccine given during pregnancy

**Methodology:** A double-blind randomized safety and immunogenicity trial was conducted on 70 women recruited from the private patients of four obstetricians in Dhaka city. A single dose of either N. meningitidis vaccine containing polysaccharide groups A, C, Y and W-135 or a 23-valent pneumococcal polysaccharide vaccine was given at 30-34 weeks of gestation and the women were then closely followed up at 8, 12, 24, and 72 hours to record adverse effects of vaccination. Serum samples were collected from mothers at the time of immunization after 1 month, and at delivery. Colostrum samples and breastmilk specimens were collected at 1 and 2 weeks and at 1, 3, and 5 months after delivery. Cord blood sera and infants sera were obtained at 6, 14, and 22 weeks. Serological assays for anti-Nm A antibodies were done in the sera of both mothers and infants and breastmilk.

**Results:** Minor local reactions to vaccination were noted which resolved completely in 72 hours. Pre-immunization anti-Nm A GMTs were similar in the vaccinated and the control groups (2.25 m g/mL vs. 2.82 m g/mL). At delivery, serum anti-Nm A GMT titres were 13.18 m g/mL and 4.91 m g/mL in the vaccinated and the control groups ( $p < 0.05$ ). Anti-Nm A antibody levels were 2.44 m g/mL at 6 weeks, 1.21 m g/mL at 14 weeks and 0.59 m g/mL at 22 weeks of the infants' life in the vaccinated group. The infant/maternal ratios were 0.49 and 0.67 in the vaccinated group and the control group respectively. IgA level was 36.25 m g/mL in colostrum ( $p < 0.05$ ) and 12.51 m g/mL in breastmilk ( $p < 0.01$ ) at 6 months.

**Conclusion:** Maternal immunization with Nm vaccine is safe and immunogenic. A 5 to 6-fold rise in maternal anti-Nm A antibody titre occurred in the vaccinated group. A decline in the infants' anti-Nm A antibody level occurred after 6 weeks, but levels remained high in the breastmilk up to 6 months after delivery in the vaccinated group.

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## Safety of a Live, Attenuated *Shigella flexneri* 2a Oral Vaccine (SC602) in Bangladeshi Adults

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**Objective:** Assess the safety of three different dose levels of a live, attenuated *Shigella flexneri* 2a oral vaccine candidate (SC602) compared to a placebo among Bangladeshi adults in an inpatient setting.

**Methodology:** Twenty healthy adult Bangladeshi men aged 20-39 years were admitted to the Matlab, Bangladesh field research hospital to participate in a randomized, double-blind, placebo-controlled phase I safety trial of SC602—a live, attenuated *Shigella flexneri* 2a vaccine. After the study was explained, the volunteers were screened and those who wished to participate signed the consent document. They were randomized to receive a single oral dose of  $1 \times 10^4$ ,  $1 \times 10^5$  or  $1 \times 10^6$  colony-forming units or placebo with a bicarbonate solution. Volunteers were interviewed and examined daily by a physician till discharge. All stool samples were collected for characterization and culture.

**Results:** There were no significant side-effects in any of the volunteers. None of the volunteers had diarrhoea, fever or significant abdominal pains. Malaise and anorexia were the most commonly-reported symptoms. The symptoms usually lasted for less than a day. SC602 was isolated from 5 of the 20 volunteers beginning 24-48 hours after vaccination. All volunteers were discharged on day 6 after vaccination. Two volunteers who were shedding the vaccine on day 5 were treated with ciprofloxacin before departure. The code was opened at the completion of the clinical portion of the trial. Analysis of symptoms did not demonstrate any dose-related concerns. A dose-related increase in excretion was observed with 3 of the 5 persons in the  $1 \times 10^6$  group excreting *S. flexneri*.

**Conclusion:** The vaccine was well-tolerated. None had diarrhoea, dysentery, fever or severe symptoms. Mild symptoms were common, but there was no clear dose-related pattern of symptoms. Excretion was dose-related, but

it was not associated with symptoms. Based on these data, we recommend further evaluation of this vaccine in larger study population.

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### Distribution of Group A Rotavirus Strains in Bangladesh: Emergence of Type G9 in 1995

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**Objective:** Determine the circulating G and P type rotavirus strains in Bangladesh.

**Methodology:** One thousand five hundred thirty-four rotavirus strains collected during 1992-1997 were characterized. Characterization was done using a combination of methods: monoclonal antibody-based enzyme immunoassay, reverse transcription polymerase chain reaction, and oligonucleotide probe hybridization. Strains were collected from various parts of Bangladesh.

**Results:** Results from this study combined with our previous findings from 1987 to 1991 (n=2515 faecal specimens in total) demonstrated that the distribution of the 4 major G types varied from year to year; types G1-4 constituted 51% of all strains tested (n=1371), and type G4 was the most prevalent (21%), followed by type G2 (17%). Of the 351 strains tested for both G and P types, common types P[8]G1, P[4]G2, and P[8]G4 on a global basis comprised 45% (n=159) of the strains. Mixed G and/or P types were found in 23% (n=79) of the samples tested. Type G9 rotavirus that were genotype P[6] and P[8] emerged in 1995. G9 strains constituted 16% (n=56) of the typeable rotavirus strains.

**Conclusion:** A vaccine must provide protection against type G9 rotavirus as well as the 4 major G types.

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### Sensitivities of *Shigella* Species Isolated from Stool Samples in Gonoshasthaya Hospital, Savar

M.A. Samad Talukder<sup>1</sup>, Ayesha Khatun<sup>2</sup> and Ismat A. Begum<sup>2</sup>

**Objective:** Examine sensitivities of *Shigella* species isolated from stool specimens in Gonoshasthaya Hospital, Savar.

**Methodology:** Stool specimens were cultured on MacConkey agar, XLD agar, and TCBS agar routinely for isolation of pathogens, including *Shigella* species and incubated at 37°C overnight. Non-lactose-fermenting non-motile Gram-negative bacilli were biochemically identified and serologically confirmed as *Shigella* species. Sensitivity tests of isolated organisms were done on Mueller-Hinton media.

**Results:** Period of study was 1 January 1996 to 30 November 1998. Seven hundred and fifty stool specimens were examined, and 43 (5.73%) yielded *Shigella* species. Of these, 22 were of males and 21 of females aged six months to 65 years (mean age 9.8 years). Thirty-two patients were aged less than 15 years, and 11 patients were above 15 years. There were 22 (51.2%) *Shigella dysenteriae*, 12 (27.9%) *S. flexneri*, 5 (11.6%) *S. boydii* and 4 (9.3%) *S. sonnei*. Forty-two (97.7%) were sensitive to ciprofloxacin, 29 (67.5%) to gentamicin and nalidixic acid, 9 (21%) to cotrimoxazole, 8 (18.6%) to mecillinam, and 5 (11.6%) to ampicillin and tetracycline.

**Conclusion:** *Shigella dysenteriae* was the most commonly isolated species. Resistance to common antibiotics was high, making treatment difficult.

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### Barriers to Immunization in the Slums of Dhaka City

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**Objective:** Document the barriers to immunization services which slum women had experienced or had heard about through discussions with their friends.

**Methodology:** In a qualitative study done in late 1996, one hundred mothers living in slum households in Zone 3 of Dhaka City Corporation were interviewed by an ICDDR,B field researcher using a detailed, open-ended questionnaire.

**Results:** The most frequent barrier mentioned by the respondents was disrespectful behaviour of the vaccinators, particularly if the respondent had lost her EPI card. The second most commonly mentioned barrier was the fee which they have to pay—both official fees charged by the NGOs and “unofficial” fees charged by the government vaccinators. Other barriers reported by the respondents include their own difficulties in travelling away from home, opposition from family members, long waiting time, inconvenient hours of clinic operation, lack of information, and earlier experiences with side-effects following immunizations.

**Conclusion:** The lower coverage of immunization in the urban slums of Dhaka is of great concern, particularly because high population density, poor hygiene conditions, high frequency of home deliveries, and high levels of malnutrition contribute to the production of population “pockets” at high risk for mortality from vaccine-preventable diseases. The findings of this study point at the need to: (1) promote more respectful behaviour from the vaccinators, (2) make the payment policy for immunization more transparent and flexible, (3) provide an honourable way for those who are unable to pay for immunization, and (4) disseminate clients’ perceived obstacles to immunization to service providers and programme leaders.

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### Effect of Reducing the Number of EPI Sessions on Cost and Effectiveness of the Programme

M. Mahmud Khan, Richard Yodar, Abdullah Al Mamun and Suhaila H. Khan

**Objective:** Evaluate the financial implication of reducing the number of EPI sessions per month in Bangladesh and discuss possible impacts on immunization coverage.

**Methodology:** This exercise was carried out from the perspective of the providers, i.e. the EPI programme, the Ministry of Health and Family Welfare, and agencies collaborating in the delivery of EPI services. The raw data for this study were obtained from the annual expenditure statements provided by the EPI Unit for 1995-1998. From this, a three-year average of annual expenditure was calculated in 1997 prices. The EPI’s accounting system has twenty separate line items or categories of expenditure. These were reclassified into standard categories typically used in immunization costing studies. Current prices of vaccines were obtained from UNICEF. To estimate the target population, the demographic data and parameters were obtained from BBS reports and yearbooks.

**Results:** In Bangladesh, vaccinations are done through 110,000 sessions held every month. To reduce the cost of delivering EPI services, reduction in the number of sessions has been proposed. This study examines the possible effect on costs if the sessions reduce to 55,000 or 22,000 per month. Recently-adopted health programme proposes delivery of immunization through fixed sites at the union level—each site operating five days a week, the total number of sessions organized in a month being about 22,000. The number of vials needed for DPT and OPV remained constant irrespective of the number of sessions organized in a month. This is because the number of children to be immunized with DPT and OPV is, on the average, about eight per session when the number of sessions per month is 110,000. If the number of sessions is cut to half, the average number of children to be immunized per session increases to about 16, and each session will need to open two 10-dose vials rather than one.

**Conclusion:** Reducing the number of EPI sessions will adversely affect the coverage of immunization unless more rigorous social mobilization campaign is launched. Assuming that the coverage will decline by 20 percentage

points for reducing the sessions by 50%, total cost of EPI remains more or less the same if additional cost of social mobilization is added. Policy makers should carefully evaluate the savings generated by reduction in the number of sessions and the additional cost needed to maintain the current coverage.

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### Child Immunization: Trends and Determinants in Bangladesh

Abdullah Al Mamun and M. Mahmud Khan

**Objective:** Examine the coverage of immunization in Bangladesh and identify important determinants of child immunization from household-level data.

**Methodology:** Data from the Bangladesh Demographic and Health Survey 1996-1997 have been used in this analysis. The survey gathered information on immunization of all children of less than 5 years. The influence of regional, demographic and socioeconomic variables was examined using differential analysis in the bivariate approach and logistic regression in the multivariate approach in order to identify the factors affecting child immunization coverage.

**Results:** According to the information from both vaccination records and mothers' recall, only 54% of Bangladeshi children (12-23 months) can be considered fully immunized, although the level of coverage for BCG and the first two doses of DPT and polio exceeds 80%. Only 12% had no vaccination at all. Various factors, including mothers' age, parity, sex of the child, place of residence, region of residence, religion, mothers' education, economic status, electricity in the household, and exposure to mass media, appear to be significant in influencing the levels of child immunization in Bangladesh. It was observed that child immunization is substantially higher in urban than in rural areas. There is a sharp regional differential in child immunization in Bangladesh. It was also observed that immunization coverage is better for births to younger women and those of lower birth order. Among the socioeconomic variables, the educational levels of mother and father had the strongest positive effect on child immunization.

**Conclusion:** Results suggest that higher age at marriage of women, improving educational status of mothers, better communication, and media coverage as well as intensive immunization activities in rural areas will improve the coverage of immunization.

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### Bangladesh's First "Mop-up" Campaign for Polio Eradication: An Assessment

Syed Izaz Rasul, S. Mizan Siddiqi and Henry B. Perry

**Objective:** Assess the results of a programme to ensure that 100% children aged less than 5 years in high-risk areas receive oral polio vaccine (OPV) on or soon after the National Immunization Day (NID) on 14 December 1998.

**Methodology:** In three high-risk areas where live wild polio virus had been isolated during the past 12 months (Madaripur, Shariatpur and Bhola municipalities), a week-long house-to-house campaign was conducted from 17 to 23 December 1998. The purpose of this campaign was to identify all children aged less than 5 years in these municipalities, who had not participated in the NID on 14 December and to administer OPV and vitamin A to them. In each of the three municipalities, 18 personnel (municipal health staff, NGO workers, and NID volunteers) worked for 5 days to complete the campaign. A team comprising personnel from the national EPI headquarters, BASICS, and the Civil Surgeon's Office, supervised the campaign.

**Results:** In total, 21,810 households were visited; 2,250 children aged less than 5 years were given OPV; and 1,575 received vitamin A capsule containing 200,000 international units (IU). The percentage of children who did not receive OPV during the NID on 14 December 1998 ranged between 5.9% and 17.8%. Shariatpur municipality had the highest coverage (94.1%), while Bhola had the lowest (82.2%).

**Conclusion:** Bangladesh is entering a new phase in its polio eradication programme. Since high levels of OPV coverage have been maintained nationwide for several years, the time has come to begin intensive immunization in

pockets of low-coverage areas and in areas where live polio virus has been identified. The study indicates that "mop-up" campaigns are feasible and can be effective in ensuring that all children in high-risk areas are reached with OPV and vitamin A supplementation.

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### Financing of the EPI in Bangladesh

M. Mahmud Khan<sup>1</sup>, Richard Yodar<sup>1</sup>, Abdullah Al Mamun<sup>1</sup> and K.M.A. Aziz<sup>2</sup>

**Objective:** Analyze the current and projected costs of delivery of EPI services to determine the resource gap and implications for future of the programme.

**Methodology:** This study examines the resource requirement for providing EPI services in Bangladesh from the perspective of the providers. In costing EPI, all costs incurred by households and communities in obtaining immunizations were not considered. Recurrent and capital costs were used and obtained from the EPI accounts office and supplemented with cost data obtained from the Directorate of Health Services, EPI officials, and other individuals knowledgeable about EPI activities.

**Results:** This study found that the EPI in Bangladesh is expected to fully immunize 1.56 million infants aged less than one year by using resources equivalent to about \$18.3 million in 1997-1998. Therefore, the total cost per fully-immunized child was about \$11.76—much lower than the developing country average of \$15. Immunization is estimated to prevent 134,000 deaths during the current year at a cost of \$136 per death prevented. It is estimated that about 1.15 million deaths have been averted since 1987 due to immunization activities. At the current level of costs, the gap between total resources needed (\$18.3 million) and the resources provided through government funding (\$8.3 million) approximates \$10 million. This gap must be closed either through generating additional resources, or through cost containment, or both. Current EPI cost represents approximately 0.06% of GDP, 0.05% of GoB revenue, and 4.95% of the budget of the Ministry of Health and Family Welfare.

**Conclusion:** In general, the EPI programme was found to be highly cost-effective in Bangladesh, especially when compared with the costs in other developing countries and with the cost-effectiveness ratios of various preventive and curative interventions. Thus, one of the best ways to generate savings in the entire health sector is to reduce the needs in the highly-visible curative sector by reducing its demand through prevention of common childhood illnesses. Although the EPI is not an expensive strategy, it is unlikely that the Ministry of Health and Family Welfare will be able to allocate 5% of its budget for immunization in the near future. Therefore, cost containment strategies should be emphasized.

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### LQAS Assessment of EPI Coverage in the Slums of Dhaka City

S. Mizan Siddiqi<sup>1</sup>, Henry B. Perry<sup>1</sup>, Izaz Rasul<sup>1</sup> and Md. Ashrafuddin<sup>2</sup>

**Objective:** Document the current immunization coverage levels in slum households of Dhaka city and identify areas of low coverage.

**Methodology:** Two lot quality assurance sampling (LQAS) surveys were carried out in slum households of Dhaka city to assess childhood immunization coverage among children of 12-23 months and to assess maternal tetanus toxoid (TT) coverage among women who had given birth during the previous 12 months. In each survey, information was collected from 170 respondents. Seventeen slum households were randomly selected for each survey from each of the 10 zones of Dhaka city.

**Results:** Fifty-eight percent of the children (12-23 months) were fully immunized before reaching their first birthday. Seventy-five percent of the mothers who had given birth during the previous 12 months had obtained two doses of TT, but only 23% had fully obtained the recommended five doses. Five zones were identified by the survey as having childhood immunization coverage not meeting pre-set threshold levels, and three zones were identified as

not meeting pre-set TT levels. Only 71% of the children aged less than one year participated in the most recent National Immunization Day.

**Conclusion:** The findings of the study indicate that EPI coverage is increasing in slum households of Dhaka city compared to earlier surveys, but the goal of 90% coverage by the year 2000 will probably not be reached. In addition to providing information about coverage levels, the LQAS methodology identified zones of Dhaka city which could benefit from focused programmatic assistance.

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### Status of Immunization in Bangladesh

A.M.R. Chowdhury<sup>1</sup>, Abbas Bhuiya<sup>2</sup>, K.M.A. Aziz<sup>1</sup>, F. Karim<sup>1</sup>, Shamim Ara<sup>1</sup>, Shagufta Sultana<sup>1</sup> and Insana Begum<sup>1</sup>

**Objective:** Estimate the progress of EPI since its introduction in 1979.

**Methodology:** In estimating the progress, secondary sources of data were used.

**Results:** From a mere 2% in 1986, the vaccination coverage quickly rose to 61% in 1991. Difference in coverage between routine monitoring by EPI programme implementers and independent surveys is presented. Reliability of the surveys of independent coverage is corroborated by other surveys. Surveys show that the coverage reached its peak in the early 1990s. An analysis of the national coverage according to different antigens suggest a very high rate for BCG and a sharp dropout between BCG and the third dose of DPT/OPV. A recent review by international experts mentioned 91% coverage of BCG and labelled it as an example of a laudable performance in the Bangladesh context. There was a high dropout rate of 30% between BCG and measles vaccines. Difference in coverage among selected geographical regions is noted. The highest coverage of nearly 94% was recorded in Kushtia, and the lowest was a meagre 28% in Kishoreganj.

**Conclusion:** The matters of concern include high dropout rate between BCG and measles vaccines, and wide difference in vaccine coverage reporting by different sources. Replacement of the target-oriented approach relating to low performance by acceptance of actually-achievable performance with improved supervision is recommended. It will also contribute to accurate reporting.

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### Cultural Concepts Reflected in Disease Terms in Local Dialect Regarding Vaccine-preventable Diseases at Bhairab, Bangladesh

K.M.A. Aziz<sup>1</sup>, A.M.R. Chowdhury<sup>1</sup>, Abbas Bhuiya<sup>2</sup>, Dineke Mol<sup>1</sup>, Shagufta Sultana<sup>1</sup>, Insana Begum<sup>1</sup>, Shamim Ara<sup>1</sup> and F. Karim<sup>1</sup>

**Objective:** Explore the implications of disease terms found in local dialect of rural Bhairab, Bangladesh, with reference to dealing with the vaccine-preventable diseases, like tetanus, polio pertussis, diphtheria, measles, and tuberculosis.

**Methodology:** Information was obtained through in-depth interview with 48 mothers of rural Bhairab having child(ren) <2 years of age during December 1996-February 1997. The mothers were selected through stratified sampling.

**Results:** A number of traditional terms in the local dialect were in use among the mothers. The terms identified were related to tetanus, polio, diphtheria, tuberculosis, and measles. The beliefs associated with the explanation of these disease occurrences are reflected in the disease terms, like *alga batas*, *alga dos*, *thaura*, *daira laga*, and *lula batas*. Through use of local dialect, members of the community get an occasion to discuss the culturally prescribed ways of getting relief from these diseases. As a result, some mothers gave greater attention to the traditional prescription without giving importance to the modern recommended methods.

**Conclusion:** The perceptions of immunization workers and mothers are based on two modes: science and tradition. These two modes must meet. This cannot take place if one tells the other to listen without listening to the others' views. Both must try to understand each other, and then only a greater compliance to immunization can occur.

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### Demand Aspects Relating to Immunization at Bhairab, Bangladesh

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F. Karim<sup>2</sup>, Shagufta Sultana<sup>2</sup>, Dineke Mol<sup>2</sup>, Shamim Ara<sup>2</sup> and Insana Begum<sup>2</sup>

**Objective:** Explore the factors associated with the demand of immunization for young children by investigating mothers' knowledge, opinions, and beliefs regarding compliance with the vaccine schedule.

**Methodology:** The investigation was undertaken using in-depth interview at urban and rural Bhairab among 97 mothers selected through stratified sampling during December 1996-February 1997.

**Results:** The rural and urban mothers held the view: vaccination is good for the child, and it protects from diseases. Majority of them did not consider vaccine to be harmful. Many of them referred to the side-effects of vaccines. The urban mothers considered TB as one of the most serious diseases. Most rural mothers considered all vaccine-preventable diseases to be severe. The sources of knowledge on vaccine were: mass media, neighbours, relatives, friends, and health workers. Mothers considered other preventive measures alongside vaccination. The causes of vaccine-preventable diseases were sometimes confusing with the symptoms. Side-effect was a major factor for not completing the vaccinations.

**Conclusion:** All the sources of knowledge failed to create enough impetus in creating inspiration for high demand for vaccination. Consideration of other preventive measures and side-effects kept the demand for vaccination low. Improvement in demand for vaccination requires emphasis on the function of vaccine in preventing specific life-threatening diseases resulting in the control of deaths and thus enabling the growing up of children.

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### Supply Aspects Relating to Immunization at Bhairab, Bangladesh

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K.M.A. Aziz<sup>1</sup>, Shamim Ara<sup>1</sup>, Shagufta Sultana<sup>1</sup> and Dineke Mol<sup>1</sup>

**Objective:** Examine the quality of supply in implementing immunization services at the EPI sessions at Bhairab and their implications.

**Methodology:** Observations and interviews were conducted in 5 urban and 4 rural sessions. The informants and the sessions were purposively selected. The study was undertaken during December 1996-February 1997 at Bhairab.

**Results:** Vaccines were sometimes received very late by the providers which delayed the vaccination session. Except DPT, all other vaccines were available in all the sessions. To maintain the temperature in the carrier, health workers used ice packs. Strip thermometers were not used in outreach sessions, and supervision was generally lacking. EPI cards for children were not available in any of the sessions for several months. Re-usable needles were used, and they were not always sharp. Two registers and tally sheets were used in every vaccination sessions. Supervisors and health workers always got cooperation from local people in getting supply of furniture and provision of a place to hold vaccination sessions.

**Conclusion:** Late arrival of supply, short supply of DPT, inadequate number of needles, and no supply of EPI card for children sometimes hampered the smooth functioning of the EPI sessions. Community members were extraordinarily cooperative in offering logistics support. Use of strip thermometers in the vaccine carriers might have a problem in maintaining the potency of vaccines. The quality of supervision and performance and continued supply of vaccines and materials are the critical areas that require remedial measures.

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## Immunization Status of Children in Chittagong Hill Tracts

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F. Karim<sup>2</sup>, and K.M.A. Aziz<sup>2</sup>

**Objective:** Evaluate the immunization programmes conducted by EPI among children aged less than 24 months in Chittagong Hill Tracts in the background of prolonged period of political instability.

**Methodology:** A survey was conducted during May-July 1998, following signing of the Peace Treaty, among 656 mothers with at least one child aged less than 24 months. Equal numbers of mothers were selected representing the Chakma, Marma, Murang, Tripura and Bangalee communities.

**Results:** Wide variation in EPI coverage was observed among the tribes. Proportion of the fully-immunized children was the highest for the Bangalees (42.64%), followed by Tripura (18.2%), Marma (19.3%), Chakma (12.3%), and Murang (8.9%). Separate analyses for 12-23 months age cohort also revealed the same pattern. Most frequent reasons for non- or partial immunization included irregular visits by the health providers, lack of mothers' knowledge about immunization, long distance of the immunization centres.

**Conclusion:** There were wide gaps in immunization coverage between the national and the study areas. After the Peace Treaty, the movement in the area has become safe. Effort to increase the immunization coverage is a critical need of the hour.

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## Health and the Environment

### Arsenic Levels in Drinking Water and Prevalence of Skin Lesions

Mahfuzar Rahman<sup>1</sup>, Martin Tondel<sup>1</sup> and Ireen Akhter Chowdhury<sup>2</sup>

**Objective:** Determine the relationship of arsenic-associated skin lesions and degree of arsenic exposure.

**Methodology:** In a cross-sectional study, several wells contaminated with arsenic were identified in four villages. Arsenic water levels were measured by flow-injection hydride generation atomic absorption spectrometry (FI-HG-AAS), and arsenical skin lesions were diagnosed as the presence of one or more of these criteria: hyperpigmentation of unexposed body surfaces and/or keratosis, especially on palms and soles. We interviewed and examined 1,481 subjects by a door-to-door visit, who were depending on these wells—all in ages of 30 years or more.

**Results:** A total of 430 subjects had skin lesions and arsenic water levels ranged from 10 m g/L to 2,040 m g/L. Individual exposure assessment was estimated by levels (m g/L) and in terms of a dose-index (m g/L-kg) and a concentration-time product (m g-years/L). The crude overall morbidity from skin lesions was 29%. After age-adjustment to the world population, the prevalence was 30.1 and 26.5 per 100 for men and women respectively. There was a significant trend for the prevalence in relation to exposure levels ( $p < 0.01$ ). The age-adjusted prevalence of skin lesions in females rose from 20.1 to 30.8 per 100 in terms of dose-index and 17.9 to 30.5 per 100 in terms of concentration-time product in the lowest compared to the highest category. The age-adjusted prevalence of skin lesions in males rose from 19.3 to 34.8 per 100 in terms of dose-index and from 18.6 to 34.8 per 100 in terms of concentration-time product in the lowest compared to the highest category. The test for trend for the total population was significant ( $p < 0.001$ ) in both series of risk estimates.

**Conclusion:** The study shows a higher prevalence of arsenic skin lesions in the study villages. This is an alarming sign of arsenic exposure and requires urgent remedy, especially regarding future skin cancer.

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### Village Health Workers Can Test Tubewell Water for Arsenic

A.M.R. Chowdhury<sup>1</sup>, Mohammed Jakariya<sup>1</sup>, Ashiqul H. Tareq<sup>1</sup> and Jalaluddin Ahmed<sup>2</sup>

**Objective:** Evaluate a simple, inexpensive and rapid arsenic testing methodology and assess the level of clarity of the messages imparted to the villagers during testing tubewell water for arsenic.

**Methodology:** BRAC's Shasthya Shebikas and programme organizers were involved in a field-testing of tubewell water using a newly-developed field-kit by the National Institute of Preventive and Social Medicine. Random samples were analyzed using spectrophotometer for cross-checking the field-kit results. Household survey and focus group discussion methods were used for monitoring the programme at Hajiganj. The results obtained by simultaneous use of field-kit and spectrophotometer techniques showed a 92% matching.

**Results:** At the BRAC offices, 802 tubewell water samples were tested. Of these, 12% had contamination level of more than 0.05 mg/L. In Hajiganj 11,954 tubewells were tested with field-kits. Of these, 93% had arsenic (>0.05 mg/L). From Hajiganj samples, 193 were selected randomly for testing using spectrophotometer. Very few villagers were found to follow the advice imparted to them during arsenic testing.

**Conclusion:** BRAC has tested a simple and low-cost procedure of tubewell water which is implementable at the community level. Through involvement of community in the test, awareness of the problem was created as a by-product. This study has demonstrated that a change in the water source ensuring safe water by introducing an effective, affordable, and simple procedure can be helpful to overcome the arsenic contamination problem.

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### Groundwater Arsenic Contamination and its Effect on Human Health in Bangladesh

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**Objective:** Explore the pattern of health effects due to arsenic contamination of groundwater and evaluate the chronic arsenicosis management regimen currently being practised in Bangladesh.

**Methodology:** This paper has been prepared on the basis of fact-finding surveys conducted purposively all over Bangladesh from 1994 to 1998 and records of patients available at the Department of Occupational and Environmental Health (DOEH) at the National Institute of Preventive and Social Medicine (NIPSOM). The surveys were carried out in villages on receiving information from the locality about the presence of groundwater contamination by arsenic and/or presence of suspected patients. Evaluation of the chronic arsenicosis management regimen, currently being practised, was carried out on 43 patients in a village that is being continuously monitored by DOEH.

**Results:** Arsenic contamination of groundwater has been identified in 52 districts. In total, 6,000 cases located in 170 villages of 72 thanas of 37 districts have been identified. The most common presentations are melanosis (93.5%), keratosis (68.3%), leukomelanosis (39.1%), and hyperkeratosis (37.6%). A few patients (0.8%) with obvious skin cancers have been detected. The clinical manifestations observed in Bangladesh have been categorized in three stages, and most patients were found in the first and second stages. The cases in initial and secondary stages have shown improvement on withdrawal of further intake of the contaminated water in conjunction with vitamin A, E, and C, vitamin-rich vegetables and fruits, and keratolytic agent (where applicable).

**Conclusion:** Toxic effect arising from arsenic contamination of groundwater is a new and emerging public health problem in Bangladesh. An estimated 50 million people are at risk of developing arsenicosis. The commonest clinical manifestations are melanosis and keratosis. Patients in initial and second stages treated with vitamin A, E, and C, vitamin-rich vegetables and fruits, and keratolytic agent (where applicable) along with withdrawal of further intake of the contaminated water have shown improvement.

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## High Levels of Lead and Cadmium in Blood of Children of Dhaka

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**Objective:** Determine the blood lead (Pb) and cadmium (Cd) levels in children as an index of the exposure to these toxic elements in the environment.

**Methodology:** In total, 49 children were included. They were living in the Tejgaon industrial area (11), Mohammadpur (14), and Keraniganj (24). A group of 9 children from other parts of Dhaka city admitted in the CRSC of ICDDR,B was used for comparison. Determination of Pb and Cd in blood was done by atomic absorption spectrophotometer (AAS) attached with graphite furnace. Blood was collected from antecubital vein after careful cleaning with swabs containing isopropanol in Venojets<sup>®</sup> tubes containing EDTA.

**Results:** The concentrations of Pb (mean±SD 176±49 µg/L) in the children from 3 study areas together were significantly higher than those from hospital (mean±SD 126±84 µg/L). Blood Pb levels in children from the Tejgaon industrial area (215.6±51.8 µg/L) were significantly higher compared to children from Mohammadpur (153.1±48.4 µg/L), Keraniganj (170.9±36.9 µg/L), and hospital. All children from 3 study areas showed high blood Pb levels (>100 µg/L) at which adverse health effects had been demonstrated. Also the blood Cd levels were higher in the children (mean+SD 1.1±0.6 µg/L) from the study areas than those from the hospital (mean+SD 0.33±.31 µg/L). Cd levels were significantly higher in children from Keraniganj than all other areas.

**Conclusion:** Both Pb and Cd levels in the blood of children from high-risk areas are alarmingly high. These could be due to high lead in the environment from gasoline, paints, ceramics, batteries, etc. High Pb in hospitalized children indicates general contamination in the Dhaka city. Young children are mostly exposed to Cd through inhalation of smokes and contaminated soils and dust from industrial emissions and sewage sludge. There appears to be differences in the extent of contamination in different high-risk areas and the factors responsible should be investigated.

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## Symptomatic Lead Poisoning in Bangladeshi Children

Naila Z. Khan, Reaz Mobarak and Mohammad Wahed

**Objective:** Study the effects of lead poisoning on psychomotor and behavioural functions in children.

**Methodology:** Children presenting with a range of development problems with no specific neurological cause for their cerebral dysfunction were screened for blood lead levels. High levels were considered to be pathognomonic of lead poisoning, and were supported by other symptoms and signs. Case reports were compiled, including social history.

**Results:** Blood lead levels were extremely high and at toxic levels in children presenting with psychomotor delay and behavioural problems, indicating lead poisoning.

**Conclusion:** Lead poisoning may cause extensive damage to the brain and nervous systems of children in Bangladesh. The effects may be more extensive for malnourished children from lower-income families living close to heavy traffic.

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## Medical Waste Disposal in Dhaka City: An Environmental Evaluation

Nasima Akter<sup>1</sup>, Noor Mohammad Kazi<sup>2</sup> and A.M.R. Chowdhury<sup>1</sup>

**Objective:** Examine the medical waste management system in Bangladesh, and identify the potential impacts that medical wastes pose to both humans and the environment. Also assess the health and environmental risk of

medical waste and provide recommendations and guidelines on methods to handle medical wastes with a minimum impact on human health and the environment.

**Methodology:** In total, 28 government, private, and diagnostic centres were included in this study. These were selected as large, medium, and small categories. Data were obtained from field observations, interviews, and laboratory analyses.

**Results:** Hospital wastes which are disposed of in common dustbins in the city are hazardous and toxic. This study showed that the hospital staff, at all levels, were not aware of the methods of safe disposal and handling of hospital wastes. Moreover, waste disposal practice was found to be quite unsafe, and both clinical and non-clinical wastes were found to be thrown together. The laboratory analyses of wastes showed the presence of infectious wastes and the risk of severe contamination of the environment. Waste collectors: cleaners, ayas and tokais are vulnerable to health hazards.

**Conclusion:** Hospital wastes pose a significant threat to human health and environment. Proper waste management strategy is needed to ensure health and environmental safety. There is an urgent need for awareness-raising programmes on *health and* environmental hazards of hospital wastes.

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### Environmental Health Education Intervention in Selected Poor Settlements

Bilqis Amin Hoque<sup>1</sup>, Robert E. Black<sup>2</sup>, Golam Morshed<sup>1</sup>, Shafiul Azam Ahmed<sup>3</sup> and Hashmat Ara<sup>1</sup>

**Objective:** Evaluate the impact of an environmental health education intervention in terms of water, sanitation, hygiene, and solid waste (WSHS) issues.

**Methodology:** This study is a part of an integrated water, sanitation, hygiene, and solid waste disposal needs assessment, education, and provision improvement project. The WSHS needs of a permanent settlement of the poor in Bauniabad and a temporary settlement of the poor in Ward 48 of Dhaka city were assessed using two cross-sectional surveys. The first survey was conducted to obtain baseline information about these settlements, and the second survey to evaluate the effectiveness of a preliminary educational programme (of six months duration) implemented in these settlements. Data on relevant knowledge and practices about WSHS were collected using interview, focus group discussion, and environmental analysis techniques. The educational intervention included courtyard meetings with female and male members of families. The results of the two surveys were compared for determining the needs before and after making people aware of the basic WSHS issues.

**Results:** Almost all people in these settlements had access to tubewell or tap water. About 80% of the people in the permanent settlement in Bauniabad used sanitary latrines compared to 30% of the people in the temporary settlement in Ward 48. Dustbins were accessible to 93% and 2% of the people in Bauniabad and Ward 48 respectively. About 50% of the Bauniabad people used dustbins. None of the pits of latrines was desludged in a sanitary way when full. All these practices remained similar after the intervention. Hands of more than 80% of the mothers were found contaminated in both areas before and after the intervention. Knowledge on WSHS issues, relevant to diarrhoea transmission and actual WSHS practices, were also poor in both the settlements. However, these parameters improved after the educational intervention. The rate of satisfactory responses about the modes of diarrhoea transmission improved from 50% to 75% and 46% to 68% in Bauniabad and Ward 48 people respectively. The lack of improvement in practices, in their opinion, was due to the lack of (i) appropriate WSHS services, (ii) appropriate technology, (iii) coordination, (iv) knowledge, and (v) community involvement.

**Conclusion:** Findings of the study indicate that the WSHS conditions and the requirements for improvement were similar in both the areas. The preliminary educational intervention helped the people and the project assess the needs following improved awareness among the people.

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## 1998 Flood: Nutrition Situation, Coping Mechanism, and Rehabilitation Plan

S.M. Moazzem Hossain<sup>1</sup> and Muhammad Shuaib

**Objective:** Assess nutrition status of children (6-59 months) in the flood-affected communities; and identify major problems encountered, coping mechanisms, and the priorities for suitable rehabilitation.

**Methodology:** A cross-sectional survey was conducted in six rural areas of Bangladesh from 26 August to 5 September 1998. In each area, 10 randomly-chosen villages were divided into clusters of 35-40 households. All the households with children (aged less than 5 years) of a randomly-chosen cluster were interviewed, and all children were measured for nutrition status. In total, 1988 households were visited, 1,313 respondents interviewed, and 1,595 children measured. Thirty-six focus group discussions (21 with male and 15 with female) were conducted to know the coping mechanisms and priority for rehabilitation plan.

**Results:** 16.5 percent of the children were moderately malnourished (-2 to -2.99 Z-scores of wt-for-ht) and 2.6% were severely malnourished (below -3 Z-scores of wt-for-ht). Major problems encountered by the affected populations include: unemployment leading to food scarcity (inability to buy), unhealthy environment leading to diseases, communication disruptions, accommodation problems, disrupted schooling of children, loss of crops, etc. Leg sores and children's sickness (diarrhoea, ARI, conjunctivitis, etc.) were the most important health-related issues reported. All apprehended a major outbreak of diseases immediately after recession of water. To cope with the shortage of food during flood, most people reduced the frequency of meals and amount of food. Many started fishing (for sales or consumption), operating boats, selling daily labour, fetching and selling fodder for cattle, etc. Sales of assets at an unusually low price as well as attempts to find jobs in the nearest urban areas were common. Affected people who had lost their jobs due to flood did not wish to have 'relief'—rather they preferred long-term interest-free loans and were ready to work and return the money.

**Conclusion:** Nutrition situation did not warrant immediate nutrition intervention, but other findings showed potential signs of deterioration. Consultations with community members may help design the most appropriate relief and rehabilitation interventions.

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## Rapid Assessment of Health Status of Children and Women in a Flood-affected Area in Bangladesh

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**Objective:** Determine the health status of children (aged less than 6 years) and women during the 1998 flood in a rural community in Bangladesh.

**Methodology:** A cross-sectional study was carried in Sonargaon thana of Narayanganj district to determine the health status of children (aged less than 6 years) and women during the 1998 flood. Ten flood shelters, one from each union, were randomly selected as clusters. In total, 284 women and 269 children were included in the study. Data were collected between 20 September and 27 September 1998 by using an interviewer-administered questionnaire.

**Results:** The mean ages of the study children and mothers were 32 months (3 days to 6 years) and 25 years (15-48 years) respectively. At the time of interview, the average duration of stay in the flood shelters was 50 days (3 to 90 days). Most respondents (98.2%) used tubewell as their source of drinking water. Only 39.4% of the population had access to sanitary toilets, and the rest used open places or flood-water. Among the population investigated, 127 (44.7%) women and 252 (93.7%) children suffered from various types of illness. The common illnesses encountered by the women during the study period were: fever 24 (18.9%), cough and cold 23 (18.1%), respiratory tract infection 5 (3.9%), diarrhoea 10 (7.9%), skin infection in foot 9 (7.1%), angular stomatitis 3 (2.3%), other skin infections 3 (2.3%), and others 79 (62.20%). The common illnesses of the children were: cough and cold 97 (38.5%), fever 94 (37.3%), diarrhoea 70 (27.7%), respiratory infections 14 (5.5%), angular stomatitis 14 (5.5%), skin infection 11

(4.4%), skin infection in foot 7 (2.8%), and other diseases 36 (14.3%). Although no death was reported among women during the study period, 7 children died during the same period. Of them, 6 deaths were due to drowning.

**Conclusion:** The results indicate that the children were more affected during the flood. The prevalence of morbidity and mortality were high among them. Although the source of drinking water was reported to be tubewell, the sanitary facility was very poor in the flood shelters.

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### Assessment of Environmental Degradation and Health Hazards Due to Sericulture Programme Activities in Bangladesh

Nasima Akter, Arnina Rahman and Mohammed Jakariya

**Objective:** Assess the potential environmental problems of the sericulture programme activities, including environmental and health concerns; identify remedial measures to reduce the adverse impact on the environment and public health; and establish monitoring criteria to maintain the environmental quality and sustainability of these programmes.

**Methodology:** The study was done under the guidance of Ayesha Abed Foundation. Data were collected through interviews, discussion with programme personnel, field observations, and laboratory analyses of water and soil samples. Variables included (separately for each programme): process, activities, energy, chemical and material uses, waste generation and disposal, and observed health and environmental problems.

**Results:** The major findings of the study were categorized as "health effects" and "environmental assessment" of the programme. Employees working in different sericulture programmes were found to suffer from various health problems. These can be caused by direct or indirect exposure to environmental pollution and contamination. The most important concern for environmental and public health issues is the disposal of liquid and solid wastes. Most chemicals found from laboratory analyses of waste water and sediment are persistent in nature and have long residual effects. The results of the analyzed chemical samples showed a higher value than the maximum allowable DoE (Department of Environment) standards for those chemicals.

**Conclusion:** The results indicate that the health and environmental problems, resulting from the sericulture programmes, are acute. Based on the findings, recommendations were made to lessen environmental degradation and health hazards, and make the programmes more environmentally sustainable.

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### Hearing Loss among Autorickshaw Drivers of Dhaka City

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**Objective:** Determine the prevalence of hearing loss among autorickshaw drivers of Dhaka city.

**Methodology:** A cross-sectional study on 113 autorickshaw drivers was carried out during April-May 1998 in the Department of ENT, Bangladesh Medical College, Dhaka. The drivers who appeared at the gate of the College between 8:00 a.m. and 10:00 a.m. were primarily chosen for the study. After screening in the Department of ENT, they were finally selected. Drivers having ear pathology or receiving streptomycin or quinine or crossing 55 years of age were excluded. Hearing function was measured by Pure Tone Audiometry. Data analysis was done by SPSS PC+ software package.

**Results:** Of the subjects studied, 34 (30.1%) had hearing loss and 37 (32.7%) had V-notch, while 62 (54.9%) had normal audiogram findings. Thirteen (11.5%) subjects had only V-notch without hearing loss. Mean durations of job among drivers with normal hearing and with hearing loss were 7.25 years and 16.59 years respectively. This difference was significant ( $p < 0.001$ ). Significant correlation ( $p < 0.005$ – $p < 0.05$ ) was observed between duration of job and hearing threshold at different frequencies of sound.

**Conclusion:** The nuisance value of autorickshaw is well-known to all city-dwellers of Bangladesh. It causes both noise pollution and air pollution. This study concludes that hearing loss is very common among autorickshaw drivers, and that the risk of developing hearing loss increases with increase in duration of job. In Bangladesh, no previous study was done in this field. Further studies are required to assess the ill effects of autorickshaw on general population in regard to noise pollution.

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### Survival of *Vibrio cholerae* O139 with *Anabaena* sp. and Detection of Toxigenic *Vibrio cholerae* from Samples of Aquatic Environment

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R.B. Sack<sup>3</sup>, A. Huq<sup>4</sup> and R.R. Colwell<sup>4</sup>

**Objective:** Investigate association of *Vibrio cholerae* O139 with a cyanobacterium *Anabaena* sp. in microcosms and detect toxigenic *V. cholerae* from various components of pond ecosystems in a rural cholera-endemic area of Bangladesh.

**Methodology:** Microcosms were prepared with 200 mL of autoclaved pond water, 2 g of *Anabaena* sp., and 10<sup>5</sup>/mL *V. cholerae* O139 in a 500-mL conical flask (Pyrex). Another flask with only 10<sup>5</sup>/mL *V. cholerae* O139 in 200 mL of autoclaved pond water was used as a control. Sampling of *Anabaena* sp., water on which *Anabaena* sp. was floating, and control water without *Anabaena* sp. were done at various time intervals. Culturable *V. cholerae* O139 were counted using TTGA media. Viable but non-culturable (VBNC) *V. cholerae* O139 were detected using fluorescent antibody and PCR techniques. Environmental samples, including water, plant, phytoplankton, zooplankton, snail, oyster, and sediment were collected every 15 days from 4 ponds in Matlab, Bangladesh. VBNC *V. cholerae* were detected from these samples using PCR and dot blot hybridization following standard procedures.

**Results:** The results showed that *V. cholerae* O139 survived in alga, control water, and water on which *Anabaena* sp. was floating as culturable form up to 23, 20, and 10 days respectively. *V. cholerae* O139 survived better in association with *Anabaena* sp. When *V. cholerae* O139 could no longer be cultured, the cells were assumed to enter the non-culturable state. VBNC cells were identified using fluorescent antibody technique and were found to persist till completion of the study (60 days) only in association with *Anabaena* sp. This result was supported by PCR, followed by dot blot hybridization. In total, 449 samples of aquatic environment were collected from 4 pond ecosystems of Matlab, and toxigenic *V. cholerae* were detected by PCR using *ctxA* as the target gene. Only 5 samples were found positive by PCR. However, the sensitivity of detection was intensified when PCR products were subjected to dot blot hybridization. The dot blot hybridization increased the positivity of PCR products from 5 to 41. Four of the *ctxA*-positive samples were found to contain *V. cholerae* O139 when amplified with *V. cholerae* O139-specific primers.

**Conclusion:** This study demonstrated that *V. cholerae* O139 can be associated with *Anabaena* sp. and can persist in VBNC form for a long time (60 days). The results of this study are consistent with those carried out with *V. cholerae* O1. Therefore, it may be concluded that both epidemic serotypes of *V. cholerae* O1 and O139 behave similarly in respect to their association with the cyanobacteria *Anabaena* sp. in microcosms. This study also showed the presence of toxigenic VBNC *V. cholerae* in various components of the aquatic environment in an endemic area of Bangladesh.

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### Biological Contamination of Tubewell Water

Bilqis Amin Hoque

**Objective:** Indicate the type and extent of common biological contamination of tubewell water and relate this to the conditions at the well-head.

**Methodology:** A cross-sectional survey was conducted to collect 2,000 tubewell water samples from 274 thanas of Bangladesh. The samples were tested in the Environmental Health Laboratory of ICDDR<sup>B</sup> for faecal coliform, ammonia-nitrogen, and pH. Sanitary conditions at the well-head as well as depth of wells were observed. Distances between any latrine, dirty ditch, domestic ditch, pond, river or canal visible from the tubewell sites were estimated.

**Results:** The geometric mean and median values of faecal coliform bacteria were as low as 3 cfu/100 mL and 1 cfu/100 mL respectively, but only about 46% of the water samples met the WHO standard (nil faecal coliform per 100 mL sample) for drinking water. About 65% of the samples had less than 1.5 mg/L ammonia-nitrogen concentration. Most tubewells had platforms as about 82% of the studied wells were installed by the Department of Public Health Engineering. The presence of a dirty ditch within 15 metres of the tubewell was positively and more or less significantly associated ( $p=0.09$ ) with the faecal coliform after controlling for other factors (using multivariate analysis). Presence of platform and depth of tubewell showed a negative association with the faecal coliform pollution. This was, however, not statistically significant.

**Conclusion:** The level of biological contamination of tubewells in terms of presence of faecal coliform is alarming. This was only an indicative study, and therefore, appropriate studies are required to determine the extent, risk factors, and nature of the problem.

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#### Probable Role of Mucinase in the Survival of *Vibrio cholerae* O1 in Association with a Cyanobacterium *Anabaena* sp.

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**Objective:** Investigate the role of mucinase in the survival of *Vibrio cholerae* O1 in association with a cyanobacterium *Anabaena* sp.

**Methodology:** A clinical isolate of *V. cholerae* O1, El Tor, Ogawa (3083-T), its haemagglutinin/protease (mucinase)-negative mutant (HAP-1-T) were used in microcosm studies for survival in association with plankton *Anabaena* sp. and in chemotaxis studies with porcine mucin. Simple drop-plate technique was followed to count culturable cells of *V. cholerae* O1 on taurocholate-tellurite-gelatin agar (TTGA) plate. Fluorescent antibody (FA) technique was used for counting viable but non-culturable cells of *V. cholerae* O1. Polymerase chain reaction (PCR) and southern hybridization techniques were used for detecting *V. cholerae* O1 with *Anabaena* sp. up to several months in microcosms. Capillary tube technique was used for determining the role of chemotaxis in the attachment of *V. cholerae* O1 with *Anabaena* sp.

**Results:** Wild type *V. cholerae* O1 (3083-T) survived up to 24 days in culturable form in association with *Anabaena* sp., but the mutant survived only for 10 days in microcosms. Both culture and FA results showed multiplication of wild-type strain of *V. cholerae* O1 in association with *Anabaena* sp. However, multiplication of *V. cholerae* in control water without *Anabaena* sp. was not observed. During chemotaxis study, 4% homogenates of *Anabaena* sp. showed 5.68% bacterial accumulation, but 2.98% accumulation occurred for the mutant strain at 90 minutes. Again, wild-type *V. cholerae* O1 showed 4.36% accumulation toward 2% solution of mucin, but mutant showed 1% accumulation at 90 minutes.

**Conclusion:** The significant survival of *V. cholerae* O1 in association with *Anabaena* sp. and attraction of *V. cholerae* O1 toward mucin may be the results of the activity of mucinase. These results indicate that the enzyme HA/protease (mucinase) may play an important role in association and survival of *V. cholerae* O1 with a mucilaginous cyanobacterium *Anabaena* sp.

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### Preliminary Survey of the Abundance of *Aeromonas* Phages in Ponds and Sewage Samples of Dhaka, Bangladesh

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A. Huq<sup>3</sup> and R.R. Colwell<sup>3</sup>

**Objective:** Study the abundance of *Aeromonas* phages in pond ecosystem and sewage samples of Dhaka, Bangladesh.

**Methodology:** Two water and sediment samples of IPH-pond and five water and sediment samples of sewage-contaminated pond of Mohakhali, Dhaka, were processed to isolate phages specific for *A. hydrophila*. Lytic pattern of various *Aeromonas*-phages with *Aeromonas* strains was tested by spot method.

**Results:** In pond ecosystem, concentration of *Aeromonas* phages ranged from  $6 \times 10^1$  to  $2 \times 10^4$  PFU/mL or g of sample. Concentrations of *Aeromonas* phages in sewage sample ranged from  $4 \times 10^1$  to  $2 \times 10^3$  /mL or g. Concentrations of *Aeromonas* phages were more in water than in the sediment samples in pond ecosystem, whereas in sewage sample, phages were concentrated more in sediment than in the water samples. Phages were lytic in nature and specific for *A. hydrophila*.

**Conclusion:** *A. hydrophila* phages are abundant in sewage samples and pond ecosystem of Dhaka. Further work is needed to isolate specific phage of *Aeromonas* suitable for use in diagnostic purpose.

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### Blue-green Algal Bloom in Pond Ecosystems and Seasonality of Cholera Cases in an Endemic Area of Bangladesh

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**Objective:** Investigate seasonality of blue-green algae in pond ecosystems and the seasonality of cholera in a rural endemic area of Bangladesh.

**Methodology:** The study was carried out in 4 pond ecosystems at Matlab in Bangladesh. One litre of water containing algae was collected at an interval of 15 days in a plastic bottle with Lugol's iodine solution for one year from July 1994 to June 1995. Five hundred mL of water sample was also collected for determining the various physico-chemical parameters, including pH and  $\text{NO}_3\text{-N}$  of water. The algae were enumerated using Sedgewick-Rafter counting chamber. Identification of the algal species and determination of physico-chemical parameters of water samples were carried out following standard procedures. Information on the incidence of cholera was collected from the records of ICDDR,B hospital at Matlab.

**Results:** The blue-green algae followed a strict seasonal pattern in most ponds studied. The incidence of cholera during the study period also showed a seasonal pattern. The period of algal bloom and the occurrence of high incidence of cholera took place almost at the same time. The increase in the number of algae also strongly correlated with pH and  $\text{NO}_3\text{-N}$  of water.

**Conclusion:** This study showed that the increase in the number of blue-green algae in the aquatic environment of rural Bangladesh takes place at the same time when the cholera cases increase. This study, like the previous studies, indicated a link between algal bloom and peak cholera season thus reinforcing the notion of blue-green algae as a possible reservoir of *V. cholerae*.

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### Detection of Shigellae from Rectal Swabs and Handwashing Samples Collected from the Members of Index Families Infected with Shigellosis

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M.M. Rahman<sup>1</sup>, A.H. Baqui<sup>1</sup>, M.A. Rahman<sup>1</sup>, M. John Albert<sup>1</sup> and R.B. Sack<sup>2</sup>

**Objective:** Detect Shigellae by culture technique from rectal swabs and handwashing samples collected from the members of index families infected with shigellosis.

**Methodology:** Rectal swabs and handwashing samples were collected in buffered glycerol saline (BGS) solution from the family members of index case on day 1, 3, 5, 7, and 9. Samples were inoculated onto MacConkey (MAC), Salmonella-Shigella (S-S), Xylose Lysine Deoxycholate (XLD) and Hektoe Enteric Agar (HEA) media. The plates were incubated at 37°C for 18-24 hours. Non-lactose-fermenting, tiny convex flat colonies were selected for further characterization following standard procedures. The species of Shigellae were identified by slide agglutination technique using specific antisera.

**Results:** In total, 925 rectal swabs were collected, of which 7.7%, 5.6%, 4.0%, 5.5% and 5.6% yielded Shigellae on day 1, 3, 5, 7, and 9 respectively by culture technique. In the case of handwashing samples, 0.6% and 2.5% yielded Shigellae on day 1 and 3 respectively but no Shigellae could be isolated from the handwashing samples on day 5, 7, and 9. The results showed that although some family members of index cases were harbouring Shigellae up to the 9th day of investigation, their hands became free from Shigellae from the 5th day of investigation.

**Conclusion:** This study emphasizes the need of handwashing of the family members, especially during the first few days after onset of shigellosis in a family.

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## Disease Control and Child Health

### Trends in and Determinants of Infant Mortality by Age at Death and Causes of Death in Matlab, Bangladesh during 1983-1996

Golam Mostafa and Jeroen K. van Ginneken

**Objective:** Study the trends in and causes of infant mortality in different age groups and identify the demographic and socioeconomic factors influencing mortality.

**Methodology:** Longitudinal data of the DSS from 1983 to 1996 were used for determining the trends in and causes of infant mortality. All births that occurred during 1983-1987 were followed, and deaths that took place in the first year of life were recorded. Mortality rates of infants in the age groups of 0-3 days, 4-14 days, 15-29 days, 1-2 months, 3-5 months, and 6-11 months were analyzed to determine the trends in and causes of death. Logistic regression was used for estimating the effects of age, parity, maternal education, religion, and area. Analysis was done separately for different segments of infancy.

**Results:** Overall infant mortality declined in both treatment and comparison areas. Mortality in all the age groups below one year has declined except for 0-3 days. The decline was more marked in the treatment area where carefully designed and intensive MCH-FP services are delivered through a special project. Neonatal tetanus mortality declined dramatically in the 1980s. Neonatal mortality continued to be high due to other causes even in the treatment area. Diarrhoea, acute respiratory infections, and malnutrition were major causes of death after the first month of life. The influence of maternal age in infancy was limited to 0-14 days after birth. Zero parity is a risk factor at all ages below one year. High (5+) parity is an important risk factor in the postneonatal period. Muslims had lower mortality than non-Muslims, especially in the age group where tetanus was a major source of infection. Infants of educated mothers experienced lower mortality than uneducated ones (also after controlling for other variables).

**Conclusion:** Neonatal tetanus mortality declined dramatically. Neonatal mortality continued to be high due to other causes. Diarrhoea, acute respiratory infections, and malnutrition were the major causes of death after the first month of life.

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## Importance of Community-based Programme and Village Doctors in Reducing ALRI Deaths in Rural Areas of Bangladesh

Mohammad Ali and Andres de Francisco

**Objective:** Assess the importance of community-based health service programme and other socioenvironmental in reducing ALRI deaths in rural areas of Bangladesh.

**Methodology:** The study was conducted in the Matlab Health and Demographic Surveillance area with a population of about 210,000. A community-based health service project has been operating in one half of the area (MCH-FP area) since 1978. Vital demographic events are being recorded from the entire study area. Data on children (less than 2 years of age) and their ALRI-specific mortality from 1987 to 1993 were investigated at the *bari* (cluster of households) level accordingly. Child-year at risk was considered as population exposed to the death risk. The *baris* with less than one child-year at risk were excluded. GIS was used for creating socio-environmental variables and mapping the ALRI mortality. Because of the skewed distribution of ALRI mortality, Poisson regression model was used as the analytical tool. In total, 6,116 *baris* were studied.

**Results:** The results confirmed a lower ALRI mortality (44% lower) in the intervention area. The bivariate analysis revealed that ALRI mortality was associated with lower educational status, lower doctor-population ratio, and higher distance from *baris* to the nearest treatment centre. Multivariate analysis rejected the association of the distance factor. Village doctors have shown an expected effect; within intervention area, the villages with higher doctor-population ratio ( $p=0.001$ ) have 83% lower mortality than that in the other villages. However, the higher ratio of doctor-population has no impact on the mortality in the non-intervention area. It was surprising that the southern part of intervention area where the Matlab hospital is located has 1.38 times higher mortality compared to its other part.

**Conclusion:** Village doctors can effectively reduce ALRI mortality in rural areas if they are backed by health interventions. The higher mortality rate in the southern part requires investigations of the service-delivery system in the area.

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## Outcome of Severe Pneumonia in Severely Malnourished and Well-nourished Children Aged Less Than 5 Years

N. Nahar, H.A. Begum, M.A.H. Mollah, N.C. Saha and U. Mitra

**Objective:** Compare the treatment outcomes of severe pneumonia in severely malnourished and well-nourished children aged less than 5 years.

**Methodology:** Two groups of children aged 2 months to 5 years with severe pneumonia were enrolled; 120 well-nourished and having half in each treatment group received either chloramphenicol or penicillin. Similarly, 120 severely malnourished children received either chloramphenicol or penicillin plus gentamicin. The treatment outcome was measured clinically and compared radiologically.

**Results:** In total, 76% (n=183) cases improved. The case fatality rate (CFR) was 7% (n=16), and all of them were malnourished. Compared to penicillin, better clinical response was noted with chloramphenicol in the well-nourished group ( $p=0.001$ ). The children in the malnourished group responded equally to either chloramphenicol or penicillin plus gentamicin ( $p=0.838$ ). No side-effects of chloramphenicol were noted.

**Conclusion:** Chloramphenicol is a better alternative for treatment of severe pneumonia in children irrespective of their nutritional status ( $p=0.049$ ). Chloramphenicol can be used in treating severe pneumonia in children.

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## Cyclical Pattern of Cholera in Bangladesh

Md. Yunus<sup>1</sup>, R.B. Sack<sup>2</sup> and K. Zaman<sup>1</sup>

**Objective:** Describe the cyclical phenomena in the incidence of cholera in a demographically defined population in a rural area of Bangladesh.

**Methodology:** The study was conducted in rural Matlab where ICDDR,B has been operating a diarrhoea hospital, and maintaining a longitudinal Demographic Surveillance System (DSS) covering a current population of about 212,000 in the area since 1966. There has been continued cholera surveillance in this population. All diarrhoeal patients from the DSS area, attending the Matlab hospital, had their stool cultured for *Vibrio cholerae* using standard procedures.

**Results:** Cholera patients were seen throughout the year, but there were also two epidemics of cholera a year, occurring regularly every spring and fall each lasting for 2-3 months. Because of the long duration of this surveillance, it has been possible to observe certain cyclical phenomena in the cholera incidence in this population, not previously recognized with shorter observation periods. It appeared that there had been four major cycles of cholera during this 33-year time period in Matlab, each lasting for 7-9 years and involving both biotypes (Classical and El Tor) of serogroup O1 and serogroup O139. Each cycle seemed to be characterized by a decrease in or disappearance of one biotype or serogroup and the appearance or re-appearance of a different one. Cholera incidences have ranged from a low of 0.2 per 1,000 to a high of 6.5 per 1,000 people per year. The fifth cycle may just be starting.

**Conclusion:** The cyclical phenomena may help predict years in which large number of cholera cases are anticipated; may facilitate the establishment of more timely and adequate treatment facilities, and the testing of cholera vaccines and their widespread use as public health tools later.

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## Measuring Children's Acute Morbidity in Matlab, Bangladesh: A Comparison of Perceived versus Checklist Data

Nurul Alam

**Objective:** Ascertain validity of the estimates of children's acute morbidity obtained by interview using the mothers' perceived illness questions and the checklist question.

**Methodology:** The Matlab Health and Socioeconomic Survey (MHSS) 1996 recorded acute morbidity of 3,795 children aged 1-179 months. Female interviewers asked mothers at their homes: (a) whether the child was sick (e.g. fever, cough, cold, etc.) during the last one month (mothers' perceived illness of their children) and (b) whether the child had any of the symptoms of: headache, eye infection, cough-cold-fever, diarrhoea, etc. in the last month (the checklist). Comparison of the prevalence of morbidity obtained by the above questions for the same recall period gave some estimates of the extent of inaccuracy in children's acute morbidity. Factors relating to inaccuracy included age and gender of child, mothers' education, and type of symptoms.

**Results:** Data on the mothers' perceived illness of their children yielded morbidity prevalence of 48.2%, while the checklist data yielded morbidity prevalence of 52.4% among children. The difference in the prevalence of 4.2% points could be regarded as the extent of under-reporting on self-perception. Under-reporting was 2.5% if symptoms were of cough-cold-fever, and more than 22% if symptoms were of diarrhoea, headache, stomachache, etc. Under-reporting was slightly higher for younger (<5 years) than for older children but did not vary by gender of the child and mothers' education and religion.

**Conclusion:** In the interview, morbidity symptoms whose severity could easily be understood by mothers were reported better than other symptoms. The question on mothers' perceived morbidity of their children underestimates disease burden.

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## Health Services

### Inter-bari Differences in the Use of Modern Contraception in Rural Bangladesh

Nashid Kamal

**Objective:** Investigate the causes of inter-bari variation in the use of modern contraception by evaluating both current demand and supply sides of the issue.

**Methodology:** Two known clusters of *baris* with high and low use of contraception were studied in rural areas of Dhaka and Chittagong divisions during May-August 1997. Clusters are primary sampling units (PSU) as of the 1981 Census, roughly corresponding to a village in rural Bangladesh. *Bari* is a unit of dwelling in rural Bangladesh, usually bonded by patrilineal relationship usually consisting of more than one hut in the same compound. The two clusters were purposively chosen for this study. From the low-prevalence area, 44 current users and 58 non-users were interviewed in detail. From the high-prevalence area, 66 current users and 58 non-users were interviewed in detail. The government family planning (FP) workers in both the clusters were interviewed. Detailed history of their experiences over the past 20 years was documented.

**Results:** Although in the initial days of introduction of contraception, religious sentiment of the bari head was an important deterrent to being a user, it no longer seemed to be the case in the low-prevalence cluster. The main problem for non-users was the poor performance of the FP worker. Within-bari discussion was almost non-existent, and most (48.6%) women learned about FP methods from the female FP worker. However, she seemed to restrict her visit to only known users and did not make substantial attempts to recruit new users. Some places in her catchment area were also inaccessible due to bad communication, and those baris were totally avoided by the FP worker which may explain the inter-bari differences in the use of modern methods of contraception. In the high-prevalence area, the baris are well-covered by the government and non-government FP workers. The sole reason for inter-bari differences seemed to be the absence of the husband due to high rate of migration for work outside that cluster.

**Conclusion:** The reasons for inter-bari differences in the use of modern contraceptives are different for high-use and low-use areas. In low-prevalence areas, higher motivational effort and better coverage by family planning workers should be ascertained by careful supervision.

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### Improving Early Identification of ARI at Community Level through Checklists for Field Workers

S.M. Tariq Azim<sup>1</sup>, Salahuddin Ahmed<sup>2</sup>, Tajul Islam<sup>1</sup>, Mahbubul Alam<sup>1</sup> and Humayun Kabir<sup>1</sup>

**Objective:** Assess whether the sensitivity and specificity of identifying a case of ARI by health workers could be improved by introduction of a checklist.

**Methodology:** Twenty health assistants (HAs) were randomly selected from two thanas. Initially, without using any checklist, the HAs examined 228 children aged less than 5 years coming to the Thana Health Complex (THC). Later, they examined 374 children at the THC using the ARI checklist. All the children, irrespective of HAs' diagnoses, were sent to the medical officers for assessment and were treated accordingly.

**Results:** The sensitivity of identifying a case of ARI (i.e. identify a child having cough or difficult breathing) by the HAs was 58.1% before the introduction and 64.2% after the introduction of the checklists ( $p=0.3$ ). Similarly, the specificity was 71% and 69% respectively, showing no significant difference. The ability of the HAs to correctly classify ARI cases as 'no pneumonia', 'pneumonia' or 'severe pneumonia/disease' changed from 31.4% to 37.4% ( $p=0.3$ ).

Strikingly, in 56 cases (8.5%) diagnosed as ARI, the physicians did not count the respiratory rate or record any signs of severe pneumonia to support their diagnoses. Similarly, in 114 cases (88.3%) diagnosed by the HA as ARI, the respiration rate was not counted.

**Conclusion:** The study shows that ability of the health workers to identify and classify ARI cases is low, and the introduction of a checklist aid, not complemented by training and supervision, did not help improve their skill.

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### A Qualitative Assessment of the Health and Family Planning Information, Education, and Communication Needs among the Residents of Dhaka City

Mahbub A. Mazumder, Monowar Jahan and Subrata Routh

**Objective:** Assess the existing systems of information, education, and communication (IEC) of health and family planning services among the residents of Dhaka city; identify their needs and options for better communication and community mobilization mechanisms; and recommend effective strategies of communication and community mobilization.

**Methodology:** This qualitative study was conducted in September-October 1996 in Ward no. 58 and 80 of Dhaka City Corporation where the operations research intervention on "Alternative Strategies for the Delivery of MCH-FP Services" was field-tested. Eight focus group discussions with clients and 12 formal group discussions with community representatives (school teachers, community leaders, youth club members, general practitioners, pharmacy attendants, etc., with an equal split for both the areas) were held to collect the required information.

**Results:** Field workers and radio were the major sources of information, education, and communication on health and family planning. No established community source was identified. Interpersonal communication and miking were found to be the most wanted channels of message transmission. Leaflets, posters, and billboards were also mentioned as effective means of information dissemination. They also stated that local clubs, associations, and youths of the community (if involved) could effectively and quickly disseminate health and family planning information to them. The respondents expressed mixed feelings about the prospects of formal committees in this regard. Most respondents were in favour of getting services from static clinics, if the behaviour of the staff is good. Various health and family planning services were offered by both male and female providers, with supply of required medicines at cheaper prices.

**Conclusion:** A combination of interpersonal communication and localized media (miking, banners, posters, billboards) can be used for providing IEC to the community. Local clubs and youths of the community could be involved for the information, education and communication activities. Services from static clinic could be a feasible alternative to current doorstep services with appropriate provisions for IEC.

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### Health Problems and Healthcare-seeking Behaviour in Urban Slums of Bangladesh

Muhammod Abdus Sabur and Khodadad Hossain Sarker

**Objective:** Explore the common health problems and healthcare-seeking behaviour in urban slums of Bangladesh.

**Methodology:** Focus group discussions and in-depth interviews were conducted with adult males and females separately by using checklist and semi-structured questionnaire. Recall period was for the last one month. Data were collected during April-May 1998 from two slums of Chittagong city.

**Results:** Most common health problems in all age groups and sexes were found to be: gastric pain, dysentery, and skin diseases. Diarrhoea, pain, general weakness, and jaundice were also identified as commonly prevailing problems. Menstrual problems and anaemia among females and fever, cough, pneumonia, and measles among children were also identified. Non-qualified allopathic drug sellers of the drug shops situated within and nearby the slums were found to be the main source of healthcare for slum dwellers. Even in presence of qualified practitioners, their

services are not availed due to their inability to pay consultation fees. Faith healers were also found to be consulted for specific problems as these were perceived to be caused by evil spirit.

**Conclusion:** Almost all health problems of the slum dwellers originated from the prevailing unhygienic environment of the slums and working/food habit of the slum population. Ensuring better environment in the slums and good working/food habit may prevent most diseases. Non-qualified allopathic drug sellers were found to be the main source of initial treatment for slum dwellers even when qualified practitioners were available. Drug sellers' service is associated with incorrect treatment and harmful practice. Programme aiming at improving their capacity might ensure quality of care for the slum population.

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### Delivering Family Planning Services without Home Visits: Preliminary Findings from Operations Research Activities in Dhaka City

Cristóbal Tuñón, Subrata Routh, Barkat-e-Khuda, K.A. Mazumder and Nirod Chandra Saha

**Objective:** Assess the effect of discontinuing home visits by urban field workers on selected family planning indicators.

**Methodology:** Data from the surveillance systems of ORP and the former MCH-FP Extension Project (Urban) in the Lalbagh area of Dhaka city were used for studying the rate of contracts between married women of reproductive age and providers of family planning services in this areas; contraceptive prevalence rate (CPR); family planning methods used, and the source of supplies from June 1996 to June 1998.

**Results:** Between January and March 1996, forty percent of the women of reproductive age had been visited by a family planning field worker in the previous three months. This figure dropped to 4% during the July-September 1997 after the regular home visits to distribute oral contraceptives and condoms in the area were discontinued. Nevertheless, CPR increased from 53.8% to 55.4% over the period. Although CPR remained higher in non-slum areas, the gap narrowed. The proportion of couples using pills or condoms did not vary. The total number of couples who obtained family planning methods from clinics increased slightly. At the end of the period, there was a greater dependence on pharmacies as a source of supplies.

**Conclusion:** The findings confirm previous observations about the feasibility of discontinuing the home delivery of condoms and oral contraceptives without reducing CPR, and the importance of pharmacies in urban areas such as Lalbagh. There is a need to study continuation rates among the increasing number of couples obtaining supplies from pharmacies. Since field workers also referred clients to clinics for essential maternal and child-survival services, more research is needed on the effect of reducing home services on the use of clinics for the other reproductive health and child-survival services.

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### Review of Interventions to Promote Local-level Planning and Coordination of Essential Health and Family Planning Services

Jasim Uddin, Jahanara Khatun, Mafizur Rahman, Cristóbal Tuñón and Siraj Uddin

**Objective:** Examine the existing mechanisms of local-level planning and coordination of health and family planning services and experiences of government and NGO agencies in urban and rural settings.

**Methodology:** Data on the existing local-level planning process, both in rural and urban settings, were collected through discussions, observations on field activities, analyses of organized meetings, and review of literature.

**Results:** The government health and family planning programme has a limited comprehensive local-level planning process. The national-level managers assign targets among the field workers. Formally, the Government has

established committees at the district, thana and lower levels which are supposed to meet every month for planning and reviewing local performance. The available evidence is that most of these committees are largely inactive. Local-level planning interventions undertaken by the former MCH-FP Extension Projects of ICDDR,B in rural and urban areas have revealed that systematic processes of performance review helped managers and supervisors diagnose problems and find solutions in rural areas. However, the success of the interventions was largely based on the commitment and skills of thana managers. Interventions in urban areas were effective in establishing a forum for service providers to discuss and resolve common health issues and problems. Both in urban and rural settings, the role of the external facilitator was critical. Interventions undertaken by different organizations in NGO and government settings, with special projects to support government officials, have shown promising results in small areas. The effects of these interventions are still being monitored, and the replicability of these interventions is yet to be established.

**Conclusion:** Interventions to promote local-level planning and coordination in Bangladesh need to identify mechanisms to motivate and train local managers and supervisors and seem to require external facilitation. The interventions must recognize the transition to the full range of ESP services and on the shift in emphasis from doorstep to static clinic. Realistic ways of obtaining a meaningful involvement of community representatives could enhance the availability of local resources and improve use pattern of those resources.

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## Reproductive Health

### Barriers to Emergency Obstetric Care in a Rural Setting of Bangladesh

Rukhsana Gazi<sup>1</sup>, Liz Goodburn<sup>2</sup>, A.M.R. Chowdhury<sup>3</sup> and F. Karim<sup>3</sup>

**Objective:** Explore the situation during delivery at the rural home level and identify the barriers to emergency obstetric care.

**Methodology:** This investigation formed a part of the collaborative study undertaken by BRAC and London School of Hygiene & Tropical Medicine on maternal morbidity in Manikganj district of Bangladesh (1992-1993). It included 26 pregnant women who were followed up at home twice a week for the past six months of their pregnancy. Experienced female interviewers collected data through informal discussion and participatory observation. No notes were taken during the conversations, but a detailed diary of each visit was maintained immediately after they returned to the research base.

**Results:** The study found that women themselves were not often aware of self-care. A fear of sin that was attributed to the presence of unknown male doctors in the hospital acted as a barrier. Women's relationship with her in-laws was very important. An early assessment and indication for referral by TBAs was another important component in the decision-making process. Although in a few cases the TBAs instructed to shift the complicated cases relatively early, there are many examples where the TBAs were trying to manage by themselves. The hospital is an unfamiliar place for the rural people. A common perception was that the hospital people only pay attention to the rich and educated people. When all efforts made by a TBA, a Fakir or a village doctor failed, only then the family members thought about hospitalization. In-laws and neighbours jointly undertook the decision. Physical distance of the facility and transport problems were taken into consideration by the family prior to the final decision to transfer the patient to hospitals.

**Conclusion:** All members of the community need to be educated about the danger signs of obstetric emergencies. Poster, charts, audiovisual aids, and folk drama might be used as effective tools for the campaign. TBA training interventions should give more emphasis on early recognition of complications and early indications for referral rather than attempting management at home.

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## Role of Male Clinics to Promote Reproductive Health Issues: The Matlab Experience

J. Chakraborty, N. Alam, P. Saha, F. Ahmed and Andres de Francisco

**Objective:** Explore the relative importance of reaching males with the aim at involving them in reproductive health programmes; evaluate the need for managing males' complaints of the reproductive tract; and plan for interventions to improve reproductive health programmes.

**Methodology:** Given the shortcomings of the family planning programmes, interventions to involve men in the reproductive health programmes have been initiated in various projects. In Matlab, the programme established four male clinics in 1995 at the health centre level to provide services to male clients attending the clinics. The clinics were operated one day a week between 10 a.m. and 5 p.m. Services provided included diagnosis of and treatment for sexually transmitted infections and perceived sexual illness. Management was ensured using the WHO-recommended syndromic treatment guidelines, and by providing counseling on HIV/AIDS. Services also included promotion of male family planning methods and condom demonstration. Paramedics posted at the health centres maintained records on patients' complaints, age, diagnosis, treatment, and other services provided.

**Results:** Between July 1997 and June 1998, 258 patients attended the male clinics. Of these, 32% were unmarried. In total, 42% of the patients were aged less than 30 years; and 8% were aged less than 20 years. Most patients (51%) had no formal education. Most were working in the agricultural sector. Thirteen was still in school. Complaints included condition suggesting urethra discharge (22%), genital ulcer (4%), and penile swelling (2%). Other common reasons for consultation were burning micturation (10%) and premature ejaculation (10%). Diagnoses included: psychosexual problem (27%), urinary tract infection (15%), inguinal bubo (10%), genital ulcer disease (3%), and urethritis (7%). Men were receptive to the messages on family planning, counseling, and treatment advice.

**Conclusion:** There seems to be a great demand for male clinics. Men attended the clinics spontaneously and reported their perceived illnesses. It is possible to increase the coverage by increasing the frequency and hours of work. Male clinics are important vehicles to involve men in the reproductive health initiatives in Bangladesh and in other developing countries.

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## Effect of Adolescent Family Life Education Programme of BRAC

Rukhsana Gazi<sup>1</sup>, Shamsher Ali Khan<sup>2</sup> and A.M.R. Chowdhury<sup>3</sup>

**Objective:** Evaluate the effect of Adolescent Family Life Education (AFLE) programme of BRAC on knowledge and attitude of the ninth grade students regarding reproductive health.

**Methodology:** The study was carried out in Mymensingh district in 1998. A survey was conducted among 440 randomly-selected students of class nine through a self-administered structured questionnaire. Of them, 219 underwent AFLE, while 221 were comparison students who did not undergo AFLE. Data were collected on selected issues relating to reproductive health, such as contraception, pregnancy care, infant-feeding, menstruation, leucorrhoea, and sexually transmitted diseases. Bivariate analysis was performed, and chi-square test was done to see the difference between the two groups of students. Qualitative information on issues, such as menstrual hygiene, healthcare-seeking behaviour, and romantic relationships were obtained through informal discussions and focus group discussions.

**Results:** Significantly higher proportion of the AFLE participants, than their peers, could correctly answer the questions on minimum age of female (90% vs. 76.5%,  $p < 0.001$ ), temporary and permanent methods of contraception (56.9% vs. 39.3%,  $p < 0.001$ ), method options for male and female (85.8% vs. 77.8%,  $p < 0.05$ ), proper birth-spacing (88.1% vs. 73.5%,  $p < 0.001$ ). A higher proportion of AFLE students (91.8% vs. 85.1%,  $p < 0.05$ ) expressed their views that a couple should take joint decision on family planning. Significantly higher proportion of comparison students expected more than two children for their own family; they had misconceptions on menstruation, leucorrhoea, and transmission of sex-related diseases. AFLE students were more likely to know about additional diet during pregnancy (96.8% vs. 86.0%,  $p < 0.001$ ), colostrum feeding (91.3% vs. 75.1%,  $p < 0.001$ ), and timing of maternal immunization (43.4% vs. 33.9%,  $p < 0.001$ ). However, irrespective of whether they had undergone AFLE courses or



not, they knew about the need to immunize during pregnancy. In spite of general disapproval of pre-marital sex, some were exposed to it. In general, the students were reluctant to consult health professionals about contraception and sex-related problems as they felt a lack of privacy and confidentiality.

**Conclusion:** The study provides the insight that there is a lack of basic knowledge on issues of reproductive health among general students, and the AFLE programme has a positive effect. A major challenge would be to provide information to others who are outside the schools through peer networking.

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### Sexual Behaviours among Male Adolescents in an Urban Community of Dhaka City

Ashrafal Alam Neeloy, Tamanna Sharmin and Lazeena Muna

**Objective:** Understand the existing sexual behaviours among male adolescents of Dhaka city.

**Methodology:** In-depth interviews were conducted with 47 male adolescents aged between 15 and 24 years residing at Moghbazar. Respondents were selected using snowball sampling technique. Several group discussions were conducted.

**Results:** Majority of the respondents had pre- and/or extra-marital sexual intercourse. Sex workers were the most common partners of those who had pre- and/or extra-marital sex. Although the main sexual preference was heterosexual, homosexual practices were also found. Pornography was the major source of sexual knowledge which was shared among peers. Majority of the respondents did not have a clear knowledge about AIDS and safer sex. Condom was perceived as a method of birth control and was rarely used.

**Conclusion:** Widespread unsafe sexual practices and lack of knowledge about safer sex have put the young people at risk. A programme is needed to educate the young population about sexuality and HIV/AIDS, including risky and less risky behaviours. Identification and in-depth knowledge of the sub-culture of male adolescents can enhance the success of prevention programmes relating to HIV and STDs.

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### Assessing Risks of Contracting HIV for Female Adolescents in Dhaka City

Lazeena Muna, Tamanna Sharmin and Ashrafal Alam Neeloy

**Objective:** Explore the values, beliefs, knowledge, and behaviours of female adolescents regarding different issues of sex and sexuality; assess the risky behaviours of contracting HIV; and provide relevant policy implications.

**Methodology:** In-depth interviews with 37 female adolescents (13 to 25 years old) were conducted in greater Moghbazar area in early 1997. Opportunistic and snowball sampling procedures were employed to get the informants. Confidentiality and sensitivity have been ensured.

**Results:** Female adolescents, both engaged in sexual activities and not engaged, are at risk of contracting HIV. Sexually active girls are practising unprotected sex with multiple partners. Girls not sexually active are ignorant of transmission and prevention of HIV. None of the adolescents perceives herself at risk. Myths on contracting HIV are very much prevalent. Social rigidity and lack of services made them not seeking care in need as well as being silent about the fact.

**Conclusion:** Health behaviour change model could be employed to make female adolescents and change agents understand about the consequences of being at risk. There is a clear need of providing information and education to the adolescents to make them responsible for their own health. The caretakers and health service providers must be oriented to and sympathetic about the need of sexual health services for female adolescents.

ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

## Programmes on Males Who Have Sex with Males in Bangladesh: Necessity and Barriers

Sharful Islam Khan and Tamanna Sharmin

**Objective:** Explore and describe the reasons for prompting interventions on males who have sex with males (MSMs) in Bangladesh and identify barriers to addressing the issue.

**Methodology:** Three hundred fifty-six in-depth interviews had been conducted in two separate qualitative studies on MSMs in Dhaka and Chittagong cities. For this paper, data from 40 in-depth interviews from those previous studies were purposively used to match the objective. In addition, information from a few MSM outreach workers and officials of a local NGO was also gathered. Tape-recorded data were translated into English and entered as text files. Content, contextual, and thematic analyses were performed.

**Results:** Despite social stigma and strong religious norms against MSM acts, its existence in Bangladeshi society is being increasingly apparent in several studies. In big cities, like Dhaka and Chittagong, MSM activity is prevalent in the form of friendship and fantasy sex, situational and opportunistic sex, forced sex, group sex, alternate sex, and of course, at commercial settings. Stereotype invisibility and secrecy often frame the situation ignored, overlooked, and rejected. Selling sex is initiated and continued mainly due to comparatively easier economic subsistence and nourishment, which will probably persuade sustained and rapid growth of commercial male sex in Bangladesh. Low level of condom use without proper lubrication, strong sexual drives and practices, higher number of partners and also sex with females are commonly reported behaviours. Knowledge gap, low self-esteem and guilty feelings have made them vulnerable to getting STDs/HIV. It seems still impractical for NGOs to be registered to work on MSM issue explicitly in Bangladesh. Discrimination in terms of social and legal status, violations of human rights, and harassment by law-enforcing agencies and musclemen (*mastans*) have added extra barriers to reaching these marginalized populations. Blaming and judgmental attitudes shown by health personnel further constrain their access to health services and information.

**Conclusion:** The notion of only "heterosexual transmission of STD/HIV" in Bangladesh is under challenge. Sociocultural, political and religious dismay to work with stigmatized MSMs are urgently needed to be reconsidered. As STDs/HIV primarily affect marginalized group, denial and discrimination, legal obscurity, and human rights violation will no longer halt the transmission or protect the image of the country. Rather, acknowledging and understanding the MSM issue with scientific clarity, possible legal reformation considering cultural sensitivity, both government and NGO commitment to work from public health perspectives, are essential steps. More research and appropriate interventions have no alternatives to saving the country from the outrage of HIV/AIDS.

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## Miscellaneous Health Issues

### Green Leafy Vegetables from Natural Sources: The Case of Rural Poor in Bangladesh

Farzana Ahmed<sup>1</sup>, K.M.A. Aziz<sup>2</sup>, Md. Yunus<sup>1</sup> and R.B. Sack<sup>3</sup>

**Objective:** Explore users' choices among the natural, home-garden, and market sources of green leafy vegetables.

**Methodology:** One hundred and sixty-eight mothers having child(ren) aged 6-59 months were selected from the very poor socioeconomic groups of the community, from eight villages of the MCH-FP study area in Matlab. Data were collected on the sources of green leafy vegetables rich in beta-carotene consumed by the mothers and children during the preceding three days before interview using dietary recall method. Mothers were interviewed once in every two months representing all the six seasons of Bangladesh ranging from April 1994 to March 1995. The green leafy vegetables were categorized into high and low contents of beta-carotene.

**Results:** The proportions of mothers and children who consumed green leafy vegetables with high and low content of beta-carotene are presented according to the sources: home-garden, market, and natural sources. In four of the six seasons, the natural source was the prime one followed by home-garden and market. Overall, the vegetables

containing high beta-carotene were more often chosen for consumption.

**Conclusion:** In choosing vegetables, those with high beta-carotene content and simplicity were preferred. In promoting consumption of green leafy vegetables, the tapping of natural resources must be emphasized, particularly among the poor communities. The successful pursuit of green leafy vegetables was mainly possible due to freedom of movement and urge for procuring suitable foods by the poor women.

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### Zinc Supplementation during Pregnancy in Bangladeshi Urban Poor: Effect on Infant Growth and Morbidity during the First Six Months of Life

Saskia J.M. Osendarp<sup>1,2</sup>, Joop M.A. van Raaij<sup>2</sup>,  
Shams El Arifeen<sup>1</sup>, A.H. Baqui<sup>1</sup> and George Fuchs<sup>1</sup>

**Objective:** Investigate the effect of maternal zinc supplementation during pregnancy on infant growth and morbidity during the first 6 months of life.

**Methodology:** The study was done on 381 infants of poor urban Bangladeshi mothers who had been randomized to receive daily 30 mg elemental zinc or cellulose placebo during pregnancy. Supplementation started between 12 and 16 weeks gestation and continued till delivery. During weekly home visits, information on infant morbidity during the past week was collected by mothers' recall. Weight, length, head-, chest-, and arm-circumference of the infants were measured monthly.

**Results:** At 6 months of age, no significant differences in Z-scores were found between the two treatment groups for weight-for-height, height-for-age or weight-for-age. Weight gain between birth and 6 months of age also did not differ between the two groups (3489±771 g vs. 3561±772 g; NS). Male infants in the zinc group experienced fewer episodes and days of dysentery compared to the placebo group (p<0.05). No other differences were observed in morbidity (total days or total number of episodes of respiratory tract infections, cough, acute diarrhoea, persistent diarrhoea or fever) among all infants from zinc- or placebo-supplemented mothers.

**Conclusion:** It is concluded that zinc supplementation during the last two trimesters of pregnancy in poor urban Bangladeshi women was associated with reduced morbidity due to dysentery in boys but had no apparent impact on infant growth during the first six months of life.

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### Seroepidemiology of Hepatitis B Virus Infection in Bangladesh

M. Hasan Zaki<sup>1,4</sup>, C. Rafiqul Ahsan<sup>1</sup>, Tarek-Al-Nasir<sup>2,4</sup> and Samir K. Saha<sup>3</sup>

**Objective:** Study the seroepidemiology of hepatitis B virus (HBV) infection in Bangladesh to determine the burden of HBV infection and the target population for vaccination.

**Methodology:** Five hundred thirty-five healthy subjects of both sexes, aged between one month and 35 years, living in different districts of Bangladesh, were enrolled in the study during June 1997-June 1998. The cases were referred to Popular Diagnostic Centre by a non-government organization as part of the routine health check-up of their newly-appointed employees. HBsAg and anti-HBc markers were detected by ELISA method. Information was collected by a questionnaire to find out the relation of HBV infection with age, sex, blood group, socioeconomic status, and place of living. Data were analyzed by EPI Info 6.02 software package.

**Results:** Of the 535 subjects, 16 (3%) were found to be HBsAg-positive and 113 (21.1%) were positive for anti-HBc. Analysis of HBV markers in different age groups showed that the prevalence of HBsAg was higher in children (5.52%) than adults (1.7%), whereas it was reverse in the case of anti-HBc (14.9% vs. 24.3%). Further segmental

analysis of age group showed that none of the children aged 0-3 years (n=55) was positive for HBsAg. However, the positivity of HBsAg was highest (10.2%) in the 7-9 years age group. Prevalence of HBsAg and anti-HBc in male (3.23% and 23.83%) was more than in female (1.87% and 10.28%). No significant relationship of HBV infection with socioeconomic status, blood group, and Rh factor was observed. The northern (n=73) and the south-east (n=84) regions of Bangladesh were found to have higher rate of infection than the south-west (n=39) and the central region (n=139).

**Conclusion:** Bangladesh is moderately endemic for HBV infection. The study revealed that preschool children and early adolescents are the high-risk age group, and horizontal transmission is possibly the major mode of transmission. Therefore, to prevent the HBV infection, children of three years old or below should be targeted for vaccination which suits well with the existing EPI schedule.

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### Spread of Multidrug-resistant Salmonella Group B in Dhaka

M. Monir Hossain, Maksuda Islam, M. Ruhul Amin, M. Hanif and Samir K. Saha

**Objective:** Determine prevalence of Salmonella Group B infection in children.

**Methodology:** Salmonella strains isolated from the clinical specimens of paediatric patients were identified by biochemical tests, analytical profile index (API), and agglutination with specific antisera. The sensitivity pattern of the strains was determined by disc diffusion method following the NCCLS recommendations. Clinical features were recorded by taking previous history and subsequent every-day clinical evaluation of patients. Clinical and microbiological information was collected by a questionnaire and analyzed by EPI Info 6.04 software package.

**Results:** In total, 53 strains of Salmonella Group B were isolated from 52 children during 1997-1998. Of these, 25 cases were of nosocomial origin, and 28 were community-acquired. The strains were isolated from stool (45), CSF (3), blood (4), and urine (2). Most isolates (96%) were multidrug-resistant, and these were mainly sensitive to ceftazidime, ciprofloxacin, and pivmecillinam. Among these cases, 45 patients were cured (26 community-acquired and 19 nosocomial), and 7 died (2 community-acquired and 5 nosocomial). The sensitivity pattern and API score of the nosocomial and community acquired strains were similar.

**Conclusion:** Salmonella Group B is an emerging pathogen in Bangladesh, which can cause varied type of invasive and non-invasive diseases in children. Resistance of this organism to most antibiotics makes the treatment difficult. Similarity in drug resistance pattern and API score possibly indicate that the same clone of strain is circulating in this region. However, molecular analysis of these strains is needed to determine their similarity. Studies to examine the association between clinical features and virulence factors of Salmonella group B may be initiated.

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### Children's Chronic and Acute Morbidity in Matlab, Bangladesh: Levels and Correlates

Nurul Alam

**Objective:** Provide chronic and acute morbidity profiles of rural children aged less than 15 years living in different socioeconomic and sanitary conditions.

**Methodology:** The Matlab Health and Socioeconomic Survey (MHSS) 1996 recorded chronic and acute morbidity symptoms of 3,765 children aged 3-179 months. Mothers were asked to report any chronic disease symptoms (e.g. general weakness or anaemia, arthritis or rheumatism, asthma, other breathing difficulty, etc.) the child might have in the past three months. They were also asked to report if the child had any of the acute symptoms of: cough, fever,

cold, headache, watery diarrhoea, etc. in the last one month. MHSS recorded data on mothers' education and cleanliness of the yard. 31% reported more than one symptom of acute morbidity and 14% more than one symptom of chronic morbidity. Prevalence of specific symptoms and of any symptoms was estimated by children's age and sex, mothers' education, and cleanliness of the yard.

**Results:** The prevalence of chronic morbidity was 13.9% with little variation between age groups. The most prevalent symptoms were asthma or breathing difficulty (4.7%) followed by general weakness or anaemia (3.2%). The odds of having chronic morbidity were higher for boys than girls and for unclean yard. More than a half (52.4%) of the sample children had at least one symptom of acute illness in the past month, and the prevalence showed an inverse relationship with children's age. Sex of the child, mothers' education, and cleanliness of the yard were not associated with the prevalence of acute morbidity. The acute symptoms in order of prevalence were: cough-cold-fever (32.1%), diarrhoeal diseases (12.8%), skin infection (3.3%), stomachache (3.1%), and eye infection (2.3%).

**Conclusion:** Both chronic and acute morbidity were widely prevalent among rural children. Household socioeconomic and sanitary conditions were inversely associated with the prevalence of chronic, but not with acute morbidity.

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### Road Traffic Accidents in Dhaka

Moslem Uddin Khan<sup>1</sup> and Nasrin Parvin Khan<sup>2</sup>

**Objective:** Evaluate the casualties of travellers on the roads of Dhaka.

**Methodology:** Information on road traffic accidents was obtained prospectively from a widely-circulated daily newspaper. Data for 1994-1995 were analyzed by seasons of accidents, types of offense, affected vehicles, and the age and sex of travellers dead and injured.

**Results:** There were 1121 traffic accidents, 1600 deaths, and 5982 injuries during the study period. Accidents took place more often during the dry season. Trucks were responsible for 44.6% of the accidents, buses 26.4%, minibuses 16%, and the rest were other types of vehicles. The affected vehicles were rickshaws and cycles (25.7%), two-stroke three-wheel vehicles (16.8%), motor cycles (12.1%). 73.8% of the accidents were due to rush driving and 12% due to loss of control. 49% of the deaths occurred in the 20-35 years age group, 16.9% in 10-19 years age group, and 17.1% in 40-59 years age group. There were 3.1 accidents, 4.4 deaths, and 16.4 injuries per day and 1.2 deaths per accident.

**Conclusion:** Rush driving, lack of valid licence, overloading, bad road and traffic conditions, lack of road partition, crowding on traffic roads, and poor understanding of the people about traffic rules are thought to be the main reasons of accident.

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## Posters

### Observation on National Immunization Day in Slums of Mohakhali, Dhaka

Suhaila H. Khan

**Objective:** Explore in-depth knowledge, attitude, and practice of the slum dwellers regarding immunization of their children and the process of the National Immunization Day (NID) in urban slums.

**Methodology:** This case study made observations on the January 1998 NID in selected slums of Mohakhali, Dhaka. Data were collected by observing two NID centres and interviewing 24 purposively selected respondents. Eight mothers and eight *matobbars* (headmen) from four slums were interviewed pre-NID and post-NID, and four mothers and four vaccinators from two centres were interviewed during the NID sessions.

**Results:** People heard about the NID from neighbours and broadcasts from loudspeakers, radio, and television. The knowledge and perception of the respondents about polio and EPI were sketchy, and did not improve after the NID. The vaccinators were NGO health educators, housewife-social workers, university students, and schoolboys from the slums. NGO workers were more knowledgeable, but the quality of work of the social workers was better. Vaccination was done from both static and mobile centres. There was no shortage of vaccines, but the quality of vaccination in terms of oral drops, maintenance of hygiene, ice packs used for maintaining temperature of vaccines varied greatly. The mobile team did not use any ice pack. Vaccination was not given in an orderly manner, and no supervisors came. The interference of *mastans* (hoodlums) hampered vaccination in one centre. The vaccinators immunized two-thirds of their target population, and only half of the children were vaccinated properly. Children living outside the slums were missed. The NID sessions immunized the socioeconomically well-off and slum children who were missed during routine EPI. The slum dwellers opined that access was easier since immunization was given in the slums and that other than this one-time vaccination centre, they did not know of any EPI centres located in Gulshan thana.

**Conclusion:** Although this study had a small sample, the findings gave a picture of the status of NID and EPI in urban slums. NID sessions and EPI activities need more publicity. Interference of local leaders/hoodlums had negative consequences and need careful consideration/scrutiny. Current observations indicate that NIDs will probably be socially sustainable, but the question of their being financially sustainable remains unanswered and unexplored.

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### Perception of Rural Women on RTIs/STIs

Rukhsana Gazi<sup>1</sup> and A.M.R. Chowdhury<sup>2</sup>

**Objective:** Explore the perception of rural women on causes, transmission and prevention of reproductive tract infections and sexually transmitted infections (RTIs/STIs); obtain information on treatment patterns for these problems; and assess the needs of the community to combat the RTI/STI problems.

**Methodology:** This study was conducted among rural women in four areas of Mymensingh district in March 1998. Data were collected through Participatory Rapid Appraisal (PRA) methods which included focus group discussions, body mapping, ranking, and listing. In total, 8 focus group discussions (2 from each area) were conducted. In each area, one focus group discussion was conducted with younger mothers (having a child of two years or less) aged less than 30 years and another with older mothers (grand-mothers, aged more than 40 years). In body mapping technique, the participants were first given papers with an outline of the female body and asked to draw pictures of how the disease enters the body and involves different organs. Finally, the participants were asked to explain their drawings.

**Results:** The RTI/STI-related diseases were termed as *gupon roag/gupta roag* (private disease) by the rural women that might or might not be *hachor* (contagious). The rural women identified the number of such diseases. These were *gormi*, *siblis*, *pocha ghao*, *pripir/pipree*, and *orsho*. The perceived causes of the diseases were grouped on the basis of hygiene behaviour, pollution, general contact, and supernatural factors. They believed that some behaviours,

such as lack of hygiene after sex, sex with bad women or persons having leucorrhoea and bad semen, sex during menstruation, and sharing underwear with affected persons might cause diseases. Gender, social status, marital status, education, occupation, and age of a person were believed to be the leading factors in disease causation. As preventive measures, hygiene behaviour, condom use, obeying religious rules, protection against contamination, and pollution were mentioned. Significant difference in perception was found between younger and older mothers regarding the consequences of diseases, their transmission, prevention, and treatment-seeking. Sources of treatments sought were both traditional and modern. Choices of treatment were based on risk perception, presentation of diseases, and gender. The rural women gave emphasis on male education, involvement of elderly women, and intervention for perceived risk-group (women in brothels) to overcome the problem.

**Conclusion:** Most participants were illiterate, but they were not ignorant about the magnitude of the problems relating to RTIs/STIs. They provided a wide range of useful information and shared their views and valuable experiences which would be helpful in addressing this issue. Understanding local terminology for RTIs/STIs will help develop a communication strategy for the local people.

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### Lactating Mothers and Contraception: Some Points to Ponder

Rumana A. Saifi and Subrata Routh

**Objective:** Understand the contraceptive use-behaviour of lactating mothers and programmatic implications of the findings.

**Methodology:** Primary data gathered through longitudinal surveillance system of the Operations Research Project, namely the Sample Registration System, were the basis of analyses done to explore the contraceptive use-behaviour of the lactating mothers of Mirsarai and Abhoynagar rural sites for the period from July 1997 to June 1998. The sample comprised 2,800 and 3,400 married women of reproductive age (MWRA) for Abhoynagar and Mirsarai respectively.

**Results:** On an average, 20% of the MWRA were found to be lactating mothers, i.e. eligible women having living child(ren) aged two years or below. Two of every five lactating mothers were using family planning methods. Pills and injectables were the most predominant modern methods of family planning used by them. More than 90% of the lactating mothers under contraception practised breast-feeding with supplementation. Fifty percent of the lactating mothers were found to be non-contraceptors. Most of them were found to represent "unmet need" with potentials of being spacers or limiters.

**Conclusion:** Despite rapid increase in contraceptive prevalence rate (CPR) during the last two decades, the CPR is still far below the desired level in Bangladesh. Data from the 1996-1997 BDHS show that 16% of the currently married women represent "unmet need" for family planning services. The contraceptive use-behaviour of lactating mothers is an important aspect in this regard. This has considerable implication for "unmet need" for family planning and unwanted pregnancies. The present lack of attention to postpartum family planning service provision is a serious loophole in the national family planning programme. The findings, therefore, suggest for adoption of a comprehensive programme for the lactating mothers. This would contribute to reduced fertility through enhanced contraception of lactating mothers, and also contribute to better maternal and child health through reduction of unwanted pregnancies.

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### Assessment of Post-flood Health Status of Pregnant Women in a Rural Community of Bangladesh

Sameena Chowdhury, Aminur Rahman, Ferdousi Islam,  
M. Quamrul Hassan, A.K.M. Fazlur Rahman and Rowshan A. Begum

**Objective:** Determine the post-flood health problems of pregnant women in a rural community of Bangladesh.

**Methodology:** A cross-sectional study was carried out to determine the post-flood health problems of pregnant women. Two hundred and ninety-three pregnant women were randomly selected from two unions of Bandar thana of Narayanganj, badly affected by the 1998 flood. Data were collected through questionnaire between 5 October and 12 November 1998.

**Results:** The mean duration of inundation was  $52 \pm 22.2$  days. About 35% of the respondents took refuge in the flood-shelters or their relatives' house. During the flood, 70% of them used tubewell as their source of drinking water. Only 12.8% used sanitary toilets during the flood. Among the respondents, 50% suffered from various types of ailments. The common health problems were: fever 120 (41%), cough and cold 124 (42.3%), diarrhoea 45 (15.4%), and skin diseases 151 (51.5%).

**Conclusion:** The major post-flood health problems encountered by the pregnant women were fever, cough and cold, diarrhoea, and skin diseases in the study area. Breaking down of sanitary facility in the study area was also identified as one of the major cause of environmental pollution.

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### Youths and HIV/AIDS in Bangladesh

Shakeel A.I. Mahmood

**Objective:** Explore the susceptibility of the youths of Bangladesh to HIV/AIDS and recommend measures to be adopted for prevention of HIV infection in our younger generation.

**Methodology:** The study was done through the latest literature survey on global and regional prevalence of HIV/AIDS, analysis of the existing situation in Bangladesh, and discussion with the national experts.

**Results:** An epidemic of HIV/AIDS has already started in Bangladesh. Fortunately, the country continues to be a low-prevalence area. However, almost all the determinants for an explosive outbreak of an HIV/AIDS epidemic have been found to exist in the country. Here we have a very high prevalence of STDs/RTIs, indicating country's increased susceptibility to HIV/AIDS. This paper explores some factors that make the children of this country extremely vulnerable. Factors, such as ignorance, illiteracy, superstition, poverty, joblessness, malnutrition, etc. have been shown to be responsible for this vulnerability. In addition, the large number of street children in our country pose a definite threat. The incidence of injecting drug use among the youths of Bangladesh has also been a factor to increase the risk of HIV/AIDS at an alarming rate.

**Conclusion:** The paper strongly warns against the danger of 'denial' and complacency. It recommends actions almost on a war footing. Importance of appropriate education at all levels, including household, school and out-of-school education and counselling, is emphasized. Education on safe sex has to be given in conformity with our sociocultural background.

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### Distribution of Government Health Services in Different Geographical Locations in Bangladesh

Muhammod Abdus Sabur<sup>1</sup> and Shamsun Nahar<sup>2</sup>

**Objective:** Explore the distribution of government health services in different geographical locations in Bangladesh.

**Methodology:** For comparing within the cities, Dhaka, Chittagong, Rajshahi, and Khulna were selected. Among districts, two from each of the four old divisions—one being divisional headquarter and the other being situated far from the headquarter—were chosen. Thus Dhaka, Sherpur, Chittagong, Sylhet, Rajshahi, Panchagarh, Khulna, and Bhola were selected. Only services under the Directorate General of Health Services were considered. Availability of hospital bed, physician, nurse, paramedic, per-capita health and per-capita medical-surgical expenditure were analyzed. Only revenue budget for three consecutive financial years were considered. Ranking was made among the cities and the districts considering the above mentioned indicators. Secondary data were used.



**Results:** Nationally, one hospital bed is available for 3,189 people. Among cities, the corresponding figures were: 429 in Rajshahi, 1,049 in Dhaka, 1,550 in Khulna, and 1,885 in Chittagong. Among districts, the figures were found to be: 1,702 in Dhaka, 1,960 in Rajshahi, 2,390 in Sylhet, 2,854 in Khulna, 4,152 in Chittagong, 4,542 in Panchagarh, 7,143 in Sherpur, and 7,504 in Bhola. Nationally, one government physician is available for 13,715 people. Among cities, one doctor was available for 2,585 people in Rajshahi, 4,530 in Dhaka, 8,038 in Khulna, and 10,603 in Chittagong. Among districts, the same figures were found to be: 7,174 in Dhaka, 8,974 in Rajshahi, 10,423 in Sylhet, 12,550 in Khulna, 12,957 in Panchagarh, 16,820 in Chittagong, 22,409 in Bhola, and 23,016 in Sherpur. Per-capita revenue health expenditure in 1992-1993 was Tk 42.16 nationally. Average per-capita revenue health expenditure among cities was found to be: Tk 264 in Rajshahi, Tk 154 in Dhaka, Tk 70 in Chittagong, and Tk 65 in Khulna; and among districts, the figures were: Tk 96 in Dhaka, Tk 66 in Rajshahi, Tk 56 in Sylhet, Tk 44 in Khulna, Tk 35 in Chittagong, Tk 28 in Panchagarh, and Tk 27 in both Sherpur and Bhola. Among the four metropolitan cities, the availability of government health services was found to be the highest in Rajshahi followed by Dhaka, and the other two cities Chittagong and Khulna being equal and next to Dhaka. Among districts, Dhaka topped the list, while Rajshahi was second, Sylhet third, Khulna fourth, Panchagarh fifth, Chittagong sixth, Bhola seventh, and Sherpur was eighth and last in the list.

**Conclusion:** Disparity was found to exist in the distribution of government health services among the studied geographical locations, both among cities and districts. Tertiary and specialized hospitals situated in the 4 metropolitan cities serve as referral centres for the entire country. This demands urgent attention and appropriate action for ensuring equity in healthcare.

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### Knowledge and Practice of the Mostly Used 'Doctors' in Bangladesh

Muhammod Abdus Sabur, A.S.M. Mainul Hassan and Khodadad Hossain Sarker

**Objective:** Explore the level of knowledge and practice of the mostly used "doctors" in Bangladesh.

**Methodology:** Non-qualified allopathic practitioners, popularly known as quacks/village doctors, practising in rural charlands and in metropolitan slums were interviewed in-depth through a semi-structured questionnaire and were observed by using checklist. Nine from rural and six from urban areas were included in the study.

**Results:** Except a few, all were found to know and practise incorrect treatment for hepatitis, which included use of intravenous fluid, antibiotics, and vitamin. Indications for intravenous fluids were found to be perceived as weakness and diarrhoea. About the drugs not to be used in diarrhoea, in pregnancy and for children, they were found to either know nothing or know incorrect treatment pattern except a few who knew not to use tetracycline for children. They had fair and correct knowledge about taking of history for cases of diarrhoea and acute respiratory infections and so also for physical examination for diarrhoea but not for pneumonia for which majority did not know anything. Their choices of treatment for these two conditions were found to be incorrect. Lack of privacy for the clients and general uncleanliness were common features in their drug shops. In most cases, similarity was observed among those who practised in the rural area and in the urban area.

**Conclusion:** Both in rural and urban areas, particularly in hard-to-reach communities, non-qualified allopathic practitioners are the mostly used source of basic curative care. Though affordable, accessible, and credible to the community, their inadequate knowledge leading to incorrect treatment and harmful practice is of concern. Programme aiming at improving their capacity may contribute to improving the situation.

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### Profile of the Most Commonly Used Healthcare Providers of Private Sector in Bangladesh

Muhammod Abdus Sabur, Khodadad Hossain Sarker and A.S.M. Mainul Hassan

**Objective:** Explore the profile of the most commonly used healthcare providers of private sector in Bangladesh

**Methodology:** Non-qualified allopathic practitioners, popularly known as quacks/village doctors, practising in

rural charlands and in metropolitan slums were interviewed in-depth through a semi-structured questionnaire. Nine from rural and six from urban areas were included in the study.

**Results:** All healthcare providers were male. Those practising in the urban areas were found to be younger to those in the rural areas. Mean length of period in this profession in urban practitioners was 5.2 years, whereas in rural practitioners the period was 6.9 years. Basic educational qualifications were found to be higher in urban than in the rural practitioners. Most were found to enter into the profession without any formal training or apprenticeship. Their clients were more or less equally divided into treatment-seekers, drug-buyers on others' prescriptions and drug-buyers on self-medication. Besides providing consultation and selling drugs, they were also engaged in pushing injections and dressing. Average monthly income of a rural practitioner was Tk 2,500 and urban practitioner Tk 5,000. Service on credit was found to be a common practice. In rural areas, all were found to be owners of the drug shops, but in the urban areas, some were found to be the employees of the shops.

**Conclusion:** Knowledge about the profile of the quacks will help design programmes aiming at improving their capacity which may contribute to improving the healthcare situation both in the rural and urban areas.

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### Factors Influencing Hospitalization of Rural Children with ARI

G.M. Monawar Hosain<sup>1</sup>, M.A. Samad Talukder<sup>2</sup>, Md. Tariqul Islam<sup>1</sup> and Qasem Chowdhury<sup>1</sup>

**Objective:** Identify the socioeconomic and demographic factors influencing admission of children aged less than 5 years with acute respiratory tract infections in a rural hospital of Bangladesh.

**Methodology:** All patients aged less than 5 years with ALRI admitted in Gonoshasthaya Kendra (GK) Hospital from 15 April 1998 to 15 November 1998 were selected for this study. A paediatrician confirmed the diagnosis, and data were collected from the case notes of the admitted patients.

**Results:** Of the total 106 patients with ALRI, 90.6% were aged less than one year, and more than two-thirds (70.8%) were male. About three-fourths (73.6%) of the patients belonged to GK Project area, and 60.4% of the patients were insured with GK's health insurance system. Patients who were admitted in this hospital came from a distance of as high as 27 km (mean 6.6 km and median 4 km). Patients from the richer group were eight times more likely to be admitted than the poorer ones. Mean delay for admission after the onset of symptoms was 4.0 days for the richer and 5.6 days for the poorer group. Before admission, almost equal percentage (65% and 66%) of patients from both groups did not seek any treatment, and after admission three patients died.

**Conclusion:** The insured people are more likely to get admitted in this hospital (overall insurance coverage is 28.9%). The poorer people in this rural area spent more days to seek treatment for ALRI after the onset of symptoms.

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### Handwashing Practices in Urban and Rural Areas of Bangladesh

Bilqis Amin Hoque<sup>1</sup>, Shafiul Azam Ahmed<sup>2</sup> and Abdullah Al Mahmud<sup>1</sup>

**Objective:** Assess the efficiency of current handwashing practices and determine its constraints.

**Methodology:** The study was conducted in rural Matlab and in Dhaka slums. One hundred families from Matlab and Dhaka each were purposively selected for the intervention. The housewives in these families received education on improved handwashing practices over a two-month period. Communities similar in terms of socioeconomic characteristics (100 rural and 100 urban slum families) were purposively selected as comparison. Handwashing-related data were collected using observation, interview, and handwashing sampling techniques. This sample size was determined based on available logistics as the observation required special arrangement. Handwashing practice was promoted and observed in terms of four components: (i) rubbing and washing both hands, (ii) using soap, soil or ash as an agent, (iii) rinsing hands with adequate volume of safe water, and (iv) drying hands in air or on a clean piece of cloth/towel.

**Results:** The frequency of handwashing varied between 4 and 25 times/day. Various combinations of the components of handwashing were practised by women according to their perceived need for handwashing. Most women washed one hand before feeding/eating. Only water was used for handwashing before eating and feeding. About 40% used an agent (such as soil, ash or soap) for handwashing after defaecation. Knowledge about the components of handwashing practices improved after the educational intervention, but its practice remained similar. Both hands were highly contaminated. Inadequate water supply (slum), unaffordability of preferred washing agent (rural and slum), and failure to absorb knowledge relating to the components of effective handwashing practices (rural and slum) were the main barriers to improvement in their practices. The prescribed handwashing was stated as more acceptable after defaecation than before feeding/eating.

**Conclusion:** This study showed that hands were contaminated, and it is difficult to improve handwashing practice by educational intervention only. It will require improvement of the related provisions also.

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### Plesiomonas shigelloides-associated Diarrhoea in Bangladeshi Children: Clinical Features and Epidemiological Trend

A.M. Khan and A.S.G. Faruque

**Objective:** Determine clinical and epidemiological features of diarrhoea caused by *Plesiomonas shigelloides* in Bangladeshi children.

**Methodology:** The study used hospital-based surveillance system of the International Centre for Diarrhoeal Disease Research, Bangladesh which prospectively collects microbiologic and clinical information from a 4% systematic sample of all diarrhoeal patients attending the facility. Clinical and epidemiological information is obtained by interviewing the guardians of the children by trained paramedical staff, and the attending physician performs a thorough physical examination. From 1993 to 1995, data on 38 children of less than 5 years with diarrhoea and stool culture positive for *P. shigelloides* only were collected, and 128 children of the same age group who had diarrhoea only due to *Vibrio cholerae* O1 infection during the same period were randomly selected for comparison of clinical and epidemiological features. Children with *V. cholerae* O1 infection were selected for comparison because both *P. shigelloides* and *V. cholerae* O1 belong to the same family (*Vibrionaceae*).

**Results:** Of the 38 children with *Plesiomonas shigelloides*, 76% were <2 years old compared to 50% of the 128 children with *V. cholerae* O1 ( $p<0.001$ ). In *Plesiomonas* group, 85% presented with watery diarrhoea vs. 97% in cholera ( $p=0.02$ ), 15% had dysentery vs. 0% in cholera ( $p<0.001$ ), 82% had stool <16 times per day vs. 71% in cholera, and vomiting was noted in 71% vs. 88% in cholera ( $p=0.01$ ). In *Plesiomonas* diarrhoea, 78% had no dehydration vs. 34% in cholera ( $p<0.001$ ), 21% presented with some dehydration vs. 44% in cholera ( $p=0.02$ ), and 1% had severe dehydration vs. 22% in cholera. Fever was present in 3% of the children with *Plesiomonas* infection. Simultaneously, in cholera it was noted in 10%. Abdominal pain was observed both in cholera (27%) and *Plesiomonas* diarrhoea (24%). In *Plesiomonas* group, 11% had diarrhoea for >14 days (in cholera 4%). Seasonality shows that isolation of *P. shigelloides* in children is common like cholera which is endemic in Bangladesh throughout the year.

**Conclusion:** This preliminary study indicates that *P. shigelloides* may be an aetiological agent in young children presenting with diarrhoea in Bangladesh, and the clinical picture is likely to be dominated by watery diarrhoea and vomiting. Dysentery, clinically significant dehydration, fever, and abdominal pain may be occasionally observed. Further studies are needed to substantiate these findings.

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### Effect of Seasonal Change on Human Autonomic Functions

Yuki Niimi<sup>1,3</sup>, Toshiyoshi Matsukawa<sup>1</sup>, Yoshiki Sugiyama<sup>2</sup>,  
A.S.M. Shamsuzzaman<sup>4</sup>, Hiroki Ito<sup>1,3</sup>, Gen Sobue<sup>3</sup>, and Tadaaki Mano<sup>1</sup>

**Objective:** Evaluate the effect of seasonal change on the sympathetic nervous system and circulatory functions in humans.

**Methodology:** Microneurographic recording of multi-unit efferent activity of the muscle-sympathetic nerve (MSNA) to the blood vessels of the skeletal muscle, plasma catecholamines, tympanic temperature, cardiac output, mean blood pressure, and heart rate were evaluated in 9 healthy young volunteers during winter (December-January) and during summer (June-July). Data were recorded during supine rest for 15 minutes (control) and during orthostatic stress by head-up tilt (20, 40 and 60°).

**Results:** During winter, the resting MSNA and the reflex responses to head-up tilt at higher level significantly increased without a significant difference in tympanic temperature ( $p < 0.05$ ). Plasma catecholamines and heart rate also significantly increased with a tendency of mean blood pressure to increase.

**Conclusion:** Season is an important determinant of disease, particularly in tropical countries like Bangladesh. The combination of the increased MSNA and plasma catecholamines during winter have increased heat production and may have caused the redistribution of the circulatory blood volume from the surface to the core of the body, facilitating conservation of heat. The increased MSNA and catecholamines during winter may play an important role in thermoregulation and in blood pressure rise. Therefore, the higher cardiovascular and cerebrovascular mortality during winter may be caused by the increased vasoconstrictor activity.

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### Skin Involvement Pattern of Arsenicosis Patients

M. Abul Hasnat, Q. Quamruzzaman, Mahmuder Rahman and Shibtosh Roy

**Objective:** Determine the prevalence and type of melanosis and keratoses among the arsenicosis patients in view of the recent arsenic contamination in drinking water.

**Methodology:** Dhaka Community Hospital has identified 2400 arsenicosis patients during a countrywide survey for arsenic contamination of ground water. In total, 2400 patients were identified of whom 1149 were randomly selected for this study. Arsenicosis patients were examined by a dermatologist in the field.

**Results:** The mean age was  $29.86 \pm 14.28$  years, and male had a higher frequency ( $\chi^2 = 42.5$  df 1) among all the subjects. The crude morbidity for melanosis and keratoses were 99% and 66% respectively. The overall crude morbidity (both melanosis and keratoses) was 63.24% and found to be common in the 10-39 years age group for male and 10-49 years age group for female. There is a sharp fall in the prevalence of skin lesions after these age groups and could be due to increased mortality rate resulting from advanced stages of the disease.

**Conclusion:** Melanosis and keratoses are the most prominent characteristic skin lesions of arsenic ingestion. These skin lesions may also be used as an indicator of high exposure to arsenic. The keratotic lesions may eventually progress into squamous cell carcinoma. The results of this small survey show that an alarming situation is impending in Bangladesh, and warrants immediate attention.

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### Chronic Arsenicism and Squamous Cell Carcinoma: A Case Study

Mahfuzar Rahman, Martin Tondel and Olav Axelson

**Objective:** Study a case of chronic arsenicism and squamous cell carcinoma.

**Methodology:** A 45-year old man with typical arsenic skin lesions and squamous cell carcinoma was studied. He has been using the same water-well for more than 30 years and remembered having skin lesions for at least ten years. Five of the six children and his wife had also skin alterations.

**Results:** The water arsenic level was 2280  $\mu\text{g/L}$  and was measured by flow-injection hydride generation atomic absorption spectrometry (FI-HG-AAS). In February 1998, an ulcerative skin lesion on his right hand was removed

that he had had for over a year before the treatment. The histopathological examination revealed infiltrating squamous cell carcinoma, grade II.

**Conclusion:** Hutchinson first described a case of arsenic-induced skin cancer due to Fowler's solution (an arsenical medication), and it can also be observed nowadays due to environmental exposure. A potentially devastating health crisis is unfolding in Bangladesh where arsenic has leached from naturally occurring minerals into drilled wells. Today, chronic arsenic poisoning can still be observed not only in Bangladesh, but also in some other parts of the world. Bangladesh seems to have one of the biggest calamities of mass arsenic poisoning as some 30-70 million people may have been consuming arsenic-polluted water of above 50 mg/L (the current WHO standard for drinking water applied in many countries of the world) for a long period. Arsenical skin lesions were diagnosed as the presence of one or more of these criteria: hyperpigmentation of unexposed body surfaces and/or keratosis, especially on palms and soles. Hyperpigmentation may occur anywhere on the body, often showing raindrop-like pigmentation or diffuse dappling of dark brown, especially marked in non-exposed parts of the body. Hypopigmentation follows the same distribution and may be present even in the absence of hyperpigmentation. Keratosis is small, corn-like elevations, usually 0.4-1 cm in diameter and nodular, found on the lateral borders of palms, fingers, and on the soles, heels, and toes. Diffuse keratosis on the palms and soles could also be present. All these warrant appropriate interventions to address arsenic poisoning in Bangladeshi population.

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### Efficacy of Tetracycline in the Treatment of Cholera Caused by *Vibrio cholerae* O1 Resistant to the Drug *in vitro*

A.M. Khan, Uvon Gierke, M. Begum and George Fuchs

**Objective:** Explore the efficacy of tetracycline in the treatment of cholera caused by *Vibrio cholerae* O1 resistant to the drug *in vitro*.

**Methodology:** In a pilot study, 157 adult patients having cholera caused by *V. cholerae* O1 were investigated. The study was done on patients attending the Dhaka hospital of ICDDR,B. The patients were divided into two groups. One hundred and thirty patients whose stool culture isolates were sensitive to tetracycline were considered 'the sensitive group' and 27 patients whose stool culture isolates were resistant to the drug constituted the 'resistant group'. Each patient was treated with tetracycline (500 mg 6 hourly) given orally for 3 days in addition to rehydration fluid.

**Results:** Though the means of total watery stool volume ( $175 \pm 116$  vs.  $119 \pm 107$  mL/kg,  $p=0.1$ ), duration of diarrhoea ( $36 \pm 22$  vs.  $28 \pm 16$  h,  $p=0.1$ ), ORS ( $187 \pm 152$  vs.  $158 \pm 104$  mL/kg,  $p=0.5$ ) and intravenous fluid requirements ( $57 \pm 96$  vs.  $35 \pm 58$  mL/kg,  $p=0.3$ ) were higher in the resistant group compared to the sensitive group, the differences were not statistically significant. These minor differences could be of epidemiological importance in the control of the disease.

**Conclusion:** The results of the study indicate that conventional dose of tetracycline may be clinically efficacious in the treatment of cholera caused by *V. cholerae* O1 resistant to the drug *in vitro*. So, further studies with increased dose of tetracycline and large sample size are needed to evaluate the exact range of efficacy of tetracycline.

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### Arsenic-induced Keratosis and Squamous Cell Carcinoma

Mahfuzar Rahman<sup>1</sup>, Peter Söderkvist<sup>2</sup>, Barbro Lundh-Rozell<sup>3</sup> and Olav Axelsson<sup>1</sup>

**Objective:** Determine how well the macroscopic and microscopic observations relate to arsenic-induced skin lesions (melanosis and keratosis) as these are the most prominent characteristic hallmarks of arsenic ingestion.

**Methodology:** Skin biopsies from nine individuals with keratosis due to arsenic were collected by punch method with local anaesthesia. All tissue specimens were divided into two parts—one part was fixed in ethanol, and another

in formalin—for further DNA isolation and paraffin-embedding. The histopathological diagnoses were confirmed by an experienced dermatopathologist in Stockholm. Arsenic measurements from well-water used by nine subjects were analyzed by flow-injection hydride generation atomic absorption spectrometry (FI-HG-AAS).

**Results:** Three of the nine skin samples had infiltrative growth with irregular apoptotic cells and squamous cell carcinoma. Two of the remaining six samples were of hyperkeratosis, one atypical hyperkeratosis, one orthohyperkeratosis, and two parakeratosis with apoptotic cells.

**Conclusion:** This very small-scale study suggests that macroscopic investigations are not good enough for diagnosing skin cancer. This study indicates that if wide-scale examinations were conducted in keratotic individuals, more cutaneous carcinomas would likely be found.

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### Immunological Effect of Vitamin A and Zinc in a Placebo-controlled Four-cell Trial

S.K. Roy, Tasnim Azim, George Fuchs, S.M. Akramuzzaman,  
S.M. Ahmad, K.E. Islam, D. Mahalanabis and M. Rahman

**Objective:** Evaluate the immunological effects of zinc and vitamin A.

**Methodology:** In a double-blind study in 1-3 years old children without acute illness and wt-for-age between 61-75% of NCHS standard, 147 children were randomly allocated to 4 groups: (1) Vitamin A 3,000 IU daily, (2) 40 mg elemental zinc daily, (3) Vitamin A and zinc, and (4) Placebo. Each child received intervention for 7 days. Serum zinc, vitamin A, RBP and TTR assays were done. Vitamin A status was also determined by RDR test. Estimations of IL-2 and IL-10 were done on supernatants collected from whole blood stimulated with PHA. Proliferation of Peripheral Blood Mononuclear Cells (PBMCs) in response to PHA, Con-A, PWM, and PPD was measured. Granulocyte polarization in response to chemo-attractant was also measured. Measles IgG titre was assayed before and after the intervention. CMI had also been done. All tests were done at baseline and were repeated after 8 weeks.

**Results:** Significant increase in granulocyte polarization at the level of  $10^6$  and  $10^8$  ( $p=0.05$ ) was observed only after zinc supplementation ( $p=0.05$ ). Measles antibody titre increased significantly after one week with zinc supplementation ( $p=0.01$ ). Significant height gain was observed in the vitamin A group compared to the zinc or zinc+ vitamin A group ( $p=0.01$ ) and significant weight gain was observed in all children except placebo group ( $p<0.000$ ). The incidence of diarrhoeal episodes was significantly lower ( $p=0.01$ ) in the zinc-supplemented children and positive RDR was observed in significantly higher proportion of children in the vitamin A group as well as zinc plus vitamin A group.

**Conclusion:** Zinc supplementation increased antibody titre of measles IgG and proportion of granulocyte polarization. No other benefits were seen in any other groups.

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### Risk Factors for Mortality in Severely Malnourished Children with Diarrhoea in Bangladesh

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**Objective:** Identify risk factors for mortality in severely malnourished children with diarrhoea in Bangladesh.

**Methodology:** One hundred and three children who died were included as cases and 103 children who survived were included as controls in a case-control study. Children of less than 3 years of age with wt-for-age less than 60% or wt-for-height less than 70% of the median (NCHS) standard were selected. Sex distribution was equal in the two groups. Variables considered as possible risk factors for death: socioeconomic status, disease history, associated complications, signs and symptoms of infections, and laboratory values were compared between the two groups.

**Results:** In univariate analysis, 16 factors: age, dehydration, hyponatraemia, acidosis, hypoglycaemia, leukocytosis, bacteraemia, bacterimia, pneumonia, clinically diagnosed septicaemia, presence of more than two complications, low to imperceptible pulse volume, hypothermia, kwashiorkor, duration of hospitalization, and clinically diagnosed severe anaemia were found to be significantly associated with death. Patients presenting with hypo or hypernatraemia, hyperkalaemia, acidosis, pneumonia, marasmic-kwashiorkor and enteric pathogens found in stool culture, and patients with leukocytosis had 2 to 2.5 times higher risk of mortality than the control. Cases who had low imperceptible pulse volume and hypoglycaemia were found to have 4 times higher risk of dying. A very strong association was found between death and hypothermia and presence of clinically diagnosed septicaemia (odds ratio 4.8 and 11.7 respectively). In logistic regression analysis, septicaemia, hypothermia, pneumonia and presence of kwashiorkor or marasmic-kwashiorkor were found to be significant risk factor after controlling for confounding variables.

**Conclusion:** The results of the study could be used as a prognostic guide by physicians treating severely malnourished children such patients in diarrhoeal disease hospitals.

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"A study was undertaken to investigate intestinal fat absorption in young children suffering from persistent diarrhea in comparison with normal children of similar age. Absorption studies were performed using the breath test technique. Following oral administration of labelled triglyceride (<sup>13</sup>C trioctanoin), interval breath sampling was done for 6 h. The time course of excretion of <sup>13</sup>CO<sub>2</sub> in the breath was determined by isotope ratio mass spectrometric analysis of the gas samples. Excretion curves were constructed from the mean values of <sup>13</sup>CO<sub>2</sub> dose (per cent) excreted at each time point in breath samples and areas under the curve were determined for diarrhoea cases as well as for normal subjects. The time course of <sup>13</sup>CO<sub>2</sub> excretions and areas under the curve thus obtained were compared with each other and also with a reference study in which data from known malabsorption cases were available. The time of peak <sup>13</sup>CO<sub>2</sub> excretion in diarrhoea cases ranged from 60 to 240 min (average 150 min) compared with 120 to 270 min (average 195 min) for normal subjects, the level of peak of <sup>13</sup>CO<sub>2</sub> excreted/h was 4.8 ± 1.2 per cent in diarrhoea cases and 5.3 ± 2.3 per cent in normal children (NS). Mean areas under the curve for the two groups were 18.9 ± 3.4 per cent for normal and 17.6 ± 4.1 per cent dose <sup>13</sup>CO<sub>2</sub> excreted/6 h for diarrhoea cases (NS). These results indicate that intestinal absorption of medium chain triglycerides was not impaired significantly in the cases of persistent diarrhoea studied."

**136 Ahmed T, Ali M, Ullah MM, Choudhury IA, Haque ME, Salam MA, Rabbani GH, Suskind RM, Fuchs GJ. Mortality in severely malnourished children with diarrhoea and use of a standardised management protocol. Lancet 1999 Jun 5;353(9168):1919-22. 12 ref, Eng.** ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

"**Background** Severely malnourished children have high mortality rates. Death commonly occurs during the first 48 h after hospital admission, and has been attributed to faulty case-management. We developed a standardised protocol for acute-phase treatment of children with severe malnutrition and diarrhoea, with the aim of reducing mortality. **Methods** We compared severely malnourished children with diarrhoea aged 0-5 years managed by non-protocol conventional treatment, and those treated by our standardised protocol that included slow rehydration with an emphasis on oral rehydration. The standardised-protocol group included children admitted to the ICDDR,B Hospital, Dhaka between Jan 1, 1997, and June

30, 1997, while those admitted between Jan 1, 1996, and June 30, 1996, before the protocol was implemented, were the non-protocol group. **Findings** Characteristics on admission of children on standardised protocol (n=334) and non-protocol children (n=293) were similar except that more children on standardised protocol had oedema, acidosis, and *Vibrio cholerae* isolated from stools. 199 (59.9%) of children on standardised protocol were successfully rehydrated with oral rehydration solution, compared with 85 (29%) in the non-protocol group (p<0.0001). Use of expensive antibiotics was less frequent in children on standardised protocol than in the other group (p<0.0001). Children on standardised protocol had fewer episodes of hypoglycaemia than non-protocol children (15 vs 30, p=0.005). 49 (17%) of children on non-protocol treatment died, compared with 30 (9%) children on standardised protocol (odds ratio for mortality, 0.49, 95% CI 0.3-0.8, p=0.003). **Interpretation** Compared with non-protocol management, our standardised protocol resulted in fewer episodes of hypoglycaemia, less need for intravenous fluids, and a 47% reduction in mortality. This standardised protocol should be considered in all children with diarrhoea and severe malnutrition."

**137 Ammon A, Petersen LR, Karch H. A large outbreak of hemolytic uremic syndrome caused by an unusual sorbitol-fermenting strain of *Escherichia coli* O157:H-. J Infect Dis 1999 May;179(5):1274-7. 15 ref, Eng.** Robert Koch Institute, Stresemannstraße 90-102, D-10963 Berlin, Germany

**138 Ananthan S, Alavandi SV. Biochemical characteristics & secretory activity of *Aeromonas* species isolated from children with gastroenteritis in Chennai. Indian J Med Res 1999 Apr;109:136-40. 26 ref, Eng.** Department of Microbiology, Dr ALM Post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600113, India

"An attempt was made to delineate the phenotypic markers for the detection of enterotoxigenic strains of *Aeromonas*. Eighteen *Aeromonas* species comprising one isolate of *A. hydrophila*, six isolates of *A. sobria* and 11 isolates of *A. caviae* were obtained from 379 children suffering from acute diarrhoea in Chennai. Nine of these isolates inclusive of three *A. sobria* and six *A. caviae* were found to produce secretory response *in vitro* in the rabbit intestinal mucosa mounted in the Ussing chambers as revealed by significant increases in the short circuit current. Eleven strains hydrolysed aesculin, 8 fermented arabinose, 6 produced acetyl methyl carbinol, 14 produced lysine decarboxylase, 3 fermented salicin, 9 produced b-haemolysin, 9 produced CAMP-like factor and only two isolates took up congo red dye. None of these phenotypic traits were found to correlate with the *in vitro* secretory activity."

**139 Anuradha P, Devi PY, Prakash MS. Effect of handwashing agents on bacterial contamination. Indian J Pediatr 1999 Jan-Feb;66(1):7-10. 6 ref, Eng.** Department of Foods and Nutrition, Post-Graduate and Research Centre, A.N.G.R. Agricultural University, Hyderabad 30, Andhra Pradesh, India

“In order to assess the effect of handwashing agents like soap and ash on the control of bacterial contamination, the study was carried out in two villages of Rajendranagar mandal of Rangareddy district, Andhra Pradesh. Twenty households belonging to high income group and twenty households belonging to low income group having 1-2 years age children were randomly selected for the study handwash samples. Before feeding the child handwash samples after washing with different agents were collected and analysed for bacterial contamination. The study revealed that use of soap or ash for washing hands before feeding the child reduced hand contamination significantly.”

**140 Ashkenazi S, Passwell JH, Harlev E, Miron D, Dagan R, Farzan N, Ramon R, Majadly F, Bryla DA, Karpas AB, Robbins JB, Schneerson R. Safety and immunogenicity of *Shigella sonnei* and *Shigella flexneri* 2a O-specific polysaccharide conjugates in children. J Infect Dis 1999 Jun;179(6):1565-8. 15 ref, Eng.** Sheba Medical Center, Tel Hashomer 52621, Israel

“O-specific polysaccharide conjugates of shigellae were safe and immunogenic in young adults, and a *Shigella sonnei* conjugate conferred protection [1-3]. Shigellosis is primarily a disease of children; therefore, the safety and immunogenicity of *S. sonnei* and *Shigella flexneri* 2a conjugates were studied in 4- to 7-year-old children. Local and systemic reactions were minimal. The first injection of both conjugates elicited significant rises in geometric mean levels of serum IgG only to the homologous lipopolysaccharide (LPS) (*S. sonnei*, 0.32-8.25 ELISA units [EU]; *S. flexneri* 2a, 1.15-20.5 EU;  $P < .0001$ ). Revaccination at 6 weeks induced a booster response to *S. flexneri* 2a LPS (20.5-30.5 EU,  $P = .003$ ). Six months later, the geometric mean levels of IgG anti-LPS for both groups were higher than the prevaccination levels ( $P < .0001$ ). Similar, but lesser, rises were observed for IgM and IgA anti-LPS. The investigational *Shigella* conjugates were safe and immunogenic in children and merit evaluation of their efficacy.”

**141 Barbieri E, Falzano L, Fiorentini C, Pianetti A, Baffone W, Fabbri A, Matarrese P, Casiere A, Katouli M, Kuhn I, Mollby R, Bruscolini F, Donelli G. Occurrence, diversity, and pathogenicity of halophilic *Vibrio* spp. and non-O1 *Vibrio cholerae* from estuarine waters along the Italian Adriatic coast. Appl Environ Microbiol 1999 Jun;65(6):2748-53. 23 ref, Eng.** Istituto di Scienze Tossicologiche, Igienistiche e Ambientali, University of Urbino, Via S. Chiara 27, 61029, Urbino, Italy

“The occurrence, diversity, and pathogenicity of *Vibrio* spp. were investigated in two estuaries along the Italian Adriatic coast. *Vibrio alginolyticus* was the predominant species, followed by *Vibrio parahaemolyticus*, non-O1 *Vibrio cholerae*, and *Vibrio vulnificus*. By using a biochemical fingerprinting method, all isolates were grouped into nine phenotypes with similarity levels of 75 to 97.5%. The production of toxins capable of causing cytoskeleton-dependent changes was detected in a large number of *Vibrio* strains. These findings indicate a significant presence of potentially pathogenic *Vibrio* strains along the Adriatic coast.”

**142 Beutin L, Strauch E, Fischer I. Isolation of *Shigella sonnei* lysogenic for a bacteriophage encoding gene for production of Shiga toxin (letter). Lancet 1999 May 1;353(9163):1498. 5 ref, Eng.** Division of Emerging Bacterial Pathogens, Department of Biological Safety, Robert Koch Institut, Nordufer 20, D-13353 Berlin, Germany

**143 Bhandari N, Bahl R, Dua T, Kumar R, Srivastava R. Role of protozoa as risk factors for persistent diarrhea. Indian J Pediatr 1999 Jan-Feb;66(1):21-6. 15 ref, Eng.** Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110029, India

“A case control study including 175 children aged 0-36 months suffering from diarrhea of  $\geq 14$  days duration was undertaken to determine whether there is an association between *Giardia lamblia*, *Entamoeba histolytica* or *Cryptosporidium* infection and persistent diarrhea (PD). Subjects were identified by ongoing household surveillance and enrolled as cases. For each case two controls were selected by survey of neighbouring households - a child with acute diarrhea and one without diarrhea. Both the controls were matched with the case for age and nutritional status. Two fresh stool samples were collected from all cases and controls at enrolment and examined for trophozoites of *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium*. *Giardia lamblia* trophozoites were detected in a significantly higher proportion of PD cases (20.0%) than acute diarrheal and non diarrheal controls (4.6% each,  $p < 0.0001$ ). There were no significant differences in the proportion of cases and controls who passed *E. histolytica* trophozoites or cryptosporidium in their stools. There was a consistent trend towards poorer weight gain in PD cases who passed giardia trophozoites in stool; the differences were statistically significant at days 14 and 21, after enrolment. *Giardia lamblia* infection is more prevalent in PD cases than in acute diarrhea or non-diarrheal controls. This prevalence is not high enough to warrant routine anti-giardia therapy in patients with PD. However, as giardiasis was observed to have adverse growth impact in PD cases, stool microscopy for detection and subsequent treatment of *Giardia lamblia* seems to be justified.”

**144 Bini EJ. Endoscopic approach to HIV-associated diarrhea: how far is far enough?**

(editorial). *Am J Gastroenterol* 1999 Mar;94(3):556-8. 26 ref, Eng. Division of Gastroenterology (111D), New York VA Medical Center, 423, East 23<sup>rd</sup> Street, New York, NY 10010, USA

**145 Bolton FJ, Surman SB, Martin K, Wareing DRA, Humphrey TJ. Presence of *Campylobacter* and *Salmonella* in sand from bathing beaches. *Epidemiol Infect* 1999 Feb;122(1):7-13. 20 ref, Eng.** Public Health Laboratory, Royal Preston Hospital, PO Box 202, Sharoe Green Lane, Preston PR2 9HG, UK

“The purpose of this study was to determine the presence of thermophilic *Campylobacter* spp. and *Salmonella* spp. in sand from non-EEC standard and EEC standard designated beaches in different locations in the UK and to assess if potentially pathogenic strains were present. *Campylobacter* spp. were detected in 82/182 (45%) of sand samples and *Salmonella* spp. in 10/182 (6%). *Campylobacter* spp. were isolated from 46/92 (50%) of samples from non-EEC standard beaches and 36/90 (40%) from EEC standard beaches. The prevalence of *Campylobacter* spp. was greater in wet sand from both types of beaches but, surprisingly, more than 30% of samples from dry sand also contained these organisms. The major pathogenic species *C. jejuni* and *C. coli* were more prevalent in sand from non-EEC standard beaches. In contrast, *C. lari* and urease positive thermophilic campylobacters, which are associated with seagulls and other migratory birds, were more prevalent in sand from EEC standard beaches. *Campylobacter* isolates were further characterized by biotyping and serotyping, which confirmed that strains known to be of types associated with human infections were frequently found in sand on bathing beaches.”

**146 Bonvin P, Ejlersen T, Jensen HD. Brain abscess caused by *Salmonella enteritidis* in an immunocompetent adult patient: successful treatment with cefotaxime and ciprofloxacin. *Scand J Infect Dis* 1998;30(6):632-4, 22 ref, Eng.** Department of Medicine, Hjørring/Brønderslev Hospital, DK-9800 Hjørring, Denmark

**147 Boudeau J, Glasser A-L, Masseret E, Joly B, Darfeuille-Michaud A. Invasive ability of an *Escherichia coli* strain isolated from the ileal mucosa of a patient with Crohn's disease. *Infect Immun* 1999 Sep;67(9):4499-509. 64 ref, Eng.** Pathogenie Bacterienne Intestinale, Laboratoire de Bacteriologie, Faculte de Pharmacie, 63001 Clermont-Ferrand, France

**148 Bras AM, Chatterjee S, Wren BW, Newell DG, Ketley JM. A novel *Campylobacter jejuni* two-component regulatory system important for temperature-dependent growth and colonization (note). *J Bacteriol* 1999 May;181(10):3298-302. 42 ref, Eng.** Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK

**149 Braun MC, He J, Wu C-Y, Kelsall BL. Cholera**

**toxin suppresses interleukin (IL)-12 production and IL-12 receptor b1 and b2 chain expression. *J Exp Med* 1999 Feb 1;189(3): 541-52. 89 ref, Eng.** Immune Cell Interaction Unit, Mucosal Immunity Section, National Institutes of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland 20892, UK

**150 Brughra R, Vipond IB, Evans MR, Sandifer QD, Roberts RJ, Salmon RL, Caul EO, Mukerjee AK. A community outbreak of food-borne small round-structured virus gastroenteritis caused by a contaminated water supply. *Epidemiol Infect* 1999 Feb;122(1): 145-54. 28 ref, Eng.** Public Health Laboratory Service Communicable Disease Surveillance Centre (Welsh Unit), Cardiff CF4 3QX, UK

“In August 1994, 30 of 135 (23%) bakery plant employees and over 100 people from South Wales and Bristol in the United Kingdom, were affected by an outbreak of gastroenteritis. Epidemiological studies of employees and three community clusters found illness in employees to be associated with drinking cold water at the bakery (relative risk 3.3, 95% CI 1.6-7.0), and in community cases with eating custard slices (relative risk 19.8%, 95% CI 2.9-135.1) from a variety of stores supplied by one particular bakery. Small round-structured viruses (SRSV) were identified in stool specimens from 4 employees and 7 community cases. Analysis of the polymerase and capsid regions of the SRSV genome by reverse transcription-polymerase chain reaction (RT-PCR) demonstrated viruses of both genogroups (1 and 2) each with several different nucleotide sequences. The heterogeneity of the viruses identified in the outbreak suggests that dried custard mix may have been inadvertently reconstituted with contaminated water. The incident shows how secondary food contamination can cause wide-scale community gastroenteritis outbreaks, and demonstrates the ability of molecular techniques to support classical epidemiological methods in outbreak investigations.”

**151 Burgos JM, Perez JL, Garcia L, Gonzalez GS, Benitez JA, Galindo F, Silberstein C, Ibarra C. Diarrheagenicity evaluation of attenuated *Vibrio cholerae* O1 and O139 strains in the human intestine ex vivo. *Vaccine* 1999 Feb 26;17(7-8):949-56. 28 ref, Eng.** Departamento de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 7 mo piso, cp 1121 Buenos Aires, Argentina

“The recent spread of El Tor cholera in Latin America highlights the need for a safe and economical vaccine. The main approach for developing live recombinant vaccines has been to disarm known pathogenic strains of cholera toxin leaving intact antigens involved in protection. These recombinant vaccine candidates do not cause severe diarrhea, but they are too reactogenic for wide scale usage. We describe here a test capable of determining the diarrheagenic potential of attenuated *V. cholerae* strains. The functional test consists in the

simultaneous recording of net water movement, electrical potential difference and short-circuit current across the human intestine *ex vivo*. We found that human tissues incubated with supernatants from the attenuated 638,413 and 251a *V. cholerae* strains caused no changes in the ion conductances and water absorption in ileal and colon tissues allowing them to be assayed in volunteers."

**152 Caeiro JP, DuPont HL, Albrecht H, Ericsson CD. Oral rehydration therapy plus loperamide versus loperamide alone in the treatment of traveler's diarrhea. Clin Infect Dis 1999 Jun;28(6):1286-9. 9 ref, Eng.** The University of Texas Medical School at Houston, Baylor College of Medicine, Houston, Texas 77030, USA

"Eighty United States students in Mexico received either loperamide (an initial dose of 4 mg, followed by 2 mg after passage of each unformed stool, up to 8 mg/d; 40 patients) or loperamide (at the same dosage schedule) plus an oral rehydration therapy (ORT) preparation (500 mL initially, followed by 250 mL after each subsequently passed unformed stool, up to 1,000 mL per 24 hours; 40 patients). The ORT preparation was a modification of the World Health Organization-recommended solution, adjusted to a sodium concentration of 60 mEq/L. All treatments were given for 48 hours. The study demonstrated equivalent clinical responses with regard to diminishment of diarrhea or subjective findings such as abdominal pain/cramps, headache, dry mouth, dizziness, or thirst. Stool number (by form) and specific gravity of urine postenrollment were similar in the groups. Administration of loperamide plus ORT for the management of traveler's diarrhea, in cases in which subjects were encouraged to drink *ad libitum*, offered no benefit over administration of loperamide alone."

**153 Cama RI, Parashar UD\*, Taylor DN, Hickey T, Figueroa D, Ortega YR, Romero S, Perez J, Sterling CR, Gentsch JR, Gilman RH, Glass RI. Enteropathogens and other factors associated with severe disease in children with acute watery diarrhea in Lima, Peru. J Infect Dis 1999 May;179(5):1139-44. 43 ref, Eng.** \*Viral Gastroenteritis Section, Mailstop G-04, Centers for Disease Control and Prevention, 1600 Clifton Rd., NE, Atlanta, GA 30333, USA

"To evaluate enteropathogens and other factors associated with severe disease in children with diarrhea, 381 children <5 years of age with diarrhea and moderate to severe dehydration (in-patients) and 381 age-, sex-, and date-of-visit-matched children with mild diarrhea (out-patients) presenting to a hospital in Peru, were studied. Rotavirus was detected in 52% of the in-patients and 35% of the out-patients (odds ratio [OR]=2.3, 95% confidence interval [95% CI]=1.6-3.2); 95% of the rotaviruses among in-patients were of serotypes G1-G4. The risk of severe diarrhea was particularly great in children who were not exclusively breast-fed in early infancy and who also lacked piped water in their homes (for children with both characteristics OR=6.8, 95% CI=3.6-12.8). The high

prevalence of rotavirus and its association with severe diarrhoea underscores the need for rotavirus vaccines. Interventions to educate mothers and improve access to safe water should augment the impact of rotavirus vaccines in preventing severe diarrhea."

**154 Campbell D, Gaucher D, Chadee K. Serum from Entamoeba histolytica-infected gerbils selectively suppresses T cell proliferation by inhibiting interleukin-2 production. J Infect Dis 1999 Jun;179(6):1495-501. 32 ref, Eng.** Institute of Parasitology of McGill University, Macdonald Campus, 21, 111 Lakeshore Rd., Ste-Anned-Bellevue, Quebec, Canada H9X 3V9

**155 Caputo C, Forbes A, Frost F, Sinclair MI, Kunde TR, Hoy JF, Fairley CK. Determinants of antibodies to Cryptosporidium infection among gay and bisexual men with HIV infection. Epidemiol Infect 1999 Apr;122(2):291-7. 20 ref, Eng.** Department of Epidemiology and Preventive Medicine, Monash University, The Alfred Hospital, Prahran, VIC 3181 Australia

"A cross-sectional serosurvey for markers of prior *Cryptosporidium* infection was conducted among homosexual or bisexual males infected with human immunodeficiency virus (HIV); of 262 individuals approached, 236 (90%) agreed to participate. Serological response to two *Cryptosporidium* antigens was measured using a Western blot assay. The intensity or detection of serological responses to two *Cryptosporidium* antigens was not associated with CD4 cell counts or tap water consumption. A number of sexual practices were related to increased serological response for only the 27-kDa marker, including having had sex within the past 2 years, having anal sex and having had a larger number of sex partners during the past 2 years. Attending a spa or sauna was related to serological response to both the 27-kDa and 17-kDa markers. Based on these results, activities related to sexual activity appear to be a significant risk factors for prior *Cryptosporidium* infection."

**156 Catassi C, Fabiani E, Spagnuolo MI, Barera G, Guarino A. Severe and protracted diarrhea: results of the 3-year SIGEP multicenter survey. J Pediatr Gastroenterol Nutr 1999 Jul;29(1):63-8. 27 ref, Eng.** University Department of Pediatrics, Via F Corridoni 11, 60123 Ancona, Italy

"**Background:** The spectrum of severe and protracted diarrhea (SPD), previously defined as intractable diarrhea, has changed during the past several decades. Despite recent advances in determining the cause of SPD and in treatment, this syndrome still represents a challenge and is becoming a major problem affecting health care resources. This study was conducted to characterize the epidemiology, spectrum of causes, and the outcome of SPD in Italy in recent years. **Methods:** All the SPD cases seen at the major centers of pediatric gastroenterology in Italy during a 3-year period (1993-1996) were recruited

in this multicenter, prospective survey. **Results:** Thirty-two children (26 boys and 6 girls; median age at the onset of SPD, 40 days) were enrolled in this study by 9 of 26 participating centers. Twelve were newly diagnosed cases, with an estimated SPD incidence rate in Italy of 0.64 to  $0.92 \times 10^{-5}$  infants per year. The most common causes were autoimmune enteropathy ( $n = 8$ ) and ultrastructural abnormalities of the enterocyte ( $n = 7$ ), whereas food intolerance and postenteritis syndrome were less frequent (3 and 2 cases, respectively). Two children with autoimmune enteropathy fulfilled the criteria for the X-linked variant of this condition. At the end of the study period, 9 of 31 patients had recovered, 15 still had diarrhea, and 7 had died. **Conclusions:** Severe and protracted diarrhea is a rare but challenging problem in Italy. Because parenteral nutrition or intestinal transplantation are the only options in a subset of cases (e.g., ultrastructural abnormalities of the enterocyte), infants with SPD should be referred to specialized centers where advanced diagnostic and therapeutic facilities are available."

**157 Chakrabarti MK, Bhattacharya J, Bhattacharya MK, Nair GB, Bhattacharya SK, Mahalanabis D. Killed oral *Shigella* vaccine made from *Shigella flexneri* 2a protects against challenge in the rabbit model of shigellosis. *Acta Paediatr* 1999 Feb;88(2):161-5. 20 ref, Eng.** Society for Applied Studies, 108 Manicktala Main Road, Flat-3/21, Calcutta 700054, India

"The protective efficacy of an orally administered heat-killed virulent strain of *Shigella flexneri* 2a (5 weekly oral doses) was evaluated in 25 rabbits (14 immunized and 11 non-immunized controls) against challenge with the same strain of *Shigella* using the rabbit model of shigellosis. All 11 non-immunized rabbits developed bloody diarrhoea following challenge and 6 (54%) died. None of the 14 immunized rabbits developed diarrhoea (all had pellet stools) but 3 (21%) died from causes not associated with diarrhoea. Protection from diarrhoea and dysentery following oral immunization with a killed *Shigella* species was 100% and highly significant. Death following challenge was 2.5-fold higher in the non-immunized group ( $p=0.115$ ) but was not significant. These promising results suggest that further studies should be undertaken to develop a killed oral vaccine against shigellosis."

**158 Chauret C, Nolan K, Chen P, Springthorpe S, Sattar S. Aging of *Cryptosporidium parvum* oocysts in river water and their susceptibility to disinfection by chlorine and monochloramine. *Can J Microbiol* 1998 Dec;44(12):1154-60. 18 ref, Eng.** Biological and Physical Sciences Unit, Indiana University Kokomo, 2300 South Washington St., Kokomo, IN 46904-9003, USA

**159 Cid D, Sanz R, Marin I, de Greve H, Ruiz-Santa-Quiteria JA, Amils R, de la Fuente R. Characterization of nonenterotoxigenic *Escherichia coli* strains producing F17 fimbriae isolated from**

**diarrheic lambs and goat kids. *J Clin Microbiol* 1999 May;37(5):1370-5. 22 ref, Eng.** Departamento Patologia Animal I, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

**160 Coster TS, Hoge CW, VanDeVerg LL, Hartman AB, Oaks EV, Venkatesan MM, Cohen D, Robin G, Fontaine-Thompson A, Sansonetti PJ, Hale TL. Vaccination against shigellosis with attenuated *Shigella flexneri* 2a strains SC602. *Infect Immun* 1999 Jul;67(7):3437-43. 37 ref, Eng.** Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC 20307, USA

"The *Shigella flexneri* 2a SC602 vaccine candidate carries deletions of the plasmid-borne virulence gene *icsA* (mediating intra- and intercellular spread) and the chromosomal locus *iuc* (encoding aerobactin) (S. Barzu, A. Fontaine, P. J. Sansonetti, and A. Phalipon, *Infect. Immun.* 64:1190-1196, 1996). Dose selection studies showed that SC602 causes shigellosis in a majority of volunteers when  $3 \times 10^8$  or  $2 \times 10^6$  CFU are ingested. In contrast, a dose of  $10^4$  CFU was associated with transient fever or mild diarrhea in 2 of 15 volunteers. All volunteers receiving single doses of  $3 \times 10^4$  CFU excreted *S. flexneri* 2a, and this colonization induced significant antibody-secreting cell and enzyme-linked immunosorbent assay responses against *S. flexneri* 2a lipopolysaccharide in two-thirds of the vaccinees. Seven volunteers who had been vaccinated 8 weeks earlier with a single dose of  $10^4$  CFU and 7 control subjects were challenged with  $2 \times 10^3$  CFU of virulent *S. flexneri* 2a organisms. Six of the control volunteers developed shigellosis with fever and severe diarrhea or dysentery, while none of the vaccinees had fever, dysentery, or severe symptoms ( $P = 0.005$ ). Three vaccinees experienced mild diarrhea, and these subjects had lower antibody titers than did the fully protected volunteers. Although the apparent window of safety is narrow, SC602 is the first example of an attenuated *S. flexneri* 2a candidate vaccine that provides protection against shigellosis in a stringent, human challenge model."

**161 Crane JK, Majumdar S, Pickhardt DF, III. Host cell death due to enteropathogenic *Escherichia coli* has features of apoptosis. *Infect Immun* 1999 May; 67(5):2575-84. 50 ref, Eng.** Center for Microbial Pathogenesis and Division of Infectious Diseases, State University of New York at Buffalo, NY 14214, USA

"Enteropathogenic *Escherichia coli* (EPEC) is a cause of prolonged watery diarrhea in children in developing countries. The ability of EPEC to kill host cells was investigated in vitro in assays using two human cultured cell lines, HeLa (cervical) and T84 (colonic). EPEC killed epithelial cells as assessed by permeability to the vital dyes trypan blue and propidium iodide. In addition, EPEC triggered changes in the host cell, suggesting apoptosis as the mode of death; such changes included early expression of phosphatidylserine on the host cell surface and internucleosomal cleavage of host cell DNA. Genistein, an inhibitor of tyrosine kinases, and

wortmannin, an inhibitor of host phosphatidylinositol 3-kinase, markedly increased EPEC-induced cell death and enhanced the features of apoptosis. EPEC-induced cell death was contact dependent and required adherence of live bacteria to the host cell. A quantitative assay for EPEC-induced cell death was developed by using the propidium iodide uptake method adapted to a fluorescence plate reader. With EPEC, the rate and extent of host cell death were less than what has been reported for *Salmonella*, *Shigella*, and *Yersinia*, three other genera of enteric bacteria known to cause apoptosis. However, rapid apoptosis of the host cell may not favor the pathogenic strategy of EPEC, a mucosa-adhering, noninvasive pathogen."

**162 Cunin P, Tedjouka E, Germani Y, Ncharre C, Bercion R, Morvan J, Martin PMV. An epidemic of bloody diarrhea: *Escherichia coli* O157 emerging in Cameroon? *Emerging Infect Dis* 1999 Mar-Apr;5(2):285-90. 34 ref, Eng. Centre Pasteur du Cameroun, BP 1274 Yaounde, Cameroun**

"Between November 1997 and April 20, 1998, bloody diarrhea sickened 298 persons in Cameroon. Laboratory investigation of the epidemic (case-fatality rate, 16.4%) documented amoebiasis in one of three patients and three types of pathogens: multidrug-resistant *Shigella dysenteriae* type 1, *S. boydii*, and enterohemorrhagic *Escherichia coli*. We report the first isolation of *E. coli* O157:H7 in Cameroon and the second series of cases in the Central African region."

**163 Czeuczulin JR, Whittam TS, Henderson IR, Navarro-Garcia F, Nataro JP. Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. *Infect Immun* 1999 Jun;67(6):2692-9. 45 ref, Eng. Center for Vaccine Development, 685 W. Baltimore St., Baltimore, MD 21201, USA**

"The phylogenetics of the various pathotypes of diarrheagenic *Escherichia coli* are not completely understood. In this study, we identified several plasmid and chromosomal genes in the pathogenic enteroaggregative *E. coli* (EAEC) prototype strain 042 and determined the prevalence of these loci among EAEC and diffusely adherent *E. coli* strains. The distribution of these genes is analyzed within an evolutionary framework provided by the characterization of allelic variation in housekeeping genes via multilocus enzyme electrophoresis. Our data reveal that EAEC strains are heterogeneous with respect to chromosomal and plasmid-borne genes but that the majority harbor a member of a conserved family of virulence plasmids. Comparison of plasmid and chromosomal relatedness of strains suggests clonality of chromosomal markers and a limited transfer model of plasmid distribution."

**164 Dalsgaard A, Forslund A, Bodhidatta L, Serichantalergs O, Pitarangsi C, Pang L, Shimada T, Echeverria P. A high proportion of *Vibrio cholerae* strains isolated from children with diarrhoea in**

**Bangkok, Thailand are multiple antibiotic resistant and belong to heterogenous non-O1, non-O139 O-serotypes. *Epidemiol Infect* 1999 Apr;122(2):217-26. 51 ref, Eng. Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Bulowsvej 13, DK 1870 Frederiksberg C, Denmark**

"Results of a surveillance on cholera conducted with patients seen at the Children Hospital in Bangkok, Thailand from August 1993 to July 1995 are presented. Annually, isolation rates for *Vibrio cholerae* varied between 1.7 and 4.4% of patients with diarrhoea. *V. cholerae* O1 serotype Ogawa accounted for between 31 and 47% of patients cultured positive for *V. cholerae*, whereas the O139 serotype dominated in early 1994 after which it disappeared. Non-O1, non-O139 strains were isolated at similar rates as serotype O1 in 1993 and 1994, but accounted for 69% of *V. cholerae* culture positive specimens in 1995. However, the annual proportion of the isolation of non-O1, non-O139 strains showed little variation and remained low between 1.0 and 1.3%. Serotyping of 69 epidemiological unrelated non-O1, non-O139 strains produced 37 different O-serotypes. *BglII* ribotyping of serotypes containing more than two strains demonstrated a high degree of heterogeneity within and between serotypes, except seven serotype O37 strains which showed an identical ribotype suggesting clonality. None of the 69 strains hybridized with a cholera toxin probe and only two strains hybridized with a heat-stable enterotoxin probe. Susceptibility testing to 12 antibiotics showed that 40 of 69 (58%) non-O1, non-O139 strains were resistant to colistin, streptomycin and sulphisoxazole and 28 of 69 (41%) were multiple antibiotic resistant (MAR; <sup>34</sup> antibiotics). Although 26 of 69 (38%) strains contained one or more plasmids, the plasmids were of low molecular weights and did not seem to encode antibiotic resistance. The results of the present study showed that a high proportion of heterogenous MAR *V. cholerae* non-O1, non-O139 strains were isolated from children at the hospital. With reference to the emergence of *V. cholerae* O139 in 1992, we suggest that non-O1, non-O139 strains should be monitored carefully to detect new serotypes with a possible epidemic potential, but also to determine the development and mechanism of antibiotic resistance."

**165 Daniels NA, Simons SL, Rodrigues A, Gunnlaugsson G, Forster TS, Wells JG, Hutwagner L, Tauxe RV, Mintz ED. First do no harm: making oral rehydration solution safer in a cholera epidemic. *Am J Trop Med Hyg* 1999 Jun;60(6):1051-5. 24 ref, Eng. Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, and Epidemic Intelligence Service Program, Epidemiology Program Office, Centers for Disease Control and Prevention, Mailstop A-38, 1600 Clifton Road, Atlanta, GA 30333, USA**

"Oral rehydration solution (ORS) is lifesaving therapy for cholera and pediatric diarrhea. During a cholera

epidemic in Guinea-Bissau, we evaluated the microbiologic quality of ORS prepared at a hospital and tested a simple intervention using special vessels for disinfecting tap water with bleach and for preparing, storing, and dispensing ORS. Few coliform bacteria and *Escherichia coli* were recovered from tap water; however, pre-intervention ORS contained numerous bacteria including *E. coli* and toxigenic *Vibrio cholerae* O1. In contrast, ORS samples from intervention vessels had few or no coliform bacteria, no *E. coli*, and no *V. cholerae*. Mean pre-intervention counts of coliform bacteria ( $3.4 \times 10^7$  colony-forming units [cfu]/100 ml) and *E. coli* ( $6.2 \times 10^3$  cfu) decreased significantly during the intervention period to  $3.6 \times 10^2$  cfu and 0 cfu, respectively ( $P < 0.001$ ). This simple system using bleach disinfectant and special storage vessels prevents bacterial contamination of ORS and reduces the risk of nosocomial transmission of cholera and other enteric pathogens."

**166 Dean HJ, Leyh R. Cross-protective efficacy of a bovine viral diarrhoea virus (BVDV) type 1 vaccine against BVDV type 2 challenge. Vaccine 1999 Mar 5;17(9-10):1117-24. 15 ref, Eng.** Endorex Corporation, 28101 Ballard Drive, Suite F, Lake Forest, IL 60045, USA

**167 Donnenberg MS. Interactions between enteropathogenic *Escherichia coli* and epithelial cells. Clin Infect Dis 1999 Mar;28(3):451-5. 40 ref, Eng.** Division of Infectious Diseases, University of Maryland at Baltimore, 10 South Pine Street, MSTF 900, Baltimore, Maryland 21201, USA

"Enteropathogenic *Escherichia coli* (EPEC) may be considered a paradigm for a multistage interaction between and host cell. EPEC strains produce a type IV pilus that is associated with initial adherence to host cells, and these strains possess a type III secretion apparatus that is necessary for transducing signals to host cells. Secretion of three Esp proteins is required for activation of a phosphotyrosine-containing receptor that allows EPEC to bind intimately to host cells via the bacterial outer membrane protein intimin. Intimately attached bacteria rest upon a pedestal composed of host cytoskeletal proteins in an arrangement recognized as the attaching and effacing phenotype. The precise molecular interactions that lead to these dramatic alterations in the host cell cytoskeleton remain to be elucidated."

**168 Dua T, Bahl R, Bhan MK\*. Lessons learnt from diarrheal diseases control program and implications for the future. Indian J Pediatr 1999 Jan-Feb;66(1):55-61. 4 ref, Eng.** \*Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

"The national Diarrheal Disease Control Program was launched with the aim of improving the knowledge and practice of appropriate case management among caretakers of young children as well as health care providers. The National Family Health Survey (1992-3)

revealed that 42.7% of mothers knew about ORS packets and 25.9% had ever used them. ORS use rates in children who suffered from diarrhea during the previous two weeks varied from 8.3% in Rajasthan to 50.1% in West Bengal. These findings represent substantial accomplishment, and also are a reminder that we still have to reach more than half of the households. Further analysis of the NFHS data showed that exposure to electronic mass media had a significant impact on mothers awareness about ORS packets (56% in exposed, 32% in unexposed) and ORT use rates (38% in exposed, 25% in unexposed). In this review, future strategies for increasing the impact of the program are discussed. These include involvement of licensed and unlicensed medical practitioners, greater use of the electronic mass media, ORS standardization, greater focus on poorly performing states and resolving residual issues in the case management of diarrhea in malnourished children and infants under 6 months of age."

**169 Eberhard ML, Nace EK, Freeman AR, Streit TG, Silva AJD, Lammie PJ. Cyclospora cayetanensis infections in Haiti: a common occurrence in the absence of watery diarrhea. Am J Trop Med Hyg 1999 Apr;60(4):584-6. 9 ref, Eng.** Division of Parasitic Diseases, Centers for Disease Control and Prevention, Mailstop F-13, 4770 Buford Highway, NE, Atlanta, Georgia 30341-3724, USA

"Stool samples from a population-based cohort of mothers and children living in Leogane, Haiti were tested for *Cyclospora cayetanensis* from January 1997 through January 1998. Data on gastrointestinal symptoms were also collected. During the winter months of January to March, the infection was detected in 15-20% of the persons sampled. Most infections did not appear to be causing diarrhea and most infected persons had few oocysts detectable in concentrates of stool. The infection appears to have marked seasonality, with highest rates during the driest and coolest time of the year. It may be that in this tropical setting, high summer temperature is the critical environmental factor that influences the seasonality of infection. This study demonstrates that *Cyclospora* infections in Haiti are common in the general population."

**170 Enweani IB, Esebelahie NO, Obroku J, Obi LC. Use of soursop and sweetsop juice in the management of diarrhoea in children (letter). J Diarrhoeal Dis Res 1998 Dec; 16(4):252-3. 5 ref, Eng.** Department of Microbiology, Faculty of Natural Sciences, Edo State University, P.M.B. 14, Ekpoma, Edo State, Nigeria

**171 Faruque ASG, Mahalanabis D, Haque SS, Fuchs GJ, Habte D. Double-blind, randomized, controlled trial of zinc or vitamin A supplementation in young children ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh.**

"In a double-blind, controlled trial with a factorial design, 684 patients (aged 6 months to 2 y; excludes 6 early



dropouts) with acute watery diarrhoea of 3 d or less and some dehydration, who were attending a hospital, were randomly assigned to 4 groups to receive: (a) vitamin A 4500 mg retinol equivalent daily for 15 d; (b) 14.2 mg elemental zinc as acetate for the first 417 patients and 40 mg of the remaining 273 patients randomized to this group for 15 d; (c) both vitamin A 4500 mg retinol equivalent and zinc at the above doses daily for 15 d; or (d) placebo mixtures for 15 d. Patients were observed in the hospital for 24 h and followed up at home for 15 d. All received ascorbic acid 30 mg with each dose of medicine or placebo. Zinc supplementation was associated with a reduced duration of diarrhoea (13%,  $p=0.03$ ) and markedly reduced rate (43%,  $p=0.017$ ) of prolonged diarrhoea ( $>7$  d). Vitamin A supplementation was associated with a non-significant trend for reduced rate of prolonged diarrhoea ( $p=0.089$ ). In conclusion, zinc supplementation as adjunct therapy had a substantial impact on the rate of prolonged diarrhoea and some impact on duration and may be beneficial in children with diarrhoea in developing countries."

**172 Faruque SM, Siddique AK, Saha MN, Asadulghani, Rahman MM, Zaman K, Albert MJ, Sack DA, Sack RB. Molecular characterization of a new ribotype of *Vibrio cholerae* O139 Bengal associated with an outbreak of cholera in Bangladesh. J Clin Microbiol 1999 May;37(5):1313-8. 36 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh**

"*Vibrio cholerae* O139 Bengal initially appeared to the southern coastal region of Bangladesh and spread northward, causing explosive during 1992 and 1993. The resurgence of *V. cholerae* O139 during 1995 after its transient displacement by a new clone of El Tor vibrios demonstrated rapid changes in the epidemiology of cholera in Bangladesh. A recent outbreak of cholera in two north-central districts of Bangladesh caused by *V. cholerae* O139 led us to analyze strains collected from the outbreak and compare them with *V. cholerae* O139 strains isolated from other regions of Bangladesh and neighboring India to investigate their origins. Analysis of restriction fragment length polymorphisms in genes for conserved rRNA (ribotype) revealed that the recently isolated *V. cholerae* O139 strains belonged to a new ribotype which was distinct from previously described ribotypes of toxigenic *V. cholerae* O139. All strains carried the genes for toxin-coregulated pili (*tcpA* and *tcpI*) and accessory colonization factor (*acfB*), the regulatory gene *toxR*, and multiple copies of the lysogenic phage genome encoding cholera toxin (CTXF) and belonged to a previously described *ctxA* genotype. Comparative analysis of the *rfb* gene cluster by PCR revealed the absence of a large region of the O1-specific *rfb* operon downstream of the *rfaD* gene and the presence of an O139-specific genomic region in all O139 strains. Southern hybridization analysis of the O139-specific genomic region also produced identical restriction patterns in strains belonging to the new ribotype and those of

previously described ribotypes. These results suggested that the new ribotype of Bengal vibrios possibly originated from an existing strain of *V. cholerae* O139 by genetic changes in the rRNA operons. In contrast to previously isolated O139 strains which mostly had resistance to trimethoprim, sulfamethoxazole, and streptomycin encoded by a transposon (SXT element), 68.6% of the toxigenic strains analyzed in the present study, including all strains belonging to the new ribotype, were susceptible to these antibiotics. Molecular analysis of the SXT element revealed possible deletion of a 3.6-kb region of the SXT element in strains which were susceptible to the antibiotics. Thus, *V. cholerae* O139 strains in Bangladesh are also undergoing considerable reassortments in genetic elements encoding antimicrobial resistance."

**173 Fayad IM, Hashem M, Hussein A, Zikri MA, Santosham M. Comparison of soy-based formulas with lactose and with sucrose in the treatment of acute diarrhea in infants. Arch Pediatr Adolesc Med 1999 Jul;153(7):675-80. 15 ref, Eng. Gastroenterology Unit, Hospital Abu El-Reeche, Cairo University, Cairo, Egypt**

"**Objective:** To evaluate the effect of feeding infants a soy-based formula with lactose compared with a soy-based formula with sucrose during an acute diarrheal episode. **Participants and methods:** Two hundred boys, aged 3 to 18 months, who were admitted to the hospital with acute diarrhea and signs of dehydration were randomly assigned to receive a soy-based formula with lactose or sucrose after initial rehydration. Intake and output (stool, urine, and vomit) were measured and recorded every 3 hours until diarrhea resolved. **Results:** The stool output during the first 24 hours of maintenance therapy, the total stool output during maintenance therapy, and the stool output during the entire illness (measured in grams per kilograms) were significantly lower among patients who received the soy-based formula with sucrose ( $P<0.05$ ,  $P<0.001$ , and  $P<0.001$ , respectively) than among patients who received the soy-based formula with lactose. The duration of diarrhea was significantly shorter among patients who received the soy-based formula with sucrose ( $P<0.001$ ). The relative risk of being withdrawn from the study increased to 1.95 (95% confidence interval, 0.65-9.2) and the relative risk of recurrence of dehydration after feeding was initiated increased significantly to 3.49 (95% confidence interval, 1.1-9.6;  $P<0.01$ ) in the group receiving the soy-based formula with lactose. **Conclusion:** During diarrheal episodes, feeding infants a soy-based formula with sucrose has a better outcome (lower stool output, shorter duration of diarrhea, and lower failure rates) than feeding infants a soy-based formula with lactose."

**174 Fitzpatrick M. Haemolytic uraemic syndrome and *E. coli* O157: prevention rests with sound public health measures. Br Med J 1999 Mar 13;318(7185):684-5. 10 ref, Eng. St. James's University Hospital, Leeds LS9 7TF, UK**

**175 Frost F, Craun GF, Calderon RL. Increasing**

**hospitalization and death possibly due to *Clostridium difficile* diarrheal disease. *Emerging Infect Dis* 1998 Oct-Dec;4(4):619-25. 13 ref, Eng.** Southwest Center for Managed Care Research, 2425 Ridgecrest Drive, S.E., Albuquerque, NM 87108, USA

“This study calculated yearly estimated national hospital discharge (1985 to 1994) and age-adjusted death rates (1980 to 1992) due to bacterial, viral, protozoal, and ill-defined enteric pathogens. Infant and young child hospitalization (but not death) rates in each category increased more than 50% during 1990 to 1994. Age-adjusted death and hospitalization rates due to enteric bacterial infections and hospitalizations due to enteric viral infections have increased since 1988. The increases in hospitalization and death rates from enteric bacterial infections were due to a more than eightfold increase in rates for specified enteric bacterial infections that were uncoded during this period (ICD9 00849). To identify bacterial agents responsible for most of these infections, hospital discharges and outpatient claims (coded with more detail after 1992) were examined for New Mexico’s Lovelace Health Systems for 1993 to 1996. Of diseases due to uncoded enteric pathogens, 73% were due to *Clostridium difficile* infection. Also, 88% of Washington State death certificates (1985 to 1996) coded to unspecified enteric pathogen infections (ICD0084) listed *C. difficile* infection.”

**176 Ghenghesh K, Bara F, Bukris B, Abeid S. *Shigella*-associated diarrhea in children in Tripoli – Libya. *Saudi Med J* 1997 Nov-Dec;18(6):557-9. 21 ref, Eng.** Department of Medical Microbiology, Faculty of Medicine, Al-Fateh University of Medical Sciences, Tripoli, Libya

“To determine the prevalence of *Shigella* species in Libyan children with diarrhea and in controls and susceptibility of the isolates to antibiotics. **Methods:** Between September, 1992 and August, 1993, stool specimens from 157 children with diarrhea (cases) and 157 age and sex matched controls were examined for the presence of *Shigella*. The children aged between a few days and 3 years. **Results:** *Shigella* spp. were isolated from 9 (5.7%) cases and 2 (1.3%) controls (odds ratio, 4.7;  $p=0.03$ ) with *Shigella flexneri* type 2 the predominating serotype. Most (56%) of *Shigella* strains from cases were isolated in autumn. The prevalence of *shigellae* were significantly higher in children older than one year and were not isolated from children less than six months old. All (100%) cases were on artificial feeding, 78% used untreated water for drinking. Blood and mucus were observed in the stool of 56% of cases. Clinical examination revealed 67% with fever and 33% with vomiting. One case diagnosed as septicemic and another one as having generalized convulsions. All isolates were sensitive to ceftriaxone, ciprofloxacin, gentamicin, nalidixic acid, and norfloxacin. Drug resistance to 3 or more drugs was found in 45% of the isolates. **Conclusions:** Because most of the isolates were resistant to trimethoprim-sulphamethoxazole (64%), this antibiotic

should no longer be considered the drug of choice in the treatment of *Shigella*-associated diarrhea in Libya. The present study shows that *Shigella*-associated diarrhea in children is still a public health problem in Libya and proper measures to combat it should be taken.”

**177 Ghosh S, Sengupta PG, Gupta DN, Mondal SK, Goswami M, Bhattacharya SK, Sircar BK. Maternal knowledge on risk behavioural practices and its association with diarrhoea in a rural community of West Bengal, India. *J Commun Dis* 1998 Dec;30(4):251-5. 10 ref, Eng.** National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

“Knowledge of rural mothers related to five diarrhoeagenic risk behaviours, identified in an earlier study, was ascertained. A high proportion of mothers (67%-79%) had knowledge about risk of bottle feeding, non-use of soap for cleaning feeding containers, storage of drinking water in wide-mouthed vessels and indiscriminate disposal of children’s faeces. However, only around 31% of mothers were aware about danger of using pond water for cleaning feeding containers. Risk behavioural practices were less amongst mothers who had knowledge about them. Risk of diarrhoea amongst children of mothers having risk practice without knowledge as compared to those who utilised their knowledge to avoid risk practice was found significantly higher ( $p<=0.005$ ) except for bottle feeding ( $p=0.330$ ). The results of this study indicate that children can be protected significantly from diarrhoea if mothers’ diarrhoeagenic behaviours can be altered through educational intervention.”

**178 Ghosh SK, Field J, Frisardi M, Rosenthal B, Mai Z, Rogers R, Samuelson J. Chitinase secretion by encysting *Entamoeba invadens* and transfected *Entamoeba histolytica* trophozoites: localization of secretory vesicles, endoplasmic reticulum, and Golgi apparatus. *Infect Immun* 1999 Jun;67(6):3073-81. 73 ref, Eng.** Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115, USA

**179 Gilbert SA, Burton KM, Prins SE, Deregt D. Typing of bovine viral diarrhea viruses directly from blood of persistently infected cattle by multiplex PCR (note). *J Clin Microbiol* 1999 Jun;37(6):2020-3. 28 ref, Eng.** Animal Diseases Research Institute, PO Box 640, Lethbridge, Alberta, Canada T1J 3Z4

**180 Girard F, Pery P, Naciri M, Quere P. Adjuvant effect of cholera toxin on systemic and mucosal immune responses in chickens infected with *E. tenella* or given recombinant parasitic antigen per os. *Vaccine* 1999 Mar 17;17(11-12):1516-24. 34 ref, Eng.** Unite de Pathologie Aviaire et de Parasitologie, Institut Nationale Recherche Agronomique, F-37380 Nouzilly, France

**181 Gluck U, Gebbers J-O, Gluck R. Phase 1**

**evaluation of intranasal virosomal influenza vaccine with and without *Escherichia coli* heat-labile toxin in adult volunteers.** *J Virol* 1999 Sep;73(9):7780-6. 27 ref, Eng. Swiss Serum & Vaccine Institute Berne, PO Box, CH-3001 Berne, Switzerland

“Virosomal vaccines were prepared by extracting hemagglutinin (HA) and neuraminidase from influenza virus and incorporating it in the membranes of liposomes composed of phosphatidylcholine. Two intranasal spray vaccine series were prepared: one series comprised 7.5 mg of HA of each of three strains recommended by the World Health Organization and 1 mg of *Escherichia coli* heat-labile toxin (HLT), and the other contained the HA without HLT. In addition, a third vaccine preparation contained 15 mg of HA and 2 mg of HLT. The parenteral virosomal vaccine contained 15 mg of HA without additional adjuvant. The immunogenicity of a single spray vaccination (15 mg of HA and 2 mg of HLT) was compared with that of two vaccinations (7.5 mg of HA with or without 1 mg of HLT) with an interval of 1 week in 60 healthy working adults. Twenty volunteers received one parenteral virosomal vaccine. Two nasal spray vaccinations with HLT-adjuvanted virosomal influenza vaccine induced a humoral immune response which was comparable to that with a single parenteral vaccination. A significantly higher induction of influenza virus-specific immunoglobulin A was noted in the saliva after two nasal applications. The immune response after a single spray vaccination was significantly lower. It could be shown that the use of HLT as a mucosal adjuvant is necessary to obtain a humoral immune response comparable to that with parenteral vaccination. All vaccines were well tolerated.”

**182 Goosney DL, Knoechel DG, Finlay BB\*, Enteropathogenic *E. coli*, *Salmonella*, and *Shigella*: masters of host cell cytoskeletal exploitation.** *Emerging Infect Dis* 1999 Apr-Jun;5(2):216-23. 63 ref, Eng. \*Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

“Bacterial pathogens have evolved numerous strategies to exploit their host’s cellular processes so that they can survive and persist. Often, a bacterium must adhere very tightly to the cells and mediate its effects extracellularly, or it must find a way to invade the host’s cells and survive intracellularly. In either case, the pathogen hijacks the host’s cytoskeleton. The cytoskeleton provides a flexible framework for the cell and is involved in mediating numerous cellular functions, from cell shape and structure to programmed cell death. Altering the host cytoskeleton is crucial for mediating pathogen adherence, invasion, and intracellular locomotion. We highlight recent advances in the pathogenesis of enteropathogenic *Escherichia coli*, *Salmonella* Typhimurium, and *Shigella flexneri*. Each illustrates how bacterial pathogens can exert dramatic effects on the host cytoskeleton.”

**183 Guerrant RL. Cryptosporidiosis: an**

**emerging, highly infectious threat.** *Emerging Infect Dis* 1997 Jan-Mar;3(1):51-5. 12 ref, Eng. Division of Geographic and International Medicine, University of Virginia School of Medicine, Health Sciences Center #485, Bldg. MR-4, Rm 3146, Charlottesville, VA 22908, USA

“*Cryptosporidium parvum*, a leading cause of persistent diarrhea in developing countries, is a major threat to the U.S. water supply. Able to infect with as few as 30 microscopic oocysts, *Cryptosporidium* is found in untreated surface water, as well as in swimming and wade pools, day-care centers, and hospitals. The organism can cause illnesses lasting longer than 1 to 2 weeks in previously healthy persons or indefinitely in immunocompromised patients; furthermore, in young children in developing countries, cryptosporidiosis predisposes to substantially increased diarrheal illnesses. Recent increased awareness of the threat of cryptosporidiosis should improve detection in patients with diarrhea. New methods such as those using polymerase chain reaction may help with detection of *Cryptosporidium* in water supplies or in asymptomatic carriers. Although treatment is very limited, new approaches that may reduce secretion or enhance repair of the damaged intestinal mucosa are under study.”

**184 Gupta S, Naik S, Naik SR. Vaccine potential of 56-66 kDa protease secreted by *Entamoeba histolytica*.** *Indian J Med Res* 1999 Apr;109:141-6. 28 ref, Eng. Department of Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India

“Excretory/secretory (ES) antigens and sub-cellular fractions of *E. histolytica* (HM1 : IMSS strain) were tested for the presence of common proteases using substrate gel electrophoresis. We obtained two *E. histolytica* proteases (56-66 kDa and 29 kDa) from ES material, soluble components and plasma membrane. Protease 56-66 kDa from ES antigen was selected for immunizing hamsters because it gave a consistent broad band. We observed 62.5 per cent protection in immunized animals, compared to 0 per cent in unimmunized controls. Although all vaccinated golden hamsters showed high antibody response, there was no correlation between antibody titres and protection. 56-66 kDa ES protease could thus prevent disease and could be a candidate molecular vaccine against amoebiasis.”

**185 Gupta V, Ray P, Sharma M. Antimicrobial resistance pattern of *Shigella* & non-typhi *Salmonella* isolated from patients with diarrhoea.** *Indian J Med Res* 1999 Feb;109:43-5. 16 ref, Eng. Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh 160012, India

“A total of 3500 stool samples from patients with diarrhoea were evaluated for Isolation of *Shigella* and non-typhi *Salmonella* organisms and their antimicrobial resistance

pattern from January 1994 to January 1998. The isolated strains included *S. typhimurium* (99), *S. senftenberg* (35), *S. enteritidis* (1), *Salmonella* groups B and C (1, 2); and *Sh. dysenteriae* (11), *Sh. flexneri* (13), *Sh. boydii* (2) and *Sh. sonne* (1). Amongst the *Shigella* species, very few strains showed resistance to the routinely used antimicrobial agents like furazolidone, nalidixic acid, gentamicin, chloramphenicol, norfloxacin etc. On the contrary, most of the *Salmonella* strains were resistant to these agents. *S. senftenberg* showed resistance to even ciprofloxacin, which has earlier not been reported in the literature; 100 per cent sensitivity was shown only to amikacin. The emergence of resistance to ciprofloxacin is a matter of concern and can be checked by limiting the use of the drug to multi-drug resistant strains of *Shigella* and *Salmonella* rather than indiscriminately using it for all patients with diarrhoea.”

**186 Gusmão RHP\*, Mascarenhas JDAP, Gabbay YB, Lins-Lainson Z, Ramos FLP, Monteiro TAF, Valente SA, Fagundes-Neto U, Linhares AC. Rotavirus subgroups, G serotypes, and electrophoretotypes in cases of nosocomial infantile diarrhoea in Belém, Brazil. J Trop Pediatr 1999 Apr;45(2):81-6. 25 ref, Eng. \***Instituto Evandro Chagas, Av. Almirante Barroso, 492, 66.090-000, Belem, Para, Brasil

“From November 1992 to November 1994 stool samples were obtained from 237 children admitted to a public hospital in Belém. Rotaviruses were detected in 19.2 per cent (60/310) of faecal samples. Of these, 32.1 per cent (18/56), 20.9 per cent (38/181), and 5.4 per cent (4/73) were recorded in cases of nosocomial diarrhoea, community-acquired diarrhoea, and controls respectively. Fifty-two (86.7 per cent) of the 60 rotavirus-positive specimens were subgrouped and the G serotypes of 55 (91.7 per cent) of them were determined. Subgroups I and II were detected in 50 per cent each of the 52 subgrouped strains. G type 2 was present in 46 (83.6 per cent) of the 55 serotyped samples; serotypes G1 and (mixed) G1 and G4 were found in 14.5 per cent and 1.8 per cent, respectively, of these specimens. Viral RNA electrophoresis showed 14 distinct patterns, including 56.7 per cent (34/60) and 43.3 per cent (26/60) of long and short profiles, respectively. In 40 (66.6 per cent) of the 60 rotavirus-positive faecal samples no enteropathogens other than rotavirus were detected. There was an increased incidence of rotavirus infection from July 1993 to February 1994. The rotavirus-related episodes of diarrhoea were more severe than those of other aetiology and greater clinical severity was not related to a specific G type, subgroup, or electrophoretotype.”

**187 Gustafsson A, Berstad A, Lund-Tønnesen S, Midtvedt T, Norin E\*. The effect of faecal enema on five microflora-associated characteristics in patients with antibiotic-associated diarrhoea. Scand J Gastroenterol 1999 Jun;34(6):580-6. 32 ref, Eng. \***Laboratory of Medical Microbial Ecology,

Department of Cell and Molecular Biology, Doktorsringen 4 A, Karolinska Institute, SE-171 77 Stockholm, Sweden

“**Background:** Patients with antibiotic-associated diarrhoea (AAD) show significant disturbances in short-chain fatty acid pattern. In the present study five more microflora-associated characteristics (MACs) were investigated before and after administration of an enema containing faecal microflora from a healthy person on a Western diet. **Methods:** The functions of the microflora were determined with gas chromatography, electrophoresis, and spectrophotometry. **Results:** The conversion of cholesterol to coprostanol and the concentration of urobilinogen and trypsin were significantly reduced in comparison with healthy persons. The pattern of mucin was altered, but b-aspartylglycine remained the same as in healthy persons. Enema treatment influenced these functions to different extents. **Conclusion:** Most MACs were significantly disturbed in patients with AAD. Administration of a human faecal enema modified these changes and relieved diarrhoea, usually within 4 days.”

**188 Haque R, Ali IM, Petri WA, Jr. Prevalence and immune response to *Entamoeba histolytica* infection in preschool children in Bangladesh. Am J Trop Med Hyg 1999 Jun; 60(6):1031-4. 16 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh**

“*Entamoeba histolytica* infection was present in 5% and *E. dispar* in 13% of asymptomatic 2-5-year-old children from an urban slum of Dhaka, Bangladesh. *Entamoeba dispar*-infected children were no more likely than uninfected children to have serum antibodies to lectin. In contrast, all children infected with *E. histolytica* had serum antibodies to lectin. This anti-lectin response included-antibodies against the carbohydrate recognition domain, which have been demonstrated in animal models to confer passive protection from amebiasis. Antibodies to lectin persisted in the sera of 17 children with *E. histolytica* infection over one year of follow-up, during which time *E. histolytica* infection cleared without treatment in 15, and anti-amebic medication in two. We conclude that half of the children in this population have serologic evidence of amebiasis by five years of age, and that an anti-lectin serum antibody response is associated with limitation of *E. histolytica* infection to the colon.”

**189 Hardie RM, Wall PG, Gott P, Bardhan M, Bartlett CLR. Infectious diarrhea in tourists staying in a resort hotel. Emerging Infect Dis 1999 Jan-Feb;5(1):168-71. 6 ref, Eng. Public Health Laboratory Service, Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ, UK**

**190 Hove H, Nørgaard H, Mortensen PB. Lactic acid bacteria and the human gastrointestinal tract. Eur J Clin Nutr 1999 May;53(5):339-50. 120 ref, Eng. Medical Department CA, Division of**

Gastroenterology, Rigshospitalet and Paediatric Department L, Gentofte University Hospital, Denmark

**191 Iqbal J, Munir MA, Khan MA. Cryptosporidium infection in young children with diarrhea in Rawalpindi, Pakistan. Am J Trop Med Hyg 1999 May;60(5):868-70. 24 ref, Eng.** Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait

"Fecal excretion of *Cryptosporidium parvum* oocysts was determined in 625 children less than five years old who presented at the pediatric clinic of a teaching hospital in Rawalpindi, Pakistan. Single stool specimens were collected from 475 children with acute diarrhea and from 150 children without diarrhea. The *Cryptosporidium* infection rate was significantly higher in children with diarrhea than in children without diarrhea (10.3% versus 3.3%). The *C. parvum* infection rate was highest in children 19-24 months of age (21.8%). There was no significant difference in the *Cryptosporidium* infection rate among male and female children of any age group studied. Socio-demographic information, drinking water supply, and contact with domestic animals had no significant role in the acquisition of *C. parvum* infection in our study population. The data suggest that *C. parvum* is relatively endemic in young children in the Rawalpindi area and that *C. parvum* may be an important pathogen associated with diarrhea."

**192 Isaka M, Yasuda Y, Kozuka S, Taniguchi T, Miura Y, Matano K, Goto N, Tochikubo K. Intranasal or subcutaneous co-administration of recombinant cholera toxin B subunit stimulates only a slight or no level of the specific IgE response in mice to tetanus toxoid. Vaccine 1999 Feb 26;17(7-8):944-8. 28 ref, Eng.** Department of Microbiology, Nagoya City University Medical School, Mizuho-ku, Nagoya 467-8601, Japan

**193 Islam MS, Hossain MS, Hasan MK, Rahman MM, Fuchs G, Mahalanabis D, Baqui AH, Albert MJ. Detection of *Shigellae* from stools of dysentery patients by culture and polymerase chain reaction techniques. J Diarrhoeal Dis Res 1998 Dec;16(4):248-51. 10 ref, Eng.** ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

"In Bangladesh, the isolation rates of *Shigella* spp. range from 11% to 12% by the conventional culture technique. Since the sensitivity of this technique is low, the polymerase chain reaction (PCR) technique was used for detecting small number of *Shigellae* from patients' stools. Sensitivity and specificity of the two techniques were also compared. Stool samples were collected from 41 patients with dysentery who attended the Clinical Research and Service Centre of the ICDDR,B: Centre for Health and Population Research. All stool specimens were directly plated onto MacConkey, Salmonella-Shigella, Xylose lysin deoxycholate and Hectoen enteric agar

media, and *Shigellae* were detected following standard procedures. DNA was extracted from the stool samples, and the target sequence of invasive plasmid antigen (*ipa*)H locus was amplified by PCR with 130 ng each of two primers (primer H8 [5'-GTTTCCTTGACCGCCTTTCCGATAC-3'] and primer H15 [5'-GCCGGTCAGCCACCCTA-3']) following standard procedures. The amplified product was hybridized using an *ipa*H probe. The isolation rates of *Shigella dysenteriae* type 1, *S. flexneri*, *S. sonnei*, and *S. boydii* were, respectively, 17.1%, 19.5%, 4.9% and 2.4% by the conventional method. The results of the PCR technique showed that 700 bp fragment was generated in 18 of the 18 culture-positive and in 7 of the 23 culture-negative stools. One hundred twenty-three strains of *Escherichia coli* were also tested by PCR for identifying the enteroinvasive *E. coli*, but none of them yielded any positive result. This study showed that the sensitivity of the culture technique is 72% and specificity is 100%, when the PCR technique was considered as gold standard. Therefore, the PCR may be considered a more sensitive and specific technique than the conventional culture technique and has the potential to be employed in routine diagnosis of dysentery in clinical centres as well as in epidemiologic studies."

**194 Jayshree RS, Acharya RS, Sridhar H. *Cyclospora cayetanensis*-associated diarrhoea in a patient with acute myeloid leukaemia. J Diarrhoeal Dis Res 1998 Dec;16(4):254-55. 9 ref, Eng.** Department of Microbiology, Kidwai Memorial Institute of Oncology, Bangalore 560029, India

**195 Jenkins MC, O'Brien C, Trout J, Guidry A, Fayer R. Hyperimmune bovine colostrum specific for recombinant *Cryptosporidium parvum* antigen confers partial protection against cryptosporidiosis in immunosuppressed adult mice. Vaccine 1999 May 14;17(19):2453-60. 42 ref, Eng.** Immunology and Disease Resistance Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705, USA

**196 Jiang B, Estes MK, Barone C, Barniak V, O'Neal CM, Ottaiano A, Madore HP, Conner ME. Heterotypic protection from rotavirus infection in mice vaccinated with virus-like particles. Vaccine 1999 Feb 26;17(7-8):1005-13. 35 ref, Eng.** Wyeth-Lederle Vaccines and Pediatrics, 401 North Middletown Road, Pearl River, NY 10965, USA

"Virus-like particles (VLPs) composed of rotavirus VP2, VP6, and VP7 of G1 or G3 serotype specificity were produced in insect cells coinfecting with recombinant baculoviruses expressing single rotavirus genes. The VLPs were purified and subsequently evaluated for immunogenicity and protection in the adult mouse model of rotavirus infection. Mice were vaccinated twice intramuscularly with G1 VLPs formulated with *Quillaja saponaria* (QS-21) or adsorbed to aluminium hydroxide (AIOH), or with G1 VLPs alone. G3 VLPs, G1 plus G3

VLPs' inactivated SA11 varions formulated with QS-21, or adjuvants were similarly inoculated as controls. Mice were examined for serum and fecal antibody responses by ELISA or microneutralization assays. Protective efficacy of the VLP vaccine formulations against oral challenge with the G3 murine EC<sub>wt</sub> rotavirus was assessed by comparing the antigen shed in stool of the VLP-vaccinated mice to that of the adjuvant-immunized mice. G1 VLPs in QS-21 induced significantly higher serum and intestinal antibody titers than G1 VLPs in AIOH or G1 VLPs alone. QS-21 also heightened serum and fecal antibody responses to G3 VLPs. These QS-21-augmented antibody responses were further characterized by equivalent IgG1 and IgG2a titers in sera, suggesting that G1 or G3 VLPs in QS-21 induced a balanced Th1/Th2 response. G1 VLPs in QS-21 induced partial protection (88%) against oral challenge with the heterotypic EC<sub>wt</sub> virus, whereas G3 VLPs in QS-21 induced complete protection (100%). In contrast, G1 VLPs when formulated with AIOH induced a predominant Th2 response and did not protect (1%) mice from virus challenge. Our results indicate that the type of adjuvant used clearly influences both antibody responses to rotavirus VLPs and the protective efficacy against rotavirus infections. These data have important implications for the development of parenteral vaccines to ameliorate rotavirus disease."

**197 Kamstrup S, Roensholt L, Jensen MH, Dalsgaard K. Production of a highly immunogenic subunit ISCOM vaccine against bovine viral diarrhoea virus. Vaccine 1999 Mar 5;17(9-10):1057-64. 25 ref, Eng.** Danish Veterinary Institute for Virus Research, Lindholm, DK-4771 Kalvehave, Denmark

**198 Karki BMS, Parija SC. Intestinal parasitic infections in Dharan (Nepal): a hospital based study. J Commun Dis 1998 Dec;30(4):295-6. 9 ref, Eng.** B.P. Koirala Institute of Health Science, Dharan, Nepal

**199 Kartalija M, Sande MA. Diarrhea and AIDS in the era of highly active antiretroviral therapy. Clin Infect Dis 1999 Apr;28(4):701-7. 41 ref, Eng.** Department of Internal Medicine, University of Utah, 50 North Medical Drive, 4C104 SOM, Salt Lake City, Utah 84132, USA

**200 Kaur H, Lal M. Reappearance of *Vibrio cholerae* O139 during March-August, 1998 in Ludhiana (Punjab), India. Indian J Med Res 1999 Jan;109:3-4. 6 ref, Eng.** Department of Microbiology, Christian Medical College & Hospital, Ludhiana 141008, India

"An outbreak of *V. cholerae* O139 was reported from Ludhiana in 1993. Strict bacteriological vigilance between 1994 and 1997 showed no isolates of O139, while O1 strains continued to be isolated. In 1998, 30 strains of *V. cholerae* O139 and 15 strains of *V. cholerae* O1 were isolated. Both strains appear to be endemic in Punjab."

**201 Kearney DJ, Steuerwald M, Koch J, Cello JP. A prospective study of endoscopy in HIV-associated diarrhoea. Am J Gastroenterol 1999 Mar;94(3):596-602. 38 ref, Eng.** Seattle VA Medical Center 111G1, 1660 South Columbian way, Seattle, WA 98108, USA

"**OBJECTIVE:** Diarrhoea commonly occurs in persons with human immunodeficiency virus (HIV) infection. The optimal use of endoscopic procedures remains poorly studied for patients with HIV-related diarrhoea. The purpose of this study is to compare the diagnostic yield of a complete endoscopic work-up including an esophagogastroduodenoscopy and colonoscopy to a more limited approach of biopsies obtainable by flexible sigmoidoscopy. **METHODS:** A prospective study of 79 patients with HIV-related diarrhoea. Upper endoscopy and colonoscopy were performed with tissue biopsies labelled according to location within the colon or small intestine. **RESULTS:** A new infection was diagnosed in 22 of 79 patients (28%). Biopsy of the left colon yielded an enteric pathogen in 17 of 22 patients (sensitivity: 77%) and in 15 of 15 patients with cytomegalovirus colitis (sensitivity: 100%). Combined left and right colonic biopsies had a sensitivity of 82%. Combined colonic and terminal ileum biopsies missed no pathogens. Duodenal biopsies yielded no additional pathogens beyond those identified by colonoscopy and terminal ileal biopsy. Patients with a new pathogen diagnosed had significantly lower CD4 lymphocyte counts as compared to patients without a new pathogen ( $p=0.001$ ). **CONCLUSIONS:** For patients with CD4 counts  $<100/\text{mm}^3$  and unexplained AIDS-related diarrhoea, flexible sigmoidoscopy with biopsy is a sufficiently thorough endoscopic evaluation."

**202 Kelkar SD, Purohit SG, Simha KV. Prevalence of rotavirus diarrhoea among hospitalized children in Pune, India. Indian J Med Res 1999 Apr;109:131-5. 30 ref, Eng.** National Institute of Virology, Abasaheb Garware College, Pune 411001, India

"Rotavirus was detected in 266 (28.15%) out of 945 faecal specimens collected between July 1992 and June 1996 from children  $\leq 5$  yr of age. Statistical analysis using odds ratios and multivariate logistic regression analysis revealed that seasonality had a strong influence on the number of rotavirus diarrhoea cases admitted to the hospital. Maximum cases occurred in the winter and minimum in the rainy season. Age was strongly associated with the prevalence of rotavirus diarrhoea. The age group of 6-24 months was the most susceptible. This disease was more predominant in males."

**203 Keskimaki M, Ratiner Y, Oinonen S, Leijala E, Nurminen M, Saari M, Siitonen A\*. Haemolytic-uraemic syndrome caused by vero toxin-producing *Escherichia coli* serotype rough: K<sup>-</sup>: H49. Scand J Infect Dis 1999;31(2):141-4. 23 ref, Eng.** \*National Public Health Institute, Laboratory of Enteric Pathogens, Mannerheimintie 166, FI-00300 Helsinki, Finland

"The first case of haemolytic-uraemic syndrome (HUS)

caused by vero toxin-producing *Escherichia coli* (VTEC) which belonged to a novel serotype, Rough: K<sup>-</sup>: H49, is reported. The case was initially diagnosed as nephropathia epidemica caused by Puumala virus, but the subsequent diagnosis of HUS caused by VTEC was made after bacteriological investigation. The strain isolated fermented sorbitol produced VT2 toxin but not enterohaemolysin, nor did it carry the *eaeA* gene. In VTEC strains, the O antigen, the *eaeA* gene and enterohaemolysin production have been characterized as virulence-associated factors and believed to have an effect on pathogenesis of these strains to cause haemorrhagic colitis or HUS. The findings of this study demonstrate that there is a need for further studies to evaluate the pathogenetic mechanism of VTEC and need for easy diagnostic methods exploiting other properties than O157 antigen and non-fermentation of sorbitol to find all VTEC in human infections."

**204 Kien CL, Murray RD, Qualman SJ, Marcon M. Lactulose feeding in piglets: a model for persistent diarrhea and colitis induced by severe sugar malabsorption. Dig Dis Sci 1999 Jul;44(7):1476-84. 55 ref, Eng.** Room W209, Children's Hospital, 700 Children's Dr., Columbus, Ohio 43205, USA

"We sought to determine, in a piglet model, whether severe sugar malabsorption causes colonic injury or inflammation. Twenty-four piglets were randomized to receive either control formula (CON) or CON supplemented with lactulose (LAC) ( $N = 12$  each group). After seven days, inflammation, apoptosis, and crypt cell proliferation were assessed in the proximal colon (cecum). Lactulose feeding caused persistent diarrhea. In both groups, breath  $H_2$  concentration was low, suggesting no increased fermentation in the LAC group. Weight gain/volume formula intake was identical in the CON and LAC groups ( $0.09 \pm 0.13$  and  $0.09 \pm 0.11$  g/ml) respectively. Injury to the colon did not occur, but inflammation of the colon (scale 0-5) was greater in LAC (score of  $1.5 \pm 1.38$ ) than in CON ( $0.42 \pm 0.79$ ;  $P < 0.05$ ). Cell proliferation at the basal 40% of the Crypt was 92% increased in CON (labeling index  $22.8 \pm 9.9$  vs  $11.9 \pm 2.8$ ;  $P < 0.05$ ). We conclude that persistent feeding during severe sugar malabsorption permits weight gain but may cause colitis."

**205 Kojima S, Yamamoto K, Kawagishi I, Homma M\*. The polar flagellar motor of *Vibrio cholerae* is driven by an  $Na^+$  motive force (note). J Bacteriol 1999 Mar;181(6): 1927-30. 39 ref, Eng.** \*Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan

**206 Kovacicova G, Skorupski K. A *Vibrio cholerae* LysR homolog, AphB, cooperates with AphA at the *tcpPH* promoter to activate expression of the ToxR virulence cascade. J Bacteriol 1999 Jul;181(14):4250-6. 38 ref, Eng.** Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA

"We describe here a new member of the LysR family of transcriptional regulators, AphB, which is required for activation of the *Vibrio cholerae* ToxR virulence cascade. AphB activates the transcription of the *tcpPH* operon in response to environmental stimuli, and this process requires cooperation with a second protein, AphA. The expression of neither *aphA* or *aphB* is strongly regulated by environmental stimuli, raising the possibility that the activities of the proteins themselves may be influenced under various conditions. Strains of the El Tor biotype of *V. cholerae* typically exhibit lower expression of ToxR-regulated virulence genes in vitro than classical strains and require specialized culture conditions (AKI medium) to induce high-level expression. We show here that expression of *aphB* from the *tac* promoter in El Tor biotype strains dramatically increases virulence gene expression to levels similar to those observed in classical strains under all growth conditions examined. These results suggest that AphB plays a role in the differential regulation of virulence genes between the two disease-causing biotypes."

**207 Levin BR. The evolution and maintenance of virulence in microparasites. Emerging Infect Dis 1996 Apr-Jun;2(2):93-102. 62 ref, Eng.** Emory University, ECLF, 1510 Clifton Road, N.E., Atlanta, GA 30322, USA

**208 Lunn PG, Erinoso HO, Northrop-Clewes CA, Boyce SA. *Giardia intestinalis* is unlikely to be a major cause of the poor growth of rural Gambian infants. J Nutr 1998 Apr; 129(4):872-7. 29 ref, Eng.** M.R.C. Dunn Nutritional Laboratory, Downham's Lane, Milton Road, Cambridge CB4 1XJ, UK

"Parasite-specific plasma immunoglobulins have been used to indicate the presence of *Giardia intestinalis* infection in 60 infants living in a rural area of the Gambia. Infants were studied longitudinally between 2 and 8 mo of age. The median age for first exposure to *G. intestinalis* was between 3 and 4 mo, and by 8 mo all but 3 infants (95%) showed a positive titer on at least one occasion. Raised *Giardia*-specific IgM titers were associated with reduced weight gain in the 2 wk preceding a positive titer, but catch-up growth occurred in the following 2 wk. IgM antibody titers were also positively associated with intestinal permeability (lactulose/mannitol ratio), urinary lactose excretion, plasma concentrations of  $\alpha$ -antichymotrypsin and total IgM, IgA and IgG immunoglobulins. However, infant growth over the whole 6-mo period (i.e., between 2 and 8 mo of age) was not related to mean *Giardia*-specific antibody titers, nor the time of first exposure to the parasite. The data suggest that giardiasis in these very young breast-fed children occurs as a mild, acute disease, and its presence could not explain the marked, long-term growth faltering observed in many of the subjects."

**209 Lysy J, Israeli E, Goldin E. The prevalence of chronic diarrhea among diabetic patients. Am J Gastroenterol 1999 Aug;94(8):2165-70. 22 ref, Eng.**

Department of Gastroenterology, Hadassah University Hospital, POB 12000, 91120 Jerusalem, Israel

**“OBJECTIVE:** The prevalence of chronic diarrhea in patients with type I and type II diabetes is uncertain, most data being available from tertiary referral centers. We report the prevalence and etiology of chronic diarrhea in 861 heterogeneous diabetic patients attending a primary care diabetic outpatient clinic. **METHODS:** All patients attending the clinic were asked to fill in a questionnaire relating to their bowel habits. Patients who fulfilled the criteria for chronic diarrhea underwent a comprehensive workup to define the cause of the diarrhea. Additional parameters were the mean duration of diabetes, hemoglobin-A1c levels, and the presence of autonomic neuropathy. **RESULTS:** Chronic diarrhea was diagnosed in 32 patients (overall prevalence of 3.7%). The prevalence of nondiabetic diarrhea was higher among type I diabetic patients than among type II patients (3.29% vs 2.3%), although it did not reach statistical significance. Diabetic diarrhea was more common among type I than type II diabetic patients (5.2% vs 0.4%, respectively,  $p < 0.01$ ). The most common cause of nondiabetic diarrhea was medication induced (metformin). **CONCLUSIONS:** Chronic diarrhea is more frequent in type I diabetic patients. The higher prevalence of diarrhea in this population can be attributed to diabetic diarrhea (which is quite rare in type II patients). The most common cause of nondiabetic diarrhea is drug therapy with metformin.”

**210 McCarthy N, Andersson Y, Jormanainen V, Gustavsson O, Giesecke J. The risk of Guillain-Barré syndrome following infection with *Campylobacter jejuni*. *Epidemiol Infect* 1999 Feb;122(1):15-7. 16 ref, Eng.** The Food Safety Authority of Ireland, Abbey Court, Abbey Street, Dublin 1, Ireland, UK

“To estimate the incidence of Guillain-Barré syndrome (GBS) following *Campylobacter jejuni* infection (CI) we studied three populations where outbreaks of CI had occurred involving an estimated 8000 cases. No case of GBS was detected in the 6 months following the outbreaks in the local populations. The point estimate for the risk of GBS following CI estimated in this study was 0 in 8000 (95% confidence interval 0-3).”

**211 McLennan JD. Knowledge and practices of preventing diarrhoea in malnourished children. *J Diarrhoeal Dis Res* 1998 Dec;16(4):235-40. 28 ref, Eng.** Behavioral Health Center, Carolinas Medical Center, 501 Billingsley Road, Charlotte NC 28211, USA

“Instructions for preventing diarrhoea, based on a knowledge-deficit model, are a common health-promotion approach aimed at the providers of child care attending nutritional rehabilitation centres. However, there is rarely an assessment of baseline knowledge to justify the need for this type of intervention and to guide its form. This study investigated the practice and knowledge of recommended diarrhoea-prevention behaviours of caregivers of 78 malnourished children consecutively

admitted to a realimentation programme. Major deficits included: 39% not boiling (or not planning on boiling) drinking water after the child reached two years of age; 35% not always washing children’s hands before meals; only 17% reporting that it was rare for their children to go barefoot; and the majority breastfeeding for less than one year. However, almost all measures of knowledge, based on open and closed questions, were not related to the corresponding practice. Several types of barriers to preventive practices were reported on open questions, including, “beliefs,” “children as barriers,” and “time.” This information may be helpful in designing more effective health-promotion programmes.”

**212 McNeal MM, Rae MN, Ward RL. Effects of different adjuvants on rotavirus antibody responses and protection in mice following intramuscular immunization with inactivated rotavirus. *Vaccine* 1999 Mar 17;17(11-12):1573-80. 28 ref, Eng.** Division of Infectious Diseases, Children’s Hospital Medical Center, 333 Burnet Avenue, Cincinnati, OH 45229-3039, USA

I.m. immunization of mice with inactivated rotavirus particles protects against subsequent infection. To optimize protection, the effects of different -adjuvants (QS-21, QS-7, QUIL A, PCPP and RAS) with potential for human use were compared. Twenty-eight days after i.m. immunization with 20 mg of purified, UV/psoralen-inactivated murine rotavirus (EDIM), either with or without adjuvant, BALB/c mice were orally challenged with live EDIM and virus shedding was measured. All five adjuvants stimulated large ( $P < 0.001$ ) increases in rotavirus antibody, but significant differences were found between adjuvants. The order of rotavirus IgG responses, i.e. no adjuvant < RAS < QS-7 < Quil A < QS-21 < PCPP, was the same as the order of protection except that QS-21 and PCPP were reversed. These results establish the importance of adjuvants during i.m. immunization with rotavirus and identify those with the greatest potential.”

**213 Makino S-i, Asakura H, Shirahata T, Ikeda T, Takeshi K, Arai K, Nagasawa M, Abe T, Sadamoto T. Molecular epidemiological study of a mass outbreak caused by enteropathogenic *Escherichia coli* O157:H45. *Microbiol Immunol* 1999;43(4):381-4. 17 ref, Eng.** Department of Veterinary Microbiology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

**214 Mascie-Taylor CGN, Alam M\*, Montanari RM, Karim R, Ahmed T, Karim E, Akhtar S. A study of the cost effectiveness of selective health interventions for the control of intestinal parasites in rural Bangladesh. *J Parasitol* 1999 Feb;85(1):6-11. 7 ref, Eng.** \*Department of Economics, University of Dhaka, Dhaka 1000, Bangladesh

“The study examined the cost effectiveness of 4 different regimens in reducing the prevalence and intensity of



infection of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm over 18-mo period in randomized community samples of children aged 2-8 yr living in rural Bangladesh. The household was the unit of randomization in each community. The 4 regimens were (1) only chemotherapy to all household members at the commencement of the study (i.e., at an interval of 18 mo), (2) same as group (1) and regular health education throughout the study period, (3) chemotherapy to all household members at the commencement of the study and subsequent chemotherapy to all children at intervals of 6 mo, and (4) same as group 3 with the addition of regular health education throughout the study period. Health education (through home and school visits and focus group discussions) was aimed at increasing awareness of worm transmission and the disabilities caused by intestinal helminths. Simple ways of improving personal hygiene and sanitation through hand washing, nail trimming, wearing of shoes, and use of a latrine and clean water supplies were encouraged. Because albendazole is a broad spectrum anthelmintic, the cost effectiveness of the 4 interventions were compared by the weighted percentage reduction in prevalence and the weighted percentage reduction in intensities of infection as measured by geometric mean egg loads of all 3 worms combined. The most cost-effective strategy was the single albendazole mass chemotherapy at an interval of 18 mo. The 2 regimens involving health education were the least cost effective."

**215 Meccas J, Strauss EJ. Molecular mechanisms of bacterial virulence: type III secretion and pathogenicity islands. *Emerging Infect Dis* 1996 Oct-Dec;2(4):271-99. 99 ref, Eng.** Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5402, USA

**216 Medrano AI, DiRita VJ, Castillo G, Sanchez J. Transient transcriptional activation of the *Vibrio cholerae* El Tor virulence regulator ToxT in response to culture conditions. *Infect Immun* 1999 May;67(5):2178-83. 36 ref, Eng.** Facultad de Medicina, Universidad Autonoma del Estado de Mexico, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Mor. Mexico 62210

"*Vibrio cholerae* El Tor require special in vitro culture conditions, consisting of an initial static growth period followed by shift to shaking (AKI conditions), for expression of cholera toxin (CT) and toxin coregulated pili (TCP). ToxT, a regulator whose initial transcription depends on the ToxR regulator, positively modulates expression of CT and TCP. To help understand control of CT and TCP in El Tor vibrios, we monitored *ctxAB* and ToxR-dependent *toxT* transcription by time course primer extension assays. AKI conditions stimulated CT synthesis with an absence of *ctxAB* transcription during static growth followed by induction upon shaking. ToxR-dependent *toxT* transcription was induced at the end of the static growth period but was transient, stopping shortly after

shaking was initiated but, interestingly, also if the static phase was prolonged. Immunoblot assays showed that ToxR protein levels were not coincidentally transient, implying a protein on/off switch mechanism for ToxR. Despite the transient activation by ToxR, transcription of *ctxAB* was maintained during shaking. This finding suggested continued *toxT* expression, possibly through relay transcription from another promoter. The 12.6-kb distant upstream *tcpA* promoter responsible for expression of the TCP operon has been proposed to provide an alternate *toxT* message by readthrough transcription. Activation of the *tcpA* promoter is supported by increased expression of TcpA protein during the shaking phase of the culture. Readthrough transcription of *toxT* from *tcpA* would be compatible with reverse transcription-PCR evidence for a *toxT* mRNA at times when ToxR-dependent transcription was no longer detectable by primer extension."

**217 Menge C, Wieler LH, Schlapp T, Baljer G. Shiga toxin 1 from *Escherichia coli* blocks activation and proliferation of bovine lymphocyte subpopulations in vitro. *Infect Immun* 1999 May;67(5):2209-17. 56 ref, Eng.** Institut für Hygiene und Infektionskrankheiten der Tiere, Frankfurter, Str. 89, D-35392 Giessen, Germany

"Shiga toxin-producing *Escherichia coli* (STEC) is widespread in the cattle population, but the clinical significance of Shiga toxins (Stx's) for the bovine species remains obscure. Since Stx's exert immunomodulating effects in other species, we examined the effect of purified Stx1 on a bovine B lymphoma cell line (BL-3) and peripheral blood mononuclear cells (PBMC) isolated from adult bovine blood by viability assays and flow cytometry analysis. Stx1 markedly induced apoptosis in stimulated BL-3 cells. The susceptibility of this B-cell-derived cell line was induced only by either lipopolysaccharide (LPS) or pokeweed mitogen, while cultures stimulated with T-cell mitogens were unaffected by the toxin. In contrast, Stx1 did not induce cellular death—neither apoptosis nor necrosis—in primary cultures of PBMC but hindered the mitogen-induced increase in metabolic activity. The influence of Stx1 on single PBMC subpopulations varied with the type of mitogenic stimulus applied. Stimulation with phytohemagglutinin P particularly induced the proliferation of bovine CD8-expressing (BoCD8<sup>+</sup>) cells, and this proliferative response was blocked by Stx1. On the other hand, Stx1 reduced the portion of viable B cells in the presence of LPS. Modulation of activation marker expression (BoCD25 and BoCD71) by Stx1 indicated that the toxin hindered the proliferation of cells by blocking their activation. In conclusion, we assume that Stx1 contributes to the pathogenesis of STEC-associated diarrhea in calves by suppressing the mucosa-associated immune response. The usefulness of cattle as a model in which to study Stx-induced immunomodulation is discussed."

**218 Morales MAG, Rosa GL, Ludovisi A, Onori AM, Pozio E. Cytokine profile induced by**

***Cryptosporidium* antigen in peripheral blood mononuclear cells from immunocompetent and immunosuppressed persons with cryptosporidiosis.** *J Infect Dis* 1999 Apr;179(4):967-73. 32 ref, Eng. Laboratory of Parasitology, Istituto Superiore di Sanita, viale Regina Elena 299, 00161 Rome, Italy

“The proliferative response of peripheral blood mononuclear cells (PBMC) to a crude extract from *Cryptosporidium parvum* (CCE) was studied in persons who acquired cryptosporidiosis in the same outbreak (15 immunocompetent subjects with prior cryptosporidiosis and 22 human immunodeficiency virus [HIV]-positive persons with various levels of immunosuppression and active cryptosporidiosis) and in individual patients (8 HIV-positive patients with active cryptosporidiosis and 15 HIV-positive persons without history of cryptosporidiosis). PBMC from HIV-positive persons showed less proliferation to CCE and mitogens than did PBMC from immunocompetent subjects with prior cryptosporidiosis, independent of CD4 cell count. In immunocompetent subjects, cytokine gene expression was consistent with cytokine production, whereas in HIV-positive subjects it was not. The production of interferon-g in CCE-stimulated PBMC from both immunocompetent and HIV-positive subjects with cryptosporidiosis and the lack of interferon-g in CCE-stimulated PBMC from HIV-positive subjects without cryptosporidiosis indicate that *C. parvum* mainly induces a Th1 response.”

**219 Morgan UM, Sturdee AP, Singleton G, Gomez MS, Gracenea M, Torres J, Hamilton SG, Woodside DP, Thompson RCA. The *Cryptosporidium* “mouse” genotype is conserved across geographic areas.** *J Clin Microbiol* 1999 May;37(5):1302-5. 20 ref, Eng. World Health Organisation Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA, 6150, Australia

**220 Mrukowicz JZ, Thompson J, Reed GW, Tollefson SJ, Kobayashi M, Araki K, Wright PF. Epidemiology of rotavirus in infants and protection against symptomatic illness afforded by primary infection and vaccination.** *Vaccine* 1999 Feb 26;17(7-8):745-53. 47 ref, Eng. II Department of Pediatrics, Polish-American Children’s Hospital, Jagiellonian University, School of Medicine, Krakow, Poland

“This study assessed the frequency of symptomatic and asymptomatic primary and secondary infections with rotavirus in children under 24 months and determined protection against symptomatic illness afforded by rhesus and human-rhesus rotavirus reassortant vaccines. Successive cohorts of children ( $n=236$ ) were followed through five winter rotavirus seasons with cultures of each reported episode of diarrheal disease and serologic determination of rotavirus exposure on paired sera bracketing the winter. An average of 46% of children experienced rotavirus infection in each season with almost

all infected by two years of age. The relative risk of rotavirus associated gastroenteritis in naïve children versus naturally immune children was 2.4 (1.1, 5.3). The relative risk of rotavirus associated gastroenteritis in naïve children versus vaccinees was 4.1 (1.6, 10.7). In a community with predominantly serotype G1 rotavirus rhesus rotavirus-based vaccines are as protective against rotavirus gastroenteritis as prior natural infection.”

**221 Nagy B, Wilson RA, Whittam TS. Genetic diversity among *Escherichia coli* isolates carrying *f18* genes from pigs with porcine postweaning diarrhea and edema disease.** *J Clin Microbiol* 1999 May;37(5):1642-5. 31 ref, Eng. Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park, PA 16802, USA

**222 Noel JS, Fankhauser RL, Ando T, Monroe SS, Glass RI. Identification of a distinct common strain of “Norwalk-like viruses” having a global distribution.** *J Infect Dis* 1999 Jun;179(6):1334-44. 64 ref, Eng. Viral Gastroenteritis Section (G-04), Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA

“Norwalk-like viruses” (NLVs) are the most common cause of outbreaks of nonbacterial gastroenteritis. During molecular surveillance of NLV strains from 152 outbreaks of gastroenteritis that occurred in the US between August 1993 and July 1997, we identified an NLV strain that predominated during the 1995-1996 season. The “95/96-US” strain caused 60 outbreaks in geographically distant locations within the US and was identified, by sequence comparisons, in an additional 7 countries on 5 continents during the same period. This is the first demonstration linking a single NLV strain globally and suggests that the circulation of these strains might involve patterns of transmission not previously considered. The diagnostic techniques are now available to establish a global network for surveillance of NLV strains that would highlight the importance of NLVs worldwide and allow molecular identification of common strains having a global distribution so as to consider interventions for their control.”

**223 Nolan TJ, Bhopale VM, Schad GA. Hyperinfective strongyloidiasis: *Strongyloides stercoralis* undergoes an autoinfective burst in neonatal gerbils.** *J Parasitol* 1999 Apr;85(2): 286-9. 9 ref, Eng. Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, 3800 Spruce St., Philadelphia, Pennsylvania 19104-6050, USA

**224 Obi CL, Coker AO, Epoke J, Ndip RN. Distributional patterns of bacterial diarrhoeagenic agents and antibiograms of isolates from diarrhoeaic and non-diarrhoeaic patients in urban and rural areas of Nigeria.** *Cent Afr J Med* 1998 Sep;44(9):223-9. 38 ref, Eng. Department of Microbiology, Delta State University, Abraka, Nigeria

**Objectives:** To determine the prevalence of bacteria that could cause diarrhoea in stool specimens of individuals with and without diarrhoea in both urban and rural areas of Nigeria. To ascertain the antibiotic susceptibilities of the bacterial diarrhoeagenic agents isolated. To document the predominant signs and symptoms associated with the various bacterial agents of diarrhoea. **Design:** Prospective study. **Setting:** Patients/individuals attending government and private clinics in Lagos, Edo and Cross-River States of Nigeria. **Subjects:** A total of 1200 stool samples were collected from patients with diarrhoea. Another total of 1 200 stool specimens were obtained from controls. **Results:** For diarrhoea cases in urban areas *Campylobacter* spp. were more predominant (28%) and were followed by enteropathogenic *Escherichia coli* (EPEC) (28%) whereas in rural areas, EPEC were the most commonly isolated bacteria (18%), closely followed by *Salmonella* spp. (16%). Controls had a similar distribution pattern. Higher rates of isolation of these enteric bacteria were recorded among diarrhoea cases than in controls ( $p < 0.05$ ). Diarrhoea due to *Vibrio*, *Yersinia*, *Aeromonas*, *Plesiomonas* and EPEC was mainly watery whereas it mainly consisted of blood/mucus for *Shigella* and *Salmonella*. All were associated with abdominal pain and fever. Results presented also indicate that over 80% of *Shigella* species, *Salmonella*, EPEC and *P. shigelloides* were susceptible to nalidixic acid and nitrofurantoin. Virtually all the enteropathogens were resistant to commonly used antibiotics such as ampicillin, erythromycin, tetracyclines and streptomycin. **Conclusion:** Results show that distributional patterns of bacterial agents of diarrhoea may vary in urban and rural areas and have revealed the effectiveness of nalidixic acid, gentamicin and nitrofurantoin, in that order against these enteropathogens.”

**225 Oyemede A, Omokhodion FO, Olawuyi JF, Sridhar MKC, Olaseha IO. Environmental and personal hygiene practices: risk factors for diarrhoea among children of Nigerian market women. J Diarrhoeal Dis Res 1998 Dec;16(4):241-7. 13 ref, Eng.** Department of Preventive and Social Medicine, University College Hospital, Ibadan, Nigeria

“A cross-sectional survey was carried out to determine the environmental and personal hygiene practices of mothers of children aged less than five years in two markets in Ibadan—one with poor sanitary conditions (Bodija) and the other one with better sanitation facilities (Gbagi). The study sought to identify the risk factors for diarrhoea among these children. Two hundred and sixty-six mothers in Bodija and 260 in Gbagi were interviewed. A questionnaire was used for collecting information on social and demographic characteristics, personal and environmental hygiene practices, including sources of food and water for their children, waste-disposal practices and occurrence of diarrhoea among their children aged less than five years. The educational status of the women in Bodija was lower than that of the women in Gbagi ( $p < 0.001$ ). Sixty (23%) women of the Bodija market

mentioned that tap water was the source of drinking water for their children, while 91 (34%) brought water from their homes, and 45 (17%) bought it from vendors in the market. The corresponding figures for women of the Gbagi market were 41 (16%), 98 (38%) and 19 (7%). Two hundred and thirty-four (90%) women in Gbagi prepared breakfast at home for their children compared to 216 (81%) women in Bodija. This difference was statistically significant ( $p < 0.05$ ). Waste disposal and personal hygiene practices were poorer among the women in Bodija. Yet the occurrence of diarrhoea was not significantly different in both the markets. Risk factors for diarrhoea identified in this study were water and food bought from vendors, child defaecation practices, mothers' cleaning up practices after child's defaecation, and refuse-disposal practices. The inherent risk of sale of unwholesome food and water by vendors is a great concern for public health authorities in Nigeria. Efforts to control diarrhoea must not only be focused on improving mothers' knowledge about food hygiene but also on environmental hygiene practices within the community.”

**226 Padilla-Vaca F, Ankri S, Bracha R, Koole LA, Mirelman D. Down regulation of *Entamoeba histolytica* virulence by monoxenic cultivation with *Escherichia coli* O55 is related to a decrease in expression of the light (35-kilodalton) subunit of the Gal/GalNAc lectin. Infect Immun 1999 May;67(5):2096-2102. 47 ref, Eng.** Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

*Entamoeba histolytica* virulence is related to a number of amebic components (lectins, cysteine proteinases, and amebapore) and host factors, such as intestinal bacterial flora. Trophozoites are selective in their interactions with bacteria, and the parasite recognition or glycoconjugates plays an important role in amebic virulence. Long-term monoxenic cultivation of pathogenic *E. histolytica* trophozoites, strains HK-9 or HM-1:IMSS, with *Escherichia coli* serotype O55, which binds strongly to the Gal/GalNAc amebic lectin, markedly reduced the trophozoites' adherence and cytopathic activity on cell monolayers of baby hamster kidney (BHK) cells. Specific probes prepared from *E. histolytica* lectin genes as well as antibodies directed against the light (35-kDa) and heavy (170-kDa) subunits of the Gal/GalNAc lectin revealed a decrease in the transcription and expression of the light subunit in trophozoites grown monoxenically with *E. coli* O55. This effect was not observed when *E. histolytica* was grown with *E. coli* 346, a mannose-binding type I pilated bacteria. Our results suggest that the light subunit of the amebic lectin is involved in the modulation of parasite adherence and cytopathic activity.”

**227 Pang X-L, Koskenniemi E, Joensuu J, Vesikari T\*. Effect of rhesus rotavirus vaccine on enteric adenovirus-associated diarrhoea in children. J Pediatr Gastroenterol Nutr 1999 Sep;29(3):366-9. 25 ref, Eng.** \*University of Tampere, Medical School, PO Box

607, 33101 Tampere, Finland

**228 Pang X-L, Vesikari T. Human astrovirus-associated gastroenteritis in children under 2 years of age followed prospectively during a rotavirus vaccine trial. Acta Paediatr 1999 May;88(5):532-6. 25 ref, Eng.** University of Tampere, Medical School, PO Box 607, 33101 Tampere, Finland

"This study evaluated the clinical significance of human astrovirus-associated gastroenteritis in young children in the community. Placebo- ( $n=1207$ ) and rhesus rotavirus tetravalent (RRV-TV) vaccine- ( $n = 1191$ ) recipient children were followed from 2 mo to 2 y of age. Stool specimens from 1528 episodes of acute gastroenteritis (805 in the placebo group and 723 in the RRV-TV vaccine group) were tested for astrovirus with a sensitive reverse transcription-polymerase chain reaction (RT-PCR) assay and positive results were confirmed by Southern hybridization using probes specific for astrovirus serotypes 1 and 2. Astroviruses were detected in 144 (9%) episodes of gastroenteritis; 92% of the findings were serotype 1 and 6% were serotype 2. The astrovirus peak season was in winter. Of the 102 children who had gastroenteritis with astrovirus as the only diarrhoea virus in the stools, 72% had watery diarrhoea, 59% had vomiting, 26% had fever, 5% needed oral rehydration and 3% were hospitalized. Overall, the clinical severity of astrovirus gastroenteritis was much lower than that of rotavirus gastroenteritis. RRV-TV rotavirus vaccine did not protect against astrovirus gastroenteritis. It is concluded that astroviruses are common causative agents in acute gastroenteritis in young children, but the symptoms of astrovirus gastroenteritis are usually mild and the illness is therefore only of minor clinical significance."

**229 Parashar UD, Bresee JS, Gentsch JR, Glass RI. Rotavirus. Emerging Infect Dis 1998 Oct-Dec;4(4):561-70. 52 ref, Eng.** Viral Gastroenteritis Section, Mail Stop G04, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Atlanta, GA 30333, USA

**230 Patra FC, Majumder RN, Eeckels R, Desjeux J-F, Mahalanabis D. Sacolene in cholera: a double blind randomized controlled trial. Scand J Infect Dis 1999;31(2): 151-4. 22 ref, Eng.** Society for Applied Studies, 108 Manicktala Main Road, Flat 3/21, Calcutta 700054, India

"Methylated casein (Sacolene), a diarrhoea remedy used in Europe, has shown an antisecretory effect in cholera-induced secretion in animals and benefit in diarrhoea in humans. In this placebo controlled trial Sacolene was evaluated in 78 male adults with severe cholera who, after initial i.v. therapy received Sacolene or placebo, 4 g at start and 2 g 4 hourly until cessation of diarrhoea, along with oral rehydration therapy and repeat i.v. therapy if indicated. No antibiotics were given during the study. The purging rate and diarrhoea duration were similar in the 2 groups. The proportion of patients requiring repeat

courses of i.v. therapy in the study group was 46% compared with 69% in controls (i.e. reduced by 23%,  $p=0.04$ ). While the severity of purging was not reduced, the proportion of patients requiring repeat i.v. therapy was reduced by Sacolene therapy. The latter finding may indicate some benefit from Sacolene in cholera, through this requires confirmation."

**231 Perera BJC, Ganesan S, Jayarasa J, Ranaweera S. The impact of breastfeeding practices on respiratory and diarrhoeal disease in infancy: a study from Sri Lanka. J Trop Pediatr 1999 Apr;45(2):115-8. 8 ref, Eng.** Unit no. 09, Ridgeway Hospital for Children (Teaching), Colombo 8, Sri Lanka

"A hospital-based descriptive recall study was conducted to assess the relationship, if any, between breastfeeding practices and morbidity from respiratory and diarrhoeal diseases in infants. A total of 343 infants (285 admitted patients and 58 controls) were recruited. Clinical and sociodemographic data and details regarding breastfeeding practices, timing of the first respiratory or diarrhoeal illness, and the timing of the first admission for a respiratory or diarrhoeal illness, were carefully documented. Three broad groups of those who were exclusively breastfed for 3 months or less, 4 months or more, and those who were never breastfed were identified. There was no significant difference in the numbers of infants who developed a respiratory or diarrhoeal illness or were admitted to hospital with a respiratory or diarrhoeal illness during the period of exclusive breastfeeding, irrespective of the period of exclusive breastfeeding. However, significant numbers of patients who were breastfed for 3 months or less developed the first respiratory infection, the first episode of diarrhoea, and the first hospital admission for respiratory or diarrhoeal disease during the first 3 months following the introduction of other foods and in the subsequent 3 months following this period. Those who were never breastfed showed the worst results. Significantly fewer of those who were breastfed for 4 months or more fulfilled the same criteria. Identical findings were noted whether the additional feeds used to terminate exclusive breastfeeding were water, herbal tea, native medicines, or formula milk. Similar results were obtained in the control group. This study reiterates the extended protective effects of exclusive breastfeeding for periods of over 4 months against respiratory and diarrhoeal diseases using a novel set of outcome measures."

**232 Perryman LE, Kapil SJ, Jones ML, Hunt EL. Protection of calves against cryptosporidiosis with immune bovine colostrum induced by a *Cryptosporidium parvum* recombinant protein. Vaccine 1999 Apr 23;17(17):2142-9. 44 ref, Eng.** Department of Microbiology, Pathology and Parasitology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA

The purpose of the study was to determine if immunization

with a recombinant protein (rC7) of *Cryptosporidium parvum* would induce immune bovine colostrum that protected calves against cryptosporidiosis following oral challenge with *C. parvum* oocysts. Late gestation Holstein cows with low titers of antibody to the p23 antigen of *C. parvum* were immunized three times with 300 mg affinity purified rC7 *C. parvum* recombinant protein (immune cows), or left nonimmunized (control cows). Colostrum was obtained from each cow in both groups and partitioned into identical aliquots of pooled immune colostrum or pooled control colostrum. Twelve calves obtained at birth received either immune or control colostrum within the first 2 h, and again at 12 and 24 h of age. Each calf was challenged orally with  $10^7$  *C. parvum* oocysts at 12 h of age and monitored for signs of cryptosporidiosis. All six calves administered pooled control colostrum developed severe diarrhea (mean total fecal volume =  $8447 \pm 5600$  ml) and shed an average of  $1.87 \pm 1.66 \times 10^{12}$  *C. parvum* oocysts. None of the six calves administered pooled immune colostrum developed diarrhea (mean total fecal volume =  $740 \pm 750$  ml,  $p < 0.05$ ), and shed significantly fewer oocysts ( $3.05 \pm 2.26 \times 10^9$ ,  $p < 0.05$ ). The absence of diarrhea and 2.79  $\log_{10}$  (99.8%) reduction in oocyst excretion indicates that immune bovine colostrum induced by immunization with *C. parvum* recombinant protein rC7 provided substantial protection against cryptosporidiosis in neonatal calves."

**233 Peruski LF, Jr., Kay BA, El-Yazeed RA, El-Etr SH, Cravioto A, Wierzba TF, Rao M, El-Ghorab N, Shaheen H, Khalil SB, Kamal K, Wasfy MO, Svennerholm A-M, Clemens JD, Savarino SJ. Phenotypic diversity of enterotoxigenic *Escherichia coli* strains from a community-based study of pediatric diarrhea in Periurban Egypt. J Clin Microbiol 1999 Sep;37(9):2974-8. 44 ref, Eng. U.S. Naval Medical Research Unit no. 3, PSC 452, Box 5000, FPO AE 09835-0007, USA**

"No past studies of diarrhea in children of the Middle East have examined in detail the phenotypes of enterotoxigenic *Escherichia coli* (ETEC) strains, which are important pathogens in this setting. During a prospective study conducted from November 1993 to September 1995 with 242 children under 3 years of age with diarrhea living near Alexandria, Egypt, 125 episodes of diarrhea were positive for ETEC. ETEC strains were available for 98 of these episodes, from which 100 ETEC strains were selected and characterized on the basis of enterotoxins, colonization factors (CFs), and O:H serotypes. Of these representative isolates, 57 produced heat-stable toxin (ST) only, 34 produced heat-labile toxin (LT) only, and 9 produced both LT and ST. Twenty-three ETEC strains expressed a CF, with the specific factors being CF antigen IV (CFA/IV; 10 of 23; 43%), CFA/II (5 of 23; 22%), CFA/I (3 of 23; 13%), PCFO166 (3 of 23; 13%), and CS7 (2 of 23; 9%). No ETEC strains appeared to express CFA/III, CS17, or PCFO159. Among the 100 ETEC strains, 47 O groups and 20 H groups were represented, with 59 O:H serotypes. The most common O

serogroups were O159 (13 strains) and O43 (10 strains). O148 and O21 were each detected in five individual strains, O7 and O56 were each detected in four individual strains, O73, O20, O86, and O114 were each detected in three individual strains, and O23, O78, O91, O103, O128, and O132 were each detected in two individual strains. The most common H serogroups were H4 (16 strains), 12 of which were of serogroup O159; H2 (9 strains), all of which were O43; H18 (6 strains); H30 (6 strains); and H28 (5 strains); strains of the last three H serogroups were all O148. Cumulatively, our results suggest a high degree of clonal diversity of disease-associated ETEC strains in this region. As a low percentage of these strains expressed a CF, it remains possible that other adhesins for which we either did not assay or that are as yet undiscovered are prevalent in this region. Our findings point out some potential barriers to effective immunization against ETEC diarrhea in this population and emphasize the need to identify additional protective antigens commonly expressed by ETEC for inclusion in future vaccine candidates."

**234 Peters HPF, Bos M, Seebregts L, Akkermans LMA, van Berge Henegouwen GP, Bol E, Mosterd WL, de Vries WR. Gastrointestinal symptoms in long-distance runners, cyclists, and triathletes: prevalence, medication, and etiology. Am J Gastroenterol 1999 Jun;94(6):1570-81. 20 ref, Eng. Department of Medical Physiology and Sports Medicine, Utrecht University, Utrecht, PO Box 80043, 3508 TA, Utrecht, The Netherlands**

**235 Poskitt EME, Cole TJ, Whitehead RG. Less diarrhoea but no change in growth: 15 years' data from three Gambian villages. Arch Dis Child 1999 Feb;80(2):115-20. 10 ref, Eng. Human Nutrition Research Centre, Downham's Lane, Cambridge CB4 1XJ, UK**

"*Aims*—To review diarrhoea presentations and nutritional status in young rural Gambian children over a 15 year period as a test of an earlier hypothesis that reduced diarrhoea prevalence would lead to improved growth and a reduced prevalence of malnutrition. *Subjects and methods*—Growth and morbidity were documented routinely in 1190 children under 2 years of age attending the Dunn Nutrition Group clinic at Keneba between 1979 and 1993. *Results*—The numbers of presentations with diarrhoea (1069 in 1979; 220 in 1993) and the proportion of clinic attendees with diarrhoea (30% in 1979; 8% in 1993) fell steadily between 1979 and 1993. However, at both 1 year old, mean weights (Z scores: 1979, -1.8; 1993, -1.8) and mean lengths (Z scores: 1979, -1.3; 1993, -1.7), and at 2 years old, mean weights (Z scores: 1979, -2.0; 1993, -1.9) and mean lengths (Z scores: 1979, -2.0; 1993, -2.1) did not change noticeably over the 15 year period. *Conclusion*—Major progressive reductions in clinic presentations with diarrhoea have not been associated with improved nutritional status in this population of young rural Gambian children."

**236 Prado V, Lagos R, Nataro JP, Martin OS, Arellano C, Wang JY, Borczyk AA, Levine MM. Population-based study of the incidence of *Shigella* diarrhea and causative serotypes in Santiago, Chile. *Pediatr Infect Dis J* 1999 Jun;18(6):500-5. 37 ref, Eng.** Center for Vaccine Development, University of Maryland School of Medicine, 685 W. Baltimore ST. 21201, USA

“Background. *Shigella* is an important cause of diarrheal disease in children in developing countries. The increasing prevalence of antibiotic resistant strains has stimulated interest in the use of multivalent *Shigella* vaccines. Because *Shigella* vaccines under development are based on eliciting immunity to O antigens, monitoring the distribution of serotypes in defined target populations is critical. We initiated health center-based surveillance in a poor semirural community in Colina, Santiago (7489 children <60 months of age) to determine the age-specific incidence of *Shigella* disease and the responsible serotypes. Findings. Surveillance was maintained at the 2 health centers during warm seasons (November 1 through April 30) for 4 successive years (1994 to 1998). *Shigella* was recovered from 54 of 243 cases of dysentery (22%) and from 215 of 3966 cases of nondysenteric diarrhea (5.4%) ( $P < 0.001$ ). The peak mean annual incidence of shigellosis occurred among children 12 to 47 months of age (9.0 to 12.6 cases/10<sup>3</sup> children), although the incidence in infants (5.2/10<sup>3</sup>) and children 48 to 59 months of age (6.2/10<sup>3</sup>) was also substantial. During the 1995 through 1996 season, an age-matched healthy control was cultured for every child <60 months of age with diarrhea. *Shigella* isolation from cases (34 of 576, 5.9%) was >8-fold higher than controls (4 of 576, 0.7%) ( $P < 0.01$ ). Four serotypes, *Shigella sonnei* (45%), *Shigella flexneri* 2b (19%), *S. flexneri* 2a (14%) and *S. flexneri* 6 (11%), accounted for 89% of all cases. Interpretation. *Shigella* remains an important pediatric pathogen in Santiago. The serotype distribution from Colina, which closely resembles data from a population-based surveillance study in Santiago in the mid-1980s, demonstrates a remarkable degree of serotype stability in Santiago during a 15-year period.”

**237 Quick RE, Venczel LV, Mintz ED, Soletto L, Aparicio J, Gironaz M, Hutwagner L, Greene K, Bopp C, Maloney K, Chavez D, Sobsey M, Tauxe RV. Diarrhoea prevention in Bolivia through point-of-use water treatment and safe storage: a promising new strategy. *Epidemiol Infect* 1999 Feb;122(1):83-90. 28 ref, Eng.** Foodborne and Diarrheal Diseases Branch, M/S A-38, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

“A novel water quality intervention that consists of point-of-use water disinfection, safe storage and community education was field tested in Bolivia. A total of 127 households in two periurban communities were randomized into intervention and control groups, surveyed and the intervention was distributed. Monthly

water quality testing and weekly diarrhoea surveillance were conducted. Over a 5-month period, intervention households had 44% fewer diarrhoea episodes than control households ( $P=0.002$ ). Infants <1 year old ( $P=0.05$ ) and children 5-14 years old ( $P=0.01$ ) in intervention households had significantly less diarrhoea than control children. *Campylobacter* was less commonly isolated from intervention than control patients ( $P=0.02$ ). Stored water in intervention households was less contaminated with *Escherichia coli* than stored water in control households ( $P<0.0001$ ). Intervention households exhibited less *E. coli* contamination of stored water and less diarrhoea than control households. This promising new strategy may have broad applicability for waterborne disease prevention.”

**238 Quiros-Tejeira RE, Ament ME\*, Rivera-Penera T, Cortina G, Vargas JH. Cytomegalovirus enterocolitis in an immunocompetent infant host: another cause of treatable intractable diarrhea in infancy. *J Pediatr Gastroenterol Nutr* 1999 Jul;29(1):86-90. 35 ref, Eng.** \*University of California at Los Angeles Medical Center, Department of Pediatrics, Division of Pediatric Gastroenterology and Nutrition, 12-383 MDCC, 10833 Le Conte Avenue, Los Angeles, CA 90095-1752, USA

**239 Raj SM, Radzi M. Should postpapillotomy screening for *Ascaris lumbricoides* infection be routine in communities endemic for ascariasis? (letter). *Am J Gastroenterol* 1999 Aug;94(8):2329. 1 ref, Eng.** University Sains Malaysia, School of Medical Sciences, Kota Bharu, Kelantan 16150, Malaysia

**240 Ramakrishna BS. Prevalence of intestinal pathogens in HIV patients with diarrhea: implications for treatment. *Indian J Pediatr* 1999 Jan-Feb;66(1):85-91. 15 ref, Eng.** Department of Gastrointestinal Sciences, Christian Medical College Hospital, Vellore 632004, India

“Patients infected with the human immunodeficiency virus (HIV) commonly experience diarrhea at some time during their illness. A variety of enteric pathogens are identified in 50-80% of these patients, depending on the intensity of the diagnostic work-up that is done. In addition to the common enteric pathogens, several unusual enteric pathogens are recognized to cause diarrhea especially in HIV patients. These include protozoan parasites such as *Cryptosporidia*, *Isospora belli*, *Cyclospora cayatanensis* and *Microsporidium* species bacteria such as enteropathogenic *Escherichia coli* and *Mycobacterium avium-intracellulare*, fungi including *Candida albicans* and *Histoplasma capsulatum*, and viruses such as astroviruses and caliciviruses. Diagnosis of these infections sometimes involves special procedures not readily available every where, and empiric therapy based on knowledge of the likely pathogens has been advocated for developing countries. This article reviews

the currently available data on geographic variation of enteric pathogens in HIV patients with diarrhea and outlines a rational strategy for empiric therapy of these patients."

**241 Raut S, Jalgaonkar SV, Tankhiwale NS, Agarwal VA. Re-emergence of *Vibrio cholerae* O139 serogroup during 1998 in Nagpur (Maharashtra), India. *Indian J Med Res* 1999 Jan;109:1-2. 4 ref, Eng.** Department of Microbiology, Government Medical College & Hospital, Nagpur 440003, India

**242 Robert FSM, Rao JP. Evidence of involvement of guanylate cyclase in the prolongation of caecocolonic transit time by indomethacin in mice (letter). *J Diarrhoeal Dis Res* 1998 Dec;16(4):256-7. 6 ref, Eng.** Department of Physiology, Christian Medical College, Vellore 632002, India

**243 Roy SK, Tomkins AM, Haider R, Behren RH, Akramuzzaman SM, Mahalanabis D, Fuchs GJ. Impact of zinc supplementation on subsequent growth and morbidity in Bangladeshi children with acute diarrhoea. *Eur J Clin Nutr* 1999 Jul;53(7):529-34. 33 ref, Eng.** ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

**“Objective:** To assess the impact of zinc supplementation during acute diarrhoea on subsequent growth and morbidity in malnourished young children. **Design:** Double blind randomized controlled clinical trial. **Setting:** International Centre for diarrhoeal Disease Research, Bangladesh. **Subjects:** Sixty-five children age 3-24 months with acute diarrhoea for less than 3 d. **Intervention:** Either elemental zinc (20 mg/d) in a multivitamin syrup or multivitamin syrup alone divided in three divided daily doses for a period of two weeks. Children were followed up weekly at home to assess subsequent growth and morbidity for a period of eight weeks. **Main outcome measures:** Gain in length and body weight and reduction in diarrhoea and respiratory tract infection. **Results:** During the follow-up, zinc supplemented children showed significantly greater cumulative length gain (18.9 mm vs 14.5 mm,  $P < 0.03$ ) and comparable body weight gain than the children of the control group. Subsequent length gain was not correlated with initial height in the zinc-supplemented group ( $r = -0.13$ ,  $P = 0.5$ ), but was significantly correlated in the control group ( $r = -0.6$ ,  $P < 0.0007$ ). Zinc-supplemented and stunted children ( $\leq 90\%$  length for age  $n = 18$ ) experienced significantly fewer episodes of diarrhoea (0.07 vs 0.6,  $P < 0.05$ ) and respiratory illness (1.0 vs 2.4,  $P < 0.01$ ) compared to the control group. The underweight children ( $\leq 71\%$  weight/age  $n = 38$ ) receiving zinc-supplementation also had fewer episodes of diarrhoea (0.4 vs 1.0,  $P < 0.04$ ) and shorter duration of diarrhoeal episodes (1.0 vs 3.0 d,  $P < 0.04$ ) compared to their counterparts in the control group. **Conclusion:** These results suggest that a short course of zinc supplementation

to malnourished children during acute diarrhoea reduces growth-faltering and diarrhoeal and respiratory morbidity during subsequent two months.”

**244 Ruiz-Pelaez JG, Mattar S. Accuracy of fecal lactoferrin and other stool tests for diagnosis of invasive diarrhea at a Colombian pediatric hospital. *Pediatr Infect Dis J* 1999 Apr;18(4):342-6. 16 ref, Eng.** Cra 7 no. 40-62, Facultad de Ciencias, Pontificia Universidad Javeriana, Santafe de Bogota, DC, Colombia

**“Objectives.** Estimate under “real life” conditions the operating characteristics of several stool tests for determining whether a diarrheal episode is invasive-inflammatory. **Design.** Determination of operating characteristics of diagnostic tests against a standard in a prospectively gathered sample. **Setting.** The emergency room of the largest Social Security Pediatric Hospital in Colombia serving referred and nonreferred patients. **Patients.** Stool samples from children attending the emergency room because of acute diarrhea (three or more loose stools per day lasting  $< 7$  days). Patients receiving antibiotics or antiparasitic medications were excluded. **Intervention.** Samples were collected in sterile containers and examined immediately for protozoa, fecal leukocytes, occult blood and lactoferrin. Specimens were inoculated onto culture media for common bacterial fecal pathogens except enteroinvasive *Escherichia coli* and *Clostridium difficile*. **Main outcome measure.** Sensitivity, specificity and likelihood ratios of several cutoff levels for fecal lactoferrin, fecal leukocytes and occult blood. **Results.** Stool samples from 500 infants and children with diarrhea were collected. Patients’ median age was 2.66 years (range, 0.5 to 13 years), and 261 (52.2%) were males. In 155 (31%) cases enteroinvasive bacteria and/or *Entamoeba histolytica* were documented. Fecal leukocytes  $> 5$  had the best sensitivity (63.2%; 95% confidence interval, 55.4 to 70.5) and specificity (84.3%, 95% confidence interval, 80.2 to 87.9), although not statistically or clinically significantly different from lactoferrin. **Conclusion.** No single test or combination had satisfactory operating characteristics. Nevertheless the use of likelihood ratios derived here can help clinicians identify invasive-inflammatory diarrheal episodes in many instances.”

**245 Ryan ET, Crean TI, John M, Butterstockon JR, Clements JD, Calderwood SB. In vivo expression and immunoadjuvancy of a mutant of heat-labile enterotoxin of *Escherichia coli* in vaccine and vector strains of *Vibrio cholerae*. *Infect Immun* 1999 Apr;67(4):1694-1701. 42 ref, Eng.** Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA 02114, USA

**“*Vibrio cholerae* secretes cholera toxin (CT) and the closely related heat-labile enterotoxin (LT) of *Escherichia coli*, the latter when expressed in *V. cholerae*. Both toxins are also potent immunoadjuvants. Mutant LT molecules that retain immunoadjuvant properties while possessing markedly diminished enterotoxic activities when**

expressed by *E. coli* have been developed. One such mutant LT molecule has the substitution of a glycine residue for arginine-192 [LT<sub>(R192G)</sub>]. Live attenuated strains of *V. cholerae* that have been used both as *V. cholerae* vaccines and as vectors for inducing mucosal and systemic immune responses directed against expressed heterologous antigens have been developed. In order to ascertain whether LT<sub>(R192G)</sub> can act as an immunoadjuvant when expressed in vivo by *V. cholerae*, we introduced a plasmid (pCS95) expressing this molecule into three vaccine strains of *V. cholerae*, Peru2, ETR3, and JRB14; the latter two strains contain genes encoding different heterologous antigens in the chromosome of the vaccine vectors. We found that LT<sub>(R192G)</sub> was expressed from pCS95 in vitro by both *E. coli* and *V. cholerae* strains but that LT<sub>(R192G)</sub> was detectable in the supernatant fraction of *V. cholerae* cultures only. In order to assess potential immunoadjuvanticity, groups of germfree mice were inoculated with the three *V. cholerae* vaccine strains alone and compared to groups inoculated with the *V. cholerae* vaccine strains supplemented with purified CT as an oral immunoadjuvant or *V. cholerae* vaccine strains expressing LT<sub>(R192G)</sub> from pCS95. We found that mice continued to pass stool containing *V. cholerae* strains with pCS95 for at least 4 days after oral inoculation, the last day evaluated. We found that inoculation with *V. cholerae* vaccine strains containing pCS95 resulted in anti-LT<sub>(R192G)</sub> immune responses, confirming in vivo expression. We were unable to detect immune responses directed against the heterologous antigens expressed at low levels in any group of animals, including animals that received purified CT as an immunoadjuvant. We were, however, able to measure increased vibriocidal immune responses against vaccine strains in animals that received *V. cholerae* vaccine strains expression LT<sub>(R192G)</sub> from pCS95 compared to the responses in animals that received *V. cholerae* vaccine strains alone. These results demonstrate that mutant LT molecules can be expressed in vivo by attenuated vaccine strains of *V. cholerae* and that such expression can result in an immunoadjuvant effect."

**246 Sagodira S, Buzoni-Gatel D, Iochmann S, Naciri M, Bout D. Protection of kids against *Cryptosporidium parvum* infection after immunization of dams with CP15-DNA. Vaccine 1999 May 14;17(19):2346-55. 36 ref, Eng.** Equipe Associee INRA d'Immunologie Parasitaire, UFR des Sciences Pharmaceutiques, 31 Avenue Monge, 37200 Tours, France

**247 Saldiva SR, Silveira AS, Philippi ST, Torres DM, Mangini AC, de Souza Dias RM, da Silva RM, Buratini MN, Massad E\*. *Ascaris-Trichuris* association and malnutrition in Brazilian children. Paediatr Perinatal Epidemiol 1999 Jan;13(1):89-98. 40 ref, Eng.** \*School of Medicine, University of São Paulo, Av. Dr. Arnaldo 455, CEP 01246-903, São Paulo, Brazil

"This work was designed to evaluate the role of intestinal parasites on nutritional status in three rural areas of Brazil.

A total of 520 children aged 1-12 years were studied through a questionnaire concerning housing, socio-economic conditions and a 24-h food intake recall. Measurements of weight and height were also performed, and three stool samples were collected on consecutive days for parasitological analysis. Scores of the standard deviation (z-scores) for the weight-for-height and height-for-age were used to characterise the growth profile. A high prevalence of intestinal parasites was detected, with *Giardia lamblia* (44%), *Endolimax nana* (43%), *Ascaris lumbricoides* (41%), and *Trichuris trichiura* (40%) being the most prevalent. Eleven per cent of the children were classified as showing stunting. Inadequate daily caloric intake was observed in 78% of the population and the proportion of those with inadequate protein intake was 34%. Logistic regression analysis was employed for the multivariate study. Stunting was significantly associated with estimators of low economic income, inadequate protein intake and polyparasitism, especially the association between *Ascaris lumbricoides* and *Trichuris trichiura*."

**248 Sandkvist M, Hough LP, Bagdasarian MM, Bagdasarian M. Direct interaction of the EpsL and EpsM proteins of the general secretion apparatus in *Vibrio cholerae*. J Bacteriol 1999 May;181(10):3129-35. 50 ref, Eng.** Department of Biochemistry, American Red Cross, Jerome H. Holland Laboratory, 15601 Crabbs Branch Way, Rockville, MD 20855, USA

"The general secretion pathway of gram-negative bacteria is responsible for extracellular secretion of a number of different proteins, including proteases and toxins. This pathway supports secretion of proteins across the cell envelope in two distinct steps, in which the second step, involving translocation through the outer membrane, is assisted by at least 13 different gene products. Two of these components, the cytoplasmic membrane proteins EpsL and EpsM of *Vibrio cholerae*, have been purified and characterized. Based on gel filtration analysis, both purified EpsM<sub>(His)6</sub> and wild-type EpsL present in an *Escherichia coli* Triton X-100 extract are dimeric proteins. EpsL and EpsM were also found to interact directly and form a Triton X-100 stable complex that could be precipitated with either anti-EpsL or anti-EpsM antibodies. In addition, when the L and M proteins were coexpressed in *E. coli*, they formed a stable complex and protected each other from proteolytic degradation, indicating that these two proteins interact in vivo and that no other Eps protein is required for their association. Since EpsL is predicted to contain a large cytoplasmic domain, while EpsM is predominantly exposed on the periplasmic side, we speculate that these components might be part of a structure that is involved in bridging the inner and outer membranes. Furthermore, since EpsL has previously been shown to interact with the autophosphorylating cytoplasmic membrane protein EpsE, we hypothesize that this trimolecular complex might be involved in regulating the opening and closing of the secretion pore and/or transducing energy to the site of outer membrane



translocation.”

**249 Sang WK, Oundo JO, Mwituria JK, Waiyaki PG, Yoh M, Iida T, Honda T. Multidrug-resistant enteroaggregative *Escherichia coli* associated with persistent diarrhea in Kenyan children. *Emerging Infect Dis* 1997 Jul-Sep;3(3):373-4. 12 ref, Eng.** Kenya Medical Research Institute, PO Box 54840, Nairobi, Kenya

“To study the association of multidrug-resistant enteroaggregative *Escherichia coli* with persistent diarrhea in Kenyan children, stool specimens were obtained from 862 outpatients under 5 years of age from July 1991 to June 1993. *E. coli* O44 was identified as the sole bacterial pathogen in four patients experiencing at least 14 days of fever, vomiting, and diarrhea. Disk diffusion testing showed *E. coli* O44 resistance to tetracycline, ampicillin, erythromycin, trimethoprim-sulphamethoxazole, and amoxicillin/clavulanate and sensitivity to chloramphenicol, nalidixic acid, azithromycin, and cefuroxime. Further studies are needed to clarify the epidemiology, clinical spectrum, and pathogenesis of enteroaggregative *E. coli* infection.”

**250 Sansonetti PJ, Nhieu GTV, Egile C. Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clin Infect Dis* 1999 Mar;28(3):466-75. 71 ref, Eng.** Unite de Pathogenie Microbienne Moleculaire, Unite 389 Institut National de la Sante et de la Recherche Medicale, Institut Pasteur, 28 Rue du Docteur Roux, 75724 Paris Cedex 15, France

“Invasion of the Intestinal barrier by *Shigella flexneri* involves complex interactions with epithelial and phagocytic cells. Major perturbation of the signals that maintain epithelial integrity permits mucosal invasion, leading to tissue destruction. Expression of this invasive phenotype depends on the secretion of Ipa proteins (invasins), which can trigger entry of the pathogen into epithelial cells by causing massive rearrangement of the host cell cytoskeleton and cause macrophage apoptotic death by direct interaction of IpaB with interleukin-1 $\beta$  (IL-1 $\beta$ )-converting enzyme. This results in the killing of defense cells and in the release of IL-1 $\beta$ . In vivo, bacteria translocate through the epithelial barrier, essentially via M cells of the follicle-associated epithelium in the colonic and rectal mucosa. Apoptotic death of macrophages in subepithelial tissues allows bacterial survival and triggers inflammation, which destabilizes epithelial structures and facilitates further bacterial entry. Once they are intracellular, bacteria multiply within the cytoplasm and move from cell to cell by an actin-dependent process.”

**251 Sauter GH, Munzing W, Ritter CV, Paumgartner G. Bile acid malabsorption as a cause of chronic diarrhea: diagnostic value of 7 $\alpha$ -hydroxy-4-cholesten-3-one in serum. *Dig Dis Sci* 1999 Jan;44(1):14-9. 21 ref, Eng.** Department of Medicine II, Klinikum Grosshadern, 81366 Munich, Germany

“To evaluate the usefulness of 7 $\alpha$ -hydroxy-4-cholesten-3-one (HCO) serum concentrations as a diagnostic marker

of bile acid malabsorption, we determined the reference range of HCO in 106 normal subjects (age  $40.2 \pm 16.8$  years; 55 women, 51 men) and conducted a utility study in 23 patients with chronic diarrhea of unknown origin (age  $49.4 \pm 15.3$  years, 13 women, 10 men). The diagnosis of bile acid malabsorption was made on the basis of a decreased retention of [ $^{75}\text{Se}$ ]homocholytaurine after oral application ( $^{75}\text{SeHCO}$  test). HCO (reference range: 6-48 ng/ml) and the  $^{75}\text{SeHCO}$  test yielded the same results in 19/23 (83%) patients. Bile acid malabsorption was identified by an increase of HCO in serum with a sensitivity of 90% and a specificity of 79%. Analysis of HCO in serum may serve as a novel, simple, and sensitive method for the detection of bile acid malabsorption in patients with chronic diarrhea of unknown origin.”

**252 Scaletsky ICA, Pedroso MZ, Oliva CAG, Carvalho RLB, Morais MB, Fagundes-Neto U. A localized adherence-like pattern as a second pattern of adherence of classic enteropathogenic *Escherichia coli* to HEp-2 cells that is associated with infantile diarrhea. *Infect Immun* 1999 Jul;67(7):3410-5. 45 ref, Eng.** Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Sao Paulo, Escola Paulista de Medicina, Rua Botucatu, 862, 04023-062 Sao Paulo, SP, Brazil

“*Escherichia coli* strains that cause nonbloody diarrhea in infants are known to present three distinct patterns of adherence to epithelial cells, namely, localized (LA), diffuse (DA), and aggregative (AA) adherence. Strains with LT (typical Enteropathogenic *Escherichia coli* [EPEC]) are well recognized as a cause of secretory diarrhea, but the role of strains with DA (DAEC) is controversial, and strains with AA (EAEC) have been more frequently related to persistent diarrhea whereas its relationship with acute diarrhea is not well defined. To determine the relationship of the different types of *E. coli* adherence patterns with acute diarrhea (lasting less than 14 days) and persistent diarrhea (lasting more than 14 days) in Sao Paulo, Brazil, we studied stool specimens from 40 infants under 1 year of age with diarrhea and 40 age-matched control infants without any gastrointestinal symptoms. Twenty-eight (35.0%) of eighty cases yielded adherent *E. coli* (HEp-2 cells). Strains with localized and aggregative adherence were associated with acute and persistent diarrhea. A total of 11.2% of the adherent strains were typical EPEC serotypes and hybridized with the enteroadherence factor probe; 5.0% were EAEC and hybridized with the EAEC probe. DAEC strains were isolated from 10.0% of patients and 7.5% of controls and did not hybridize with the two probes used (*daaC* and AIDA-I). Strains with a localized adherence-like pattern (atypical EPEC) were found significantly more frequently ( $P = 0.028$ ) in cultures from children with diarrhea (17.5%) than in controls (2.5%).”

**253 Schriefer A, Maltez JR, Silva N, Stoeckle MY, Barral-Netto M, Riley LW. Expression of a pilin subunit BfpA of the bundle-forming pilus of enteropathogenic *Escherichia coli* in an *aroA* live**

**salmonella vaccine strain. Vaccine 1999 Feb 26;17(7-8):770-8. 30 ref, Eng.** School of Public Health, University of California, Berkeley, 140, Warren Hall, Berkeley, CA 94720, USA

“Enteropathogenic *Escherichia coli* (EPEC) is a major cause of childhood diarrhea in developing countries and is a leading cause of severe diarrheal illness among Brazilian infants. As one approach to constructing a vaccine candidate against diarrhea caused by EPEC, we evaluated whether the pilin subunit (BfpA) of the bundle-forming pilus (BFP) could be expressed by a live *Salmonella* vaccine strain. Several copies of the coding region of BfpA (*bfpA*) were amplified by PCR from a preparation of the EAF plasmid of EPEC strain B171 and cloned into plasmid vectors. An intact copy of *bfpA* was subcloned into the heat inducible prokaryotic expression vector pCYTEXP1, and the resulting pBfpA was used to transform the *aroA* *S. typhimurium* strain SL3261, generating SL3261 (pBfpA). The recombinant vaccine strain was able to express, but not to process, rBfpA as evidenced by a prominent 21 kDa protein that crossreacted with anti-BFP antiserum found only in extracts of heat-treated SL3261 (pBfpA), but not in strains of untreated SL3261 (pBfpA) or SL3261 not carrying the plasmid. Furthermore, rBfpA accumulation was not toxic to the *Salmonella* host, as evidenced by similar plating efficiencies between induced and uninduced strains of SL3261 (pBfpA). Finally, SL3261 (pBfpA) orally administered to BALB/c mice was capable of eliciting a sustained and vigorous humoral immune response to BfpA, achievable even with a single oral dose of approximately 10<sup>9</sup> organisms. Therefore, this pilin product may serve as a potential immunogen as part of a live combined vaccine strategy to prevent two of the major public health problems in Brazil salmonellosis and EPEC childhood diarrhea.”

**254 Schroeder AA, Lawrence CE, Abrahamsen MS. Differential mRNA display cloning and characterization of a *Cryptosporidium parvum* gene expressed during intracellular development. J Parasitol 1999 Apr;85(2):213-20. 32 ref, Eng.** Veterinary Pathobiology, University of Minnesota, St. Paul, Minnesota 55108, USA

**255 Schuch R, Maurelli AT. The Mxi-Spa type III secretory pathway of *Shigella flexneri* requires on outer membrane lipoprotein, MxiM, for invasin translocation. Infect Immun 1999 Apr;67(4):1982-91. 55 ref, Eng.** Department of Microbiology and Immunology, F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, USA

“Invasion of epithelial cells by *Shigella flexneri* is mediated by a set of translocated bacterial invasins, the Ipa proteins, and its dedicated type III secretion system, called Mxi-Spa. We show here that *mxiM*, part of the *mxi-spa* locus in the *S. flexneri* virulence plasmid, encodes an

indispensable type III secretion apparatus component, required for both Ipa translocation and tissue culture cell invasion. We demonstrated that mature MxiM, first identified as a putative lipoprotein, is lipidated in vivo. Consistent with features of known lipoproteins, MxiM (i) can be labeled with [<sup>3</sup>H]palmitate and [2-<sup>3</sup>H]glycerol, (ii) is associated with the cell envelope, (iii) is secreted independently of the type III pathway, and (iv) requires an intact lipoprotein modification and processing site for full activity. The lipidated form of MxiM was detected primarily in the outer membrane, where it establishes a peripheral association with the inner leaflet. Through analysis of subcellular Ipa distribution in a *mxiM* null mutant background, MxiM was found to be required for the assembly and/or function of outer, but not inner, membrane regions of Mxi-Spa. This function probably requires interactions with other Mxi-Spa subunits within the periplasmic space. We discuss implications of these findings with respect to the function of MxiM and the structure of Mxi-Spa as a whole.”

**256 Schuhmacher DA, Klose KE. Environmental signals modulate ToxT-dependent virulence factor expression in *Vibrio cholerae*. J Bacteriol 1999 Mar;181(5):1508-14. 34 ref, Eng.** Department of Microbiology, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78284-7758, USA

“The regulatory protein ToxT directly activates the transcription of virulence factors in *Vibrio cholerae*, including cholera toxin (CT) and the toxin-coregulated pilus (TCP). Specific environmental signals stimulate virulence factor expression by inducing the transcription of *toxT*. We demonstrate that transcriptional activation by the ToxT protein is also modulated by environmental signals. ToxT expressed from an inducible promoter activated high-level expression of CT and TCP in *V. cholerae* at 30°C, but expression of CT and TCP was significantly decreased or abolished by the addition of 0.4% bile to the medium and/or an increase of the temperature to 37°C. Also, expression of six ToxT-dependent *TnphoA* fusions was modulated by temperature and bile. Measurement of ToxT-dependent transcription of genes encoding CT and TCP by *ctxAp*- and *tcpAp*-luciferase fusions confirmed that negative regulation by 37°C or bile occurs at the transcriptional level in *V. cholerae*. Interestingly, ToxT-dependent transcription of these same promoters in *Salmonella typhimurium* was relatively insensitive to regulation by temperature or bile. These data are consistent with ToxT transcriptional activity being modulated by environmental signals in *V. cholerae* and demonstrate an additional level of complexity governing the expression of virulence factors in this pathogen. We propose that negative regulation of ToxT-dependent transcription by environmental signals prevents the incorrect temporal and spatial expression of virulence factors during cholera pathogenesis.”

**257 Shapiro RL, Otieno MR, Adcock PM, Phillips-Howard PA, Hawley WA, Kumar L, Waiyaki P,**

**Nahlen BL, Slutsker L. Transmission of epidemic *Vibrio cholerae* O1 in rural western Kenya associated with drinking water from Lake Victoria: an environmental reservoir for cholera? *Am J Trop Med Hyg* 1999 Feb;60(2):271-6. 20 ref, Eng.** Foodborne and Diarrheal Diseases Branch, Mailstop A-38, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA

“Sub-Saharan Africa has the highest reported cholera incidence and mortality rates in the world. In 1997, a cholera epidemic occurred in western Kenya. Between June 1997 and March 1998, 14,275 cholera admissions to hospitals in Nyanza Province in western Kenya were reported. There were 547 deaths (case fatality rate = 4%). Of 31 *Vibrio cholerae* O1 isolates tested, all but one were sensitive to tetracycline. We performed a case-control study among 61 cholera patients and age-, sex-, and clinic-matched controls. Multivariate analysis showed that risk factors for cholera were drinking water from Lake Victoria or from a stream, sharing food with a person with watery diarrhea, and attending funeral feasts. Compared with other diarrheal pathogens, cholera was more common among persons living in a village bordering Lake Victoria. Cholera has become an important public health concern in western Kenya, and may become an endemic pathogen in the region.”

**258 Sodemann M, Jakobsen MS, Mølbak K, Alvarenga IC, Martins C, Aaby P. Malaria parasitemia and childhood diarrhea in a peri-urban area of Guinea-Bissau. *Am J Trop Med Hyg* 1999 Aug;61(2):336-8. 22 ref, Eng.** Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Serum Institut, Artillerivej 5, DK 2300, Copenhagen, Denmark

“To examine the association between diarrhea in early childhood and malaria parasitemia, we conducted a nested case-control study in Guinea-Bissau of 297 children with diarrhea and a similar number of children without diarrhea matched for age, season, and residential area. There were no associations between diarrhea and parasite rate, parasite density, or clinical malaria. However, anti-malarials were easily available and frequently used, which was reflected by a 0.7% prevalence of children with a parasite density >100/200 leukocytes. Thus, the findings do not preclude that diarrhea may be a sign of clinical malaria or high-parasite density in endemic areas with lower use of antimalarials.”

**259 Sodemann M, Jakobsen MS, Mølbak K, Martins C, Aaby P. Management of childhood diarrhea and use of oral rehydration salts in a suburban West African community. *Am J Trop Med Hyg* 1999 Jan;60(1):167-71. 22 ref, Eng.** Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark

“In a household survey in Guinea-Bissau, 319 episodes of diarrhea in children were followed by interviews every

second day with the aim of investigating perceived morbidity and subsequent actions taken. The majority of the mothers had good knowledge of oral rehydration salts (ORS). However, only 58% of the episodes were treated with ORS and the amount given was insufficient. Mothers with no knowledge of ORS did not use it during the observed attack of diarrhea regardless of contact with a health center, which suggests that maternal knowledge is an important determinant of whether health personnel provide ORS. Children with diarrhea considered to be caused by teething were less likely to receive ORS in the acute phase (risk ratio = 0.6, 95% confidence interval [CI] = 0.5-0.9). Univariate analyses showed that the use of ORS was related to number of reported symptoms, the mother being the care taker, consultations, previous use of ORS, good knowledge of ORS, and having ORS sachets at home. Multivariate Cox regression analyses showed that the presence of ORS sachets at home at the onset of diarrhea was the strongest predictor of use (hazard ratio = 3.3, 95% CI = 1.9-3.6). Improved health education should focus more on the quantity of ORS needed, early signs of dehydration, treatment of ‘teething diarrhea, and breast feeding, and address mothers who have no prior knowledge of ORS. Management of diarrhea may be improved by a more liberal distribution of ORS sachets.”

**260 Suzart S, Gomes TAT, Guth BEC\*. Characterization of serotypes and outer membrane protein profiles in enteroaggregative *Escherichia coli* strains. *Microbiol Immunol* 1999;43(3):201-5. 25 ref, Eng.** \*Universidade Federal de Sao Paulo, Rua Botucatu 862-3º andar, CEP 04023-062, Sao Paulo, Brazil

“Twenty-four *Escherichia coli* strains mainly isolated from children with diarrhea in São Paulo, and showing characteristics of enteroaggregative *E. coli* (EAEC), were characterized by serotyping and outer membrane protein (OMP) profiles. The relationship between these characteristics was evaluated, as well as the usefulness of OMP profiles in the clonal analysis of EAEC strains. All strains presented aggregative adherence to HeLa cells and were classified in two groups based on their interaction with the EAEC DNA probe. A diversity of serotypes and OMP profiles was observed in both groups studied. Although no significant correlation between serotypes and OMP profiles was observed, unique OMP profiles were identified in 80% of the probe-positive strains which were distributed in only 4 OMP profiles. This result may indicate the presence of a few clones in the probe-positive group. On the other hand, probe-negative strains seem to constitute a more diverse group. In general, the observed heterogeneity in serotypes and OMP profiles described in the present study suggest a great genetic diversity in EAEC isolates of either the same or different serotypes and in strains presenting the same EAEC markers identified in our community.”

**261 Tacconelli E, Tumbarello M, de Gaetano Donati K, Leone F, Mazzella P, Cauda R. *Clostridium difficile*-associated diarrhea in human**

**immunodeficiency virus infection—a changing scenario (letter).** *Clin Infect Dis* 1999 Apr;28(4):936-7. 5 ref, Eng. Departments of Infectious Diseases and Microbiology, Catholic University, Rome, Italy

**262 Udall JN, Jr., Hempe JM, Schmidt-Sommerfeld E, Scheer WD, Mannick E, Blecker U, Correa H. Longitudinal analysis of plasma cytomegalovirus DNA in a child with Crohn's disease and cytomegalovirus gastroenteritis.** *J Paediatr Gastroenterol Nutr* 1999 May;28(5): 502-5. 17 ref, Eng. Department of Pediatrics, Louisiana State University Medical Center, 1542 Tulane Avenue, New Orleans, LA 70112-2822, USA

**263 van Doorn L-J, Haperen AV-V, Burnens A, Huysmans M, Vandamme P, Giesendorf BAJ, Blaser MJ, Quint WGV. Rapid identification of the thermotolerant *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* from various geographic locations by a GTPase-based PCR-reverse hybridization assay.** *J Clin Microbiol* 1999 Jun;37(6):1790-6. 27 ref, Eng. Delft Diagnostic Laboratory, Delft, The Netherlands

“Recently, a gene from *Campylobacter jejuni* encoding a putative GTPase was identified. Based on two semiconserved GTP-binding sites encoded within this gene, PCR primers were selected that allow amplification of a 153-bp fragment from *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*. Sequence analysis of these PCR products revealed consistent interspecies variation, which allowed the definition of species-specific probes for each of the four thermotolerant *Campylobacter* species. Multiple probes were used to develop a line probe assay (LiPA) that permits analysis of PCR products by a single reverse hybridization step. A total of 320 reference strains and clinical isolates from various geographic origins were tested by the GTP-based PCR-LiPA. The PCR-LiPA is highly specific in comparison with conventional identification methods, including biochemical and whole-cell protein analyses. In conclusion, a simple method has been developed for rapid and highly specific identification of thermotolerant *Campylobacter* species.”

**264 Varley RCG, Tarvid J, Chao DNW. A reassessment of the cost-effectiveness of water and sanitation interventions in programmes for controlling childhood diarrhoea.** *Bull WHO* 1998;76(6):617-31. 31 ref, Eng. (address not available)

“Cost-effectiveness analysis indicates that some water supply and sanitation (WSS) interventions are highly cost-effective for the control of diarrhoea among under-5-year-olds, on a par with oral rehydration therapy. These are relatively inexpensive “software-related” interventions such as hygiene education, social marketing of good hygiene practices, regulation of drinking-water, and monitoring of water quality. Such interventions are needed to ensure that the potentially positive health impacts of

WSS infrastructure are fully realized in practice. The perception that WSS programmes are not a cost-effective use of health sector resources has arisen from three factors: an assumption that all WSS interventions involve construction of physical infrastructure, a misperception of the health sector's role in WSS programmes, and a misunderstanding of the scope of cost-effectiveness analysis. WSS infrastructure (“hardware”) is generally built and operated by public works agencies and financed by construction grants, operational subsidies, user fees and property taxes. Health sector agencies should provide “software” such as project design, hygiene education, and water quality regulation. Cost-effectiveness analysis should measure the incremental health impacts attributable to health sector investments, using the actual call on health sector resources as the measure of cost. The cost-effectiveness of a set of hardware and software combinations is estimated, using US\$ per case averted, US\$ per death averted, and US\$ per disability-adjusted life year (DALY) saved.”

**265 Vesikari T, Joensuu J, Baer M, Käyhty H, Olander R-M, Sormunen H, Miettinen A, Ward RL, Guillot T. Concurrent administration of rhesus rotavirus tetravalent (RRV-TV) vaccine with pentavalent diphtheria-pertussis-tetanus-*Haemophilus influenzae* b-inactivated polio and hepatitis B vaccines.** *Acta Paediatr* 1999 May;88(5):513-20. 19 ref, Eng. University of Tampere, Medical School, PO Box 607, 33101 Tampere, Finland

“To investigate the incorporation of oral rhesus-human reassortant rotavirus tetravalent (RRV-TV) vaccine into a routine immunization programme, RRV-TV or oral placebo was coadministered with a pentavalent diphtheria-tetanus-whole-cell pertussis-*Haemophilus influenzae* b (Hib)-inactivated polio vaccine and hepatitis B vaccine following a 3-4-5-mo schedule in a double-blind trial involving 249 infants. Seroconversion rates after 3 doses of rotavirus vaccine were 80% for rotavirus immunoglobulin A (IgA) and 93% for RRV neutralizing antibodies. Rotavirus vaccine did not interfere with the immune responses to diphtheria, tetanus, pertussis, Hib, poliovirus 1, 2 and 3, or hepatitis, B. Following the first, second and third doses of vaccine, fever >38°C on the day of vaccination was seen in 31%, 24% and 24%, respectively, with no difference between RRV-TV- and placebo-vaccinated children. This fever was presumably due to the whole-cell pertussis vaccine. Those vaccinees who received concomitant RRV-TV vaccine had another peak of fever around d 4 after the first dose, when 25% of them had fever >38°C and 3% >39°C. It is concluded that RRV-TV rotavirus vaccine can be given concurrently with other childhood immunizations following a 3-4-5-mo vaccination schedule. However, febrile reactions to RRV-TV rotavirus vaccine are common when the first dose is given at the age of 3 mo.”

**266 Villa S, Guiscafre H, Martinez H, Munoz O, Gutierrez G. Seasonal diarrhoeal mortality among Mexican children.** *Bull WHO* 1999;77(5):375-80. 26

**ref, Eng.** Centro Medico Nacional Siglo XXI, Coordinacion de Investigacion Medica, Edificio "B", Unidad de Congresos, 4o. Piso, Avenue, Cuauhtemoc 330, Col Doctores, C.P. 06725, Mexico, D.F. Mexico

"The study investigated the effects on diarrhoeal deaths among under-5-year-old Mexican children of the following variables: season (summer or winter), region (north versus south), age group, and place of death. Examination of death certificates indicated that the distribution of deaths in 1989-90 was bimodal, with one peak during the winter and a more pronounced one during the summer. In 1993-94, however, the winter peak was higher than that in the summer (odds ratio (OR)=2.04). These findings were due mostly to deaths among children aged 1-23 months (OR=1.86). Diarrhoeal mortality was highest among children aged 6-11 months (OR=2.23). During the winter, there was a significant increase in the number of deaths that occurred in medical care units and among children who had been seen by a physician before they died, but deaths occurring at home showed no seasonal variation. In the northern states, the reduction in diarrhoeal mortality was less in winter than in summer (OR=2.62). In the southern states, the proportional reduction during the winter was similar to that in the summer."

**267 Wanachiwanawin D, Manatsathit S, Lertlaituan P, Thakerngpol K, Gool PS. Intestinal microsporidiosis in HIV infected patients with chronic diarrhea in Thailand. South Asian J Trop Med Public Health 1998 Dec;29(4):767-71. 20 ref, Eng.** Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

"Microsporidia have been recognized as emerging opportunistic agents affecting multiple organs. Intestinal microsporidiosis caused by *Enterocytozoon bienewisi* and *Encephalitozoon intestinalis* is a common disease which is associated with gastrointestinal symptoms, particularly in AIDS patients. So far, information on, the frequency of this enteric disease in Thailand is not available. Therefore, the present study was undertaken to investigate the prevalence of intestinal microsporidiosis in HIV infected persons with chronic diarrhea. From 1995 to 1996, multiple diarrheal stool specimens were received and examined for the presence of the organism using Weber's modified trichrome staining method and transmission electron microscopy for confirmation. Twenty-two of 66 patients (33.3%) were positive for microsporidia which appeared as pink-red spores of 0.8-1.2 x 0.7-0.9 mm with the characteristic transverse or oblique band representing the coiled polar filament. Clinical features of these patients included chronic diarrhea (100%), weight loss (100%), abdominal pain (77%), fever (36%), vomiting (36%) and anorexia (18%). Transmission electron microscopic examination of fecal specimens from the 22 patients with positive staining results revealed *E. bienewisi* in 18 cases."

**268 Wanke CA, Cohan D, Thummakul T, Jongwuitiwes S, Grayson ML, Hammer SM, Hanvanich M. Diarrheal disease in patients infected with human immunodeficiency virus in Bangkok, Thailand. Am J Trop Med Hyg 1999 May;60(5):871-4. 22 ref, Eng.** Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA

"Diarrheal disease and its associated morbidities occur frequently in patients infected with human immunodeficiency virus (HIV) and may be associated with a decreased quality of life. We studied the spectrum of symptoms, measures of nutritional status, and the enteric pathogens associated with diarrheal disease in a group of 24 patients infected with HIV in Bangkok, Thailand compared with a group of 19 patients infected with HIV without diarrhea cared for at the same clinic. Patients with diarrhea appeared to have more advanced disease by CD4 cell counts and complained more frequently of symptoms such as anorexia, gas, and bloating than patients without diarrhea. Patients with diarrhea had a tendency toward a lower nutritional status, as measured by body mass index and mid arm circumference. Stool culture and examination revealed that enteric pathogens including *Salmonella* species and *Cryptosporidium parvum* sporidia were recovered at equal frequencies in patients with and without diarrhea (27% of the patients with diarrhea and 25% of the patients without diarrhea). Microsporidia was identified in one patient with diarrhea. It was not possible to identify a pathogen in 73% of the patients with diarrhea and 75% of the patients without diarrhea, suggesting that additional agents or factors may be responsible for the diarrheal symptoms in the patients with diarrhea. More extensive studies to identify potentially treatable pathogens in HIV-infected patients with diarrhea in Thailand are warranted and further attempts to better define the syndrome of pathogen-negative diarrheal disease in patients infected with HIV might result in the development of more targeted interventions in these patients."

**269 Watnick PI, Fullner KJ, Kolter R. A role for the mannose-sensitive hemagglutinin in biofilm formation by *Vibrio cholerae* El Tor (note). J Bacteriol 1999 Jun;181(11):3606-9. 23 ref, Eng.** Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA

**270 Way SS, Borczuk AC, Goldberg MB. Adaptive immune response to *Shigella flexneri* 2a *cydC* in immunocompetent mice and mice lacking immunoglobulin A. Infect Immun 1999 Apr;67(4):2001-4. 20 ref, Eng.** Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461-1602, USA

"*Shigella flexneri cydC*, which is deficient in cytochrome *bd*, was rapidly cleared from the lungs of intranasally

inoculated mice and was sereny negative, yet it induced 93% protection against challenge with wild-type *S. flexneri*. Mice that lack immunoglobulin A (IgA) were fully protected, suggesting that IgA may not be required for adaptive immunity in this model system."

**271 Weiner C, Pan Q, Hurtig M, Boren T, Bostwick E, Hammarstrom L. Passive immunity against human pathogens using bovine antibodies. Clin Exp Immunol 1999 May;116(2):193-205. 100 ref, Eng.** Department of Clinical Immunology, Huddinge Hospital, S-14186 Huddinge, Sweden

**272 Widmer G, Orbach EA, Tzipori S. Constitutive expression of small subunit ribosomal RNA transcripts in *Cryptosporidium parvum* oocysts and intracellular stages. J Parasitol 1999 Apr;85(2):229-33. 16 ref, Eng.** Tufts University School of Veterinary Medicine, Division of Infectious Diseases, North Grafton, Massachusetts 01536, USA

**273 Wingertzahn MA, Teichberg S, Wapnir RA\*. Modified starch enhances absorption and accelerates recovery in experimental diarrhea in rats. Pediatr Res 1999 Mar;45(3):397-402. 40 ref, Eng.** \*Department of Pediatrics, North Shore University Hospital, Manhasset, NY 11030, USA

"Rice gruels have been used as home remedies to treat dehydration associated with diarrheal illness in developing countries. These preparations have produced conflicting results, most likely due to the heterogeneity of starch used. We investigated whether the modified tapioca starch, Textra™ (TX), at 5.0 or 10.0 g/L added to a 90 mmol/L Na<sup>+</sup>-111 mmol glucose oral rehydration solution (ORS) enhanced water and electrolyte absorption in two models of diarrhea. To induce a secretory state (model A), the jejunum of juvenile rats was perfused with 10 mmol/L theophylline (THEO) under anesthesia and then perfused with the solutions indicated above. To produce chronic osmotic-secretory diarrhea (model B), rats had a magnesium citrate-phenolphthalein solution as the sole fluid source for 1 wk, and then were perfused as the THEO-treated rats. Water, electrolyte, and glucose absorption were measured during both perfusions. As an extension of the perfusion studies, we compared how fast rats recovered from chronic osmotic diarrhea by offering them either water, ORS, or ORS containing 5.0 g/L TX along with solid food. Recovery rate markers were measured after 24 h and included weight gain, food and fluid intake, and stool output. In model A, addition of 5.0 g/L TX to ORS reversed Na<sup>+</sup> secretion and improved net water as well as K<sup>+</sup> and glucose absorption, compared with THEO-treated rats perfused with ORS without TX. In model B, addition of TX to ORS increased water, Na<sup>+</sup>, K<sup>+</sup>, and glucose absorption, compared with rats perfused without TX. Increasing TX from 5.0 to 10.0g/L had no additional benefit. In recovery experiments, animals with free access to ORS with TX had significantly greater weight gain and decreased stool output compared with animals recovering with water or ORS without TX. Our

experiments suggest that TX may be a useful additive to standard ORS to promote fluid and electrolyte absorption and may provide additional energy without increasing ORS osmotic load."

**274 Yamamoto M, Kiyono H, Yamamoto S, Batanero E, Kweon M-N, Otake S, Azuma M, Takeda Y, McGhee JR. Direct effects on antigen-presenting cells and T lymphocytes explain the adjuvanticity of a nontoxic cholera toxin mutant. J Immunol 1999 Jun 15;162(12):7015-21. 40 ref, Eng.** Immunobiology Vaccine Center and the Department of Microbiology, University of Alabama, 761 Bevell Biomedical Research Building, 845 19<sup>th</sup> Street South, Birmingham, AL 35294-2170, USA

"The present study has elucidated two distinct mechanism that may explain how a mutant of cholera toxin (mCT), E112K, retains adjuvant effects though it lacks ADP-ribosyltransferase activity and associated toxicity. In the first mechanism, we show that mCT E112K, like native cholera toxin (nCT), enhances B7-2 expression, but, to some extent, also enhances B7-1 on Peyer's patch B cells and macrophages. Cocultivation of CD4<sup>+</sup> T cells with E112K-or nCT-treated B cells and macrophages in the presence of anti-CD3 stimulation resulted in the induction of T cell-proliferative responses. Further, the responses were blocked by mAbs to B7-1 and/or B7-2; however, the effect of anti-B7-1 was minimal. In the second mechanism, addition of mCT E112K or nCT to anti-CD3 mAb-stimulated Peyer's patch CD4<sup>+</sup> T cells inhibited proliferative responses, while recombinant CT-B subunit (rCT-B) did not. Analysis of cytokine responses showed that both mCT E112K and nCT preferentially inhibited IFN-g production. Interestingly, however, nCT, but not mCT E112K, induced apoptosis in CD4<sup>+</sup> T cells activated via the TCR-CD3 complex. These results indicate that CT uses at least two pathways for inhibition of Th1 responses and that, while nCT induces cAMP accumulation that in turn leads to apoptosis in Th1-type cells, mCT E112K, which lacks ADP-ribosyltransferase activity, inhibits IFN-g synthesis by a separate mechanism. Thus, mCT E112K, like nCT, induces adjuvant responses via up-regulation of mainly B7-2 on APCs and through preferential inhibition of Th1-type CD4<sup>+</sup> T cell responses in the absence of ADP-ribosyltransferase activity."

**275 Yu RR, DiRita VJ. Analysis of an autoregulatory loop controlling ToxT, cholera toxin and toxin-coregulated pilus production in *Vibrio cholerae*. J Bacteriol 1999 Apr;181(8):2584-92. 37 ref, Eng.** Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109, USA

"Coordinate expression of many virulence genes in the human pathogen *Vibrio cholerae* is controlled by the ToxR, TcpP, and ToxT proteins. These proteins function in a regulatory cascade in which ToxR and TcpP, two inner membrane proteins, are required to activate *toxT* and ToxT is the direct activator of virulence gene

expression. ToxT-activated genes include those whose products are required for the biogenesis of cholera toxin (CTX) and the toxin-coregulated pilus, the major subunit of which is TcpA. This work examined control of *toxT* transcription. We tested a model whereby activation of *toxT* by ToxR and TcpP is required to prime an autoregulatory loop in which ToxT-dependent transcription of the *tcpA* promoter reads through a proposed terminator between the *tcpF* and *toxT* genes to result in continued ToxT production. Primer extension analysis of RNA from wild-type classical strain O395 showed that there are two products encoding *toxT*, one of which is longer than the other by 105 bp. Deletion of the *toxT* promoter (*toxTD<sub>pro</sub>*) resulted in the abolishment of *toxT* transcription, as predicted. Deletion of the *tcpA* promoter (*tcpAD<sub>pro</sub>*) had no effect on subsequent detection of the smaller *toxT<sup>ppv</sup>* primer extension product, but the larger *toxT* product was not detected, indicating that this product may be the result of transcription from the *tcpA* promoter and not of initiation directly upstream of *toxT*. Neither mutant strain produced detectable TcpA, but the CTX levels of the strains were different. The *toxTD<sub>pro</sub>* strain produced little detectable CTX, while the *tcpAD<sub>pro</sub>* strain produced CTX levels intermediate between those of the wild-type and *toxTD<sub>pro</sub>* strains. Dependence of *toxT* transcription on TcpP and TcpH was confirmed by analyzing RNAs from strains carrying deletions in the genes encoding these regulators. The *tcpP* defect resulted in undetectable *toxT* transcription, whereas the *tcpH* mutation led to a diminishing of *toxT* RNA but not complete abolishment. Taken together, these results suggest that *toxT* transcription is dependent on two different promoters; one is directly upstream and is activated in part by TcpP and TcpH, and the other is much further upstream and is activated by ToxT."

**276 Zhang D, Honda T. Disappearance of glyceraldehyde 3-phosphate dehydrogenase from erythrocyte membrane by hemolysis with thermostable direct hemolysin of *Vibrio parahaemolyticus* or *Vibrio cholerae* El Tor hemolysin. *Microbiol Immunol* 1999;43(3):303-5. 19 ref, Eng.** Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan

"It is believed that the thermostable direct hemolysin (TDH) of *Vibrio parahaemolyticus* and El Tor hemolysin (ETH) of *Vibrio cholerae* damage erythrocytes and other cells by acting as pore-forming toxins. In this study, we

found that a protein band with a molecular weight of 37,000 daltons specifically disappeared from erythrocyte membrane after hemolysis by TDH and ETH, but not after hypotonic hemolysis. The 37 kDa band was identified as glyceraldehyde 3-phosphate dehydrogenase (G3PD), a glycolytic enzyme, based on N-terminal 14 amino acid sequencing. The role of G3PD in hemolysis is discussed."

**277 Zodpey SP, Deshpande SG, Ughade SN, Hinge AV, Shrikhande SN. Risk factors for development of dehydration in children aged under five who have acute watery diarrhoea: a case-control study. *Public Health* 1998 Jul;112(4):233-6. 13 ref, Eng.** Department of Social and Preventive Medicine, Government Medical College Hospital, Nagpur, Maharashtra State, India 440003, India

"Objective: To identify risk factors for development of dehydration in under five year olds with acute watery diarrhoea. Design: Hospital based unmatched case-control study. Setting: Diarrhoea Treatment Unit, Government Medical College Hospital, Nagpur, India. Participants: The study included 387 cases of diarrhoea having severe or moderate dehydration and 387 controls suffering from diarrhoea with mild or no dehydration. Risk factors: The study included infancy, female sex, religion, residing in urban slums or rural area, under nutrition, cessation of breast feeding during diarrhoeal episode, fluid intake decreased/stopped during diarrhoea, ORS not received, home available fluids (HAF) not received, both ORS and HAF not received, non-washing of hands by mother before preparation of food, after defaecation, after disposal of faeces, history of measles in the previous six months, frequency of stools > 8/d, frequency of vomiting more than twice per day and temperature more than 99°F, as risk factors for development of dehydration. Statistical analysis: Univariate analysis included OR, 95% CI for OR and Chi-square test. Multivariate analysis was carried out by unconditional multiple logistic regression (MLR). Results: This study identified the significance of infancy, religion, severe undernutrition, non-washing of hands by mother before preparation of food, frequency of stool > 8/d, frequency of vomiting > 2/d, history of measles in previous six months, withdrawal of breast feeding during diarrhoea, withdrawal of fluids during diarrhoea and not giving ORS, HAF or both during diarrhoea, in the outcome of development of moderate or severe dehydration. Conclusions: Timely intervention in the preventable risk factors included in this study may prevent the development of moderate or severe dehydration in the children suffering from acute watery diarrhoea."

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