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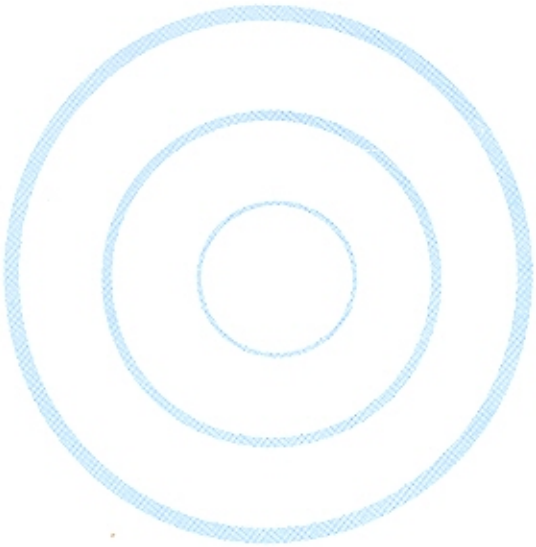

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An outbreak of group C rotavirus gastroenteritis among adults living in Valentim Gentil, São Paulo State, Brazil

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ABSTRACT

An outbreak of gastroenteritis affecting adults and children occurred in the small city of Valentim Gentil, São Paulo, Brazil, in 1993. Nineteen faecal samples (from 10 cases and 9 contacts) were examined by direct electron microscopy (DEM), immune electron microscopy (IEM), polyacrylamide gel electrophoresis (PAGE), and enzyme-linked immunosorbent assay (ELISA) for group A and C rotaviruses. DEM detected rotavirus in 6 of the 10 cases and in none of the contacts. All of the samples were negative for group A rotavirus by ELISA. Analysis by PAGE showed an electrophoretic profile suggestive of group C rotavirus in two cases. Group C rotavirus was identified by IEM in 4 of the cases and in 1 of the contacts. All of the samples were submitted to ELISA for group C rotavirus. This resulted in a total of 10 positives – 7 for diarrhoeal cases and 3 for contacts. This outbreak was strongly associated with group C rotavirus. The importance of combining different diagnostic methods is emphasised.

Key words: Gastroenteritis; Rotavirus; Disease outbreaks; Microscopy, Electron; Diagnosis, Laboratory

INTRODUCTION

Acute gastroenteritis accounts for high morbidity and mortality rates among infants and young children throughout the world, mainly in less-developed countries (1). To cope with this, the World Health Organization (WHO) has launched global programmes that include the use of oral rehydration salts, which have greatly contributed to the decrease in mortality rates, although morbidity rates have not decreased so far (2). In spite of these efforts, however, a large proportion (30% to 70%) of diarrhoeal cases still remains to be aetiologically elucidated (3). Rotavirus is the most important cause of severe diarrhoea affecting both human and young animals (1,4,5).

The rotaviruses are classified in the Reoviridae family. Mature rotavirus particles measure about 70 nm in diameter and possess a triple layered protein capsid (outer capsid, inner capsid and core). The virus genome comprises 11 segments of double-stranded RNA. To date, seven distinct antigenic groups have been identified with no antigenic cross-reactions among them (6). Rotaviruses of group A, B, and C have been found in humans, and those belonging to group A are known as the most frequent cause of infantile gastroenteritis. They lead to severe dehydration in 20% to 70% of patients hospitalised for diarrhoea worldwide (1,7).

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The lack of routine specific tests for detecting rotaviruses belonging to group B or C accounts, to a large extent, for the still high proportion of diarrhoeal cases of unknown aetiology (8). The rotavirus RNA segments can be separated by electrophoresis in polyacrylamide gel (PAGE) (9), and the pattern of migration may help in detecting different RNA profiles. Although the electrophoretic profiles of group B and C show similar patterns, it is known that they belong to distinct antigenic groups (6). The identification of distinct groups may be confirmed by enzyme-linked immunoassay (ELISA) and polymerase chain reaction (PCR) (10,11,12).

Direct electron microscopy (DEM) of the stools to detect viral particles by negative staining techniques (13) is used as a presumptive method for different human viral infections of the intestine. Viral morphology, substructure and size are important features that allow the identification of viruses in clinical samples (14). Electron microscopy is still an essential tool in the investigation of viruses causing gastroenteritis. Indeed, many of these agents do not grow in tissue cultures, and in most laboratories other specific methods for their detection are not available.

Non-group A rotaviruses have been detected either in sporadic diarrhoeal cases or in outbreaks of gastroenteritis, such as the group B rotaviruses that caused extensive epidemics of diarrhoea in China (15,16). Group C rotaviruses have been detected worldwide, in both children and adults (6,17-29,31-34).

Because of the epidemiological importance of viral infantile gastroenteritis, a "National Laboratory Surveillance of Viral Agents of Gastroenteritis in Brazil" has been set up. It aims at establishing a national network to study the prevalence, seasonality, and distribution of both rotaviruses and adenoviruses in several regions of Brazil (30).

The aim of the present study was to assess the possible role of group C rotavirus in the aetiology of an epidemic of non-bacterial gastroenteritis affecting adults and children in São Paulo State, Brazil.

SUBJECTS, MATERIALS AND METHODS

Outbreak description

The epidemic took place from July to August 1993, affecting adults and children living in Valentim Gentil, a small town in the north of São Paulo State. It has a water supply of good quality and an adequate sewage system. The population is approximately 6,000 inhabitants. During the epidemic, 154 cases were notified, of which 140 (91%) in the urban district and 14 (9%) in the rural area. Cases of diarrhoea occurred in all the urban neighbourhoods. Sixteen patients (10%) required hospitalisation. Bacteriological and parasitological examinations were carried out in the Regional Laboratories at Votuporanga and São José of Rio Preto, SP. The Adolfo Lutz Institute received 19 stool samples, of which 10 were obtained from patients with diarrhoea (7 adults and 3 children), and 9 were collected from healthy contacts. These samples were examined by DEM, ELISA for group A and C rotaviruses, RNA electrophoresis in polyacrylamide gel (PAGE) and immune electron microscopy (IEM), using a specific antiserum produced at the Adolfo Lutz Institute and sera from the patients that were positive by PAGE.

DEM and IEM

The 19 samples were analysed for viruses by negative staining technique using 2% sodium phosphotungstate, pH 6.4. The IEM reactions (35) were carried out using group C anti-rotavirus hyperimmune serum (dilution 1/1000) produced in rabbits and patient's serum (dilution. 1/50).

ELISA

An enzyme immunoassay was carried out on all the 19 samples to detect the presence of group A rotavirus antigens, using EIARA (enzyme immune assay for rotavirus and adenovirus) method (36). The ELISA technique used to detect group C rotavirus was as described by Tsunemitsu *et. al.* (12).

PAGE

The samples of cases and contacts were subjected to electrophoresis in polyacrylamide gel for characterisation of the rotavirus genome (9).

RESULTS

Table I shows the age and sex distribution of the 154 cases of diarrhoea. Thirty-eight percent of the patients were children aged from 0 to 14 years.

Table I. Age and sex distribution of diarrhoea cases in Valentim Gentil

Sex	Age (years)							
	<1	1 to 4	5 to 9	10 to 14	15 to 49	>50	Unknown	
Male	7	12	12	14	14	4	1	64
Female	7	21	7	12	30	13	0	90
Total	14	33	19	26	44	17	1	154
(%)	(9)	(21)	(12)	(17)	(29)	(11)	(1)	(100)

Table II. Comparison of the results of DEM, ELISA for group A and C, PAGE and IEM test; stools from gastroenteritis cases and their contacts from Valentim Gentil- SP, Brazil

Samples	Age (years)	DEM	ELISA group A	ELISA Group C	PAGE	IEM Patients' serum	IEM Hyperimmune serum
1 - case	27	R	-	-	ND	+	-
2 - case	37	R	-	+	ND	+	+
3 - case	13	R	-	+	ND	+	+
4 - case	14	R	-	+	+	+	+
5 - case	21	R	-	+	ND	+	-
6 - case	7	R	-	+	+	+	+
7 - case	25	ND	-	+	ND	N.T.	-
8 - case	25	ND	-	+	ND	N.T	-
9 - case	28	ND	-	-	ND	N.T	-
10 - case	76	ND	-	-	ND	N.T	-
11 - case	5	ND	-	-	ND	N.T	-
12 - case	32	ND	-	-	ND	N.T	-
13 - case	2	ND	-	-	ND	N.T	-
14 - case	2	ND	-	-	ND	N.T	-
15 - case	1	ND	-	N.T.	N.T.	N.T	N.T.
16 - case	5	ND	-	-	ND	N.T	-
17 - case	7	ND	-	+	ND	N.T	+
18 - case	10	ND	-	+	ND	N.T	-
19 - case	6	ND	-	+	ND	N.T	-

cont. = contacts; ND = not detected; N.T. = not tested; R = Rotavirus; - = negative; + = positive

None of the 154 samples were positive for bacterial or parasitic enteropathogens. The peak of major occurrence of gastroenteritis cases was in July/August, as shown in figure 1. In addition, the 19 samples tested by ELISA for group A rotaviruses were negative (Table II). However, the ELISA performed for group C rotaviruses showed 10 positive samples, 7/10 (70%) among diarrhoeic patients, and 3/9 (33%) in the contacts. DEM allowed the detection of a virus with a typical rotavirus morphology in 6/10 (60%) of the symptomatic cases but all the contact samples were negative. IEM with the hyperimmune polyclonal serum was positive in 4/10 diarrhoea cases (40%) (Fig. 2) and 1/9 contacts (11%). PAGE showed 2 diarrhoeic samples (20%) with an electrophoretic profile suggestive of group C rotavirus. The identification of bands 5, 6 and 7 making a triplet is typical of group C rotavirus RNA profile (Fig. 3).

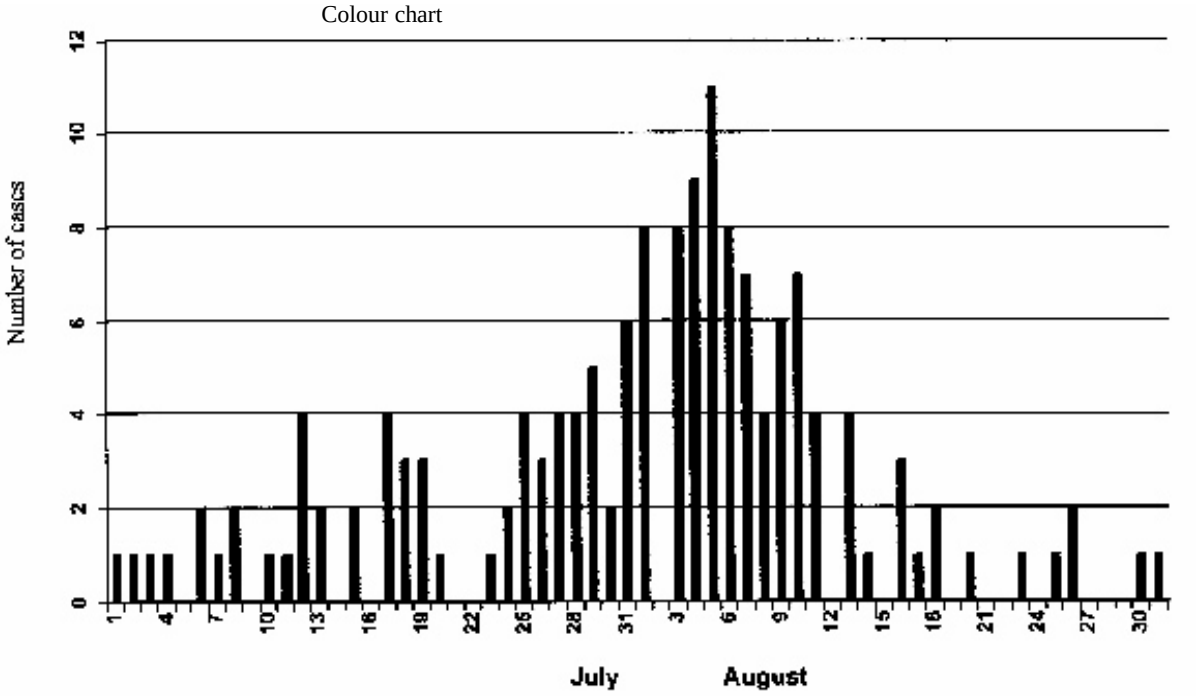

Fig. 1. Distribution of diarrhoea cases in Valentim Gentil, July-August 1993

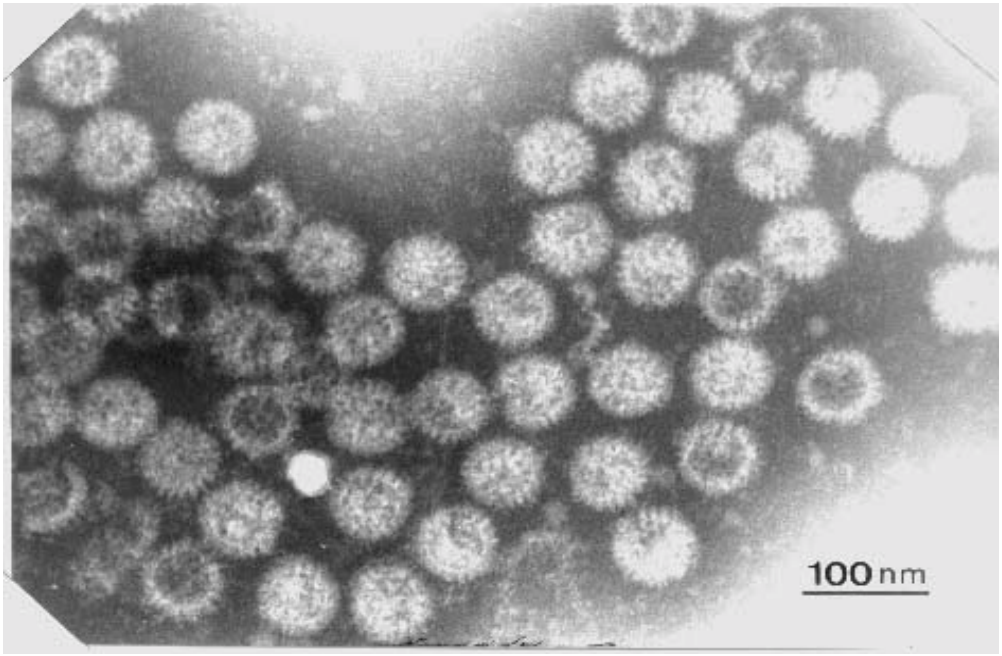

Fig. 2. Immuno-electron microscopy of sample No. 6. Immune complexes formed by the use of hyperimmune serum from rabbit (dilution 1/1000).

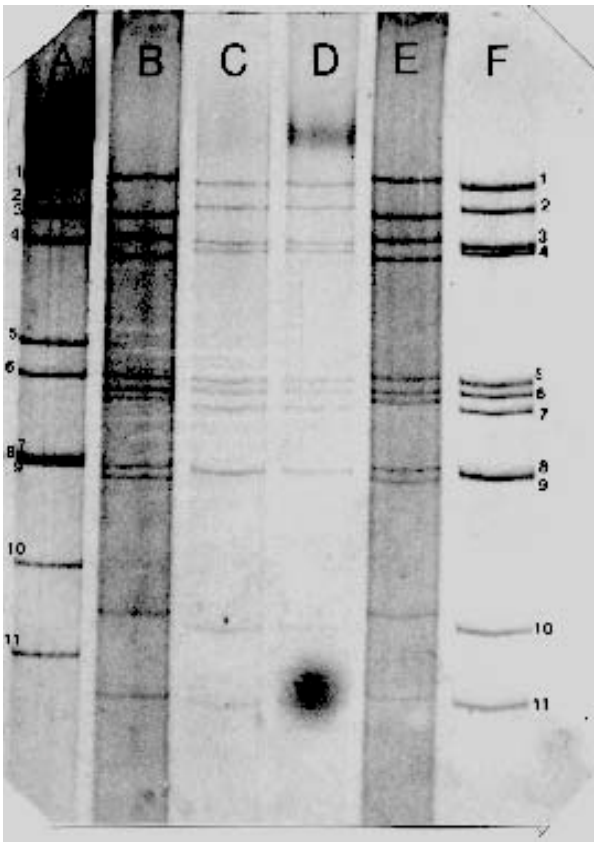

Fig. 3. Genomic profiles of group C rotavirus strains detected in samples from Valentim Gentil, São Paulo, Brazil.

Lane A: Long pattern control of group A rotavirus.

Lane B and E: Control of group C rotavirus (porcine/cow samples).

Lane C, D and F: Samples positive for group C rotavirus.

DISCUSSION

The distribution of diarrhoeal cases by age groups indicated high rates among children aged 1 to 4 years (31%), 5 to 14 years (29%) and especially in subjects of 15 years and more (40%).

The incidence of diarrhoeal cases (Figure 1) showing a peak in late July and early August suggests an infectious outbreak. Of interest is the simultaneous occurrence of group C rotaviruses associated with diarrhoea outbreaks in distinct and distant Brazilian regions, i.e. Belém of Pará State (33) and Valentim Gentil, SP (34). Another aspect of interest in our present study is that adults were also involved , unlike the finding from Gabbay *et al.* (33) who, however, studied the occurrence of this virus in a day-care centre. Our data are similar to the results published by Oishi *et al.* in Japan (26) who also detected group C rotavirus in 3 of 6 university students aged 20-21 years with severe diarrhoea.

Group C rotavirus has been described in Asia, Australia, Europe, and Central and South America, and reported in children and adults (6). The virus was mostly found associated with sporadic cases of gastroenteritis, although Matsumoto *et al*. (27) have described a gastroenteritis epidemic associated with group C rotavirus in 675 students and teachers in Japan in 1989. They hypothesise that the infection is originated and propagated by food. In our study, it was not possible to determine the origin of the epidemic, as the investigation only began in November 1993, with a bacteriological analysis of the water supply, which was negative.

Ushijima *et al.* (25) in a study carried out in Tokyo, Japan, in 1989, examined 2,599 diarrhoeal stool specimens using PAGE and detected 572 positive rotavirus results, of which only 4 were group C rotavirus. Familial outbreaks caused by group C rotavirus have been described by Caul *et al*. (28) in 1990 and Maunula *et al.* (29) in 1992. The latter publication detected the presence of specific IgM in 75% of the patients when immunofluorescence was used. An epidemic of group C rotavirus involving 23 college students was described by Oishi *et al.* (26) in 1993, in Japan, where electron microscopy and molecular biology techniques were used for diagnosing the agent. These authors suggested that group C rotavirus may be an important aetiologic agent of gastroenteritis in adults, and is transmitted by food.

The development of laboratory reagents necessary for specific diagnosis has been hampered by the difficulties in cultivating non-group A rotavirus in vitro. However, molecular techniques have made it possible to produce reagents for serotyping or genomic analyses of these groups of rotaviruses (10). Simultaneous analysis by DEM or PAGE is necessary to detect non-group A rotavirus, when the result of the routinely used enzyme immunoassay is negative.

Samples in which DEM showed only “empty” particles, i. e. capsid sheets without RNA, were negative by PAGE. DEM, based on morphological analysis, is not group-specific, and less sensitive than immunologic assays. However, in our case No. 1 (see table II), the presence of the virus could only be shown by electron

microscopy. This highlights the importance of DEM, expensive though this method is.

Rotavirus group C was detected by ELISA in 3 of the 9 samples of contacts (33%) examined. Considering the 10 symptomatic cases, another 7 samples were positive by ELISA. This confirms the sensitivity of the test and the epidemic nature of this virus. If at the time of sample collection the contacts were asymptomatic, it was not possible to ascertain that they remained so, and thus were true carriers. Two of the samples positive by DEM (Table II, sample no. 1 and 5) did not react with the polyclonal rabbit serum, but did react with the convalescent serum, forming immune complexes. The ELISA-negative result of sample No. 1 for both groups A or C, despite the detection of a large number of rotavirus particles by DEM is intriguing. With regards to sample No. 5, positive by ELISA for group C and IEM with patient's serum, but negative with polyclonal serum, the results may be related to IEM methodology problems.

Sample No. 17 was positive by ELISA for group C and by IEM, with a formation of an immune complex. It was, however, negative by DEM and PAGE. These data indicate the high sensitivity of IEM. At the time of collection, only small quantities of virus were probably excreted, leading to negative results with DEM and PAGE. The ELISA-positive results for group C rotavirus in sample nos. 17, 18 and 19 support the hypothesis that these household contacts might subsequently have developed diarrhoea.

Our results indicate that the epidemic gastroenteritis occurred in Valentim Gentil was strongly associated with group C rotavirus. Our results also show the importance of combining several sensitive techniques to diagnose group C rotaviruses.

The finding of group C rotavirus in a non- diarrhoeic sample by Timenetsky *et al*. (32) in 1993, and the presence of the virus in the contacts in our study, emphasise the need for more studies of individuals with and without diarrhoea during epidemics without apparent cause.

The results stress the importance of the Epidemiological Surveillance Program of Viral Gastroenteritis in Brazil. The relevance of agents, such as rotavirus C, is currently being underestimated. The importance of using combined methodologies (DEM, ELISA, and PAGE) that increase diagnostic sensitivity is strongly emphasised.

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Endemic Cholera in Delhi, 1995: Analysis of Data from a Sentinel Centre

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ABSTRACT

Data on cholera cases admitted to the Delhi Infectious Diseases Hospital (IDH) are presented to describe the pattern of occurrence of cholera in Delhi in 1995. Rectal swabs from 4082 cases of acute diarrhoea admitted to the IDH were examined for excretion of *Vibrio cholerae*. Of them, 2004 (49%) and 4 (0.1%) were positive for *V. cholerae* O1 biotype El Tor and *V. cholerae* O139 respectively. Most cholera cases occurred during May - September (summer and monsoon months). The period from January to March (winter) was completely free from cholera. The urban areas were not affected uniformly. Of the 80 PIN (Postal Index Number) code areas, 10 contributed to 57% of the cases. The early cases were scattered in PIN code areas distant from one another. The hospitalisation rates for cholera were the highest in children aged less than five years and declined significantly with increasing patients' age. Males had significantly higher rates than females aged up to 20 years, whereas the situation was reversed in the 20 to 39 year age group. Four per cent of the affected families had multiple cases. An estimated 1% of the household contacts of hospitalised cases of cholera were themselves hospitalised for cholera within 2 days of the first admission. Of the 260 *V. cholerae* O1 isolates tested, 4%, 7%, 8%, 89%, 91% and 95% were resistant to tetracycline, nalidixic acid, chloramphenicol, co-trimoxazole, streptomycin, and furazolidone respectively. The study highlights the usefulness of surveillance data to identify groups, urban areas and seasons with increased risk for cholera and to allow control measures to be focussed on those in greatest need.

Key words: Cholera; *Vibrio cholerae;* Disease outbreaks; Epidemiology; Drug resistance, Microbial; Microbial sensitivity tests.

INTRODUCTION

Vibrio cholerae O1 biotype El Tor remained confined for several decades to its original home in the Celebes islands in Indonesia. It began to spread in pandemic form in 1961 (1). It invaded India in 1964 and had almost replaced *V. cholerae* O1 biotype classical by the end of 1965 (1, 2). The organism appeared in Delhi for the first time in June 1965 and became endemic thereafter (3,4).

The epidemiology of cholera took a dramatic turn in the later months of 1992. *V. cholerae* O139 Bengal emerged as a new strain of cholera in Madras (5), rapidly spread far and wide and became a major cause of epidemics in India (5-8), Bangladesh (9) and other countries (10). It was first isolated in Delhi on 6 April 1993 and accounted for 54% and 22% of cholera cases in 1993 and 1994 respectively. How cholera behaved in Delhi in 1995 is described in this article.

About Delhi

Delhi, the capital of India, is situated on the banks of the river Yamuna at approximately 28° latitude north and 77° longitude east. The city covers an area of 1483 sq. km. It has a population of more than 9.4 million (1991 census), with an average population density of around 6350 per sq. km. For more than 4 decades, the population of Delhi has been growing rapidly, at a constant rate of about 50%

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per decade (11). More than 25% of the population (1991 census) live in slums. The literacy rate is 75% (12). The overall sex ratio in Delhi is very low (827 females per 1000 males) (l2). It is particularly so in the adult population aged 20 years and more (797 females per 1000 males) because of the immigration of adult males seeking employment.

The city has a piped water supply that is mostly intermittent and inadequate. The millions living in slums, resettlement and unauthorised colonies use water from shallow tubewells for drinking, cooking and other domestic purposes. These people do not have access to sewerage. They usually defecate in the open places because community latrines, even if available in some slum colonies, are usually poorly maintained.

Delhi has four main seasons: dry and cold (December-March), dry and hot (April-June), hot and wet (July-September), and again dry and cool (October-November). Average annual rainfall is 714 mm, almost all of it during the south-west monsoon from July through September. In 1995, there was less rain than usual in July (46 mm; normal 211 mm) and more in January-March (138 mm; normal 63 mm) (11).

SUBJECTS AND METHODS

The National Institute of Communicable Diseases (NICD), Delhi, has been monitoring the epidemiology of cholera in Delhi since 1965. As a part of the surveillance system, cases of acute diarrhoea clinically suspected to be cholera are referred to the Infectious Diseases Hospital (IDH), Delhi. Two rectal swabs from each of these cases are collected, one in Venkatraman-Ramakrishnan fluid and one in Cary-Blair medium (13). The samples – with the patient's name, age, sex, father's or husband's name and residential address – are sent within 24 hours to the laboratories of NICD, about 5 km away from the IDH. The samples are cultured on Bile Salt Agar after enrichment in Alkaline Peptone Water. Suspected colonies are confirmed by serology for *V. cholerae* O1; biochemical tests are performed whenever necessary. Since 1993, all the isolates of *V. cholerae* non O1 are also tested with an antiserum against *V. cholerae* O139 Bengal (7). Each year, a subset of isolates is tested for antimicrobial sensitivity using the Kirby-Bauer method.

To examine the seasonal relationship of cholera with climatic factors (temperature [means of monthly minimum and maximum values], relative humidity [%] and rainfall [mm]), we collected meteorological data for the year 1995 from published documents (11). Geographical distribution of cases was described in terms of PIN (Postal Index Number) codes (14).

To calculate age- and sex-specific hospitalisation rates for cholera in Delhi, data on population were obtained from the 1991 census. No adjustment was made for population changes from 1991 to 1995.

The analyses were done with Epi Info software, version 6.02.

RESULTS

In 1995, 4082 stool samples from IDH were processed for isolation of *V. cholerae* in the laboratories of NICD. Of them, 2004 (49.1%) and 4 (0.1%) yielded *V. cholerae* O1 biotype El Tor and *V. cholerae* O139 Bengal respectively. All but one of the *V. cholerae* O1 isolates were serotype Ogawa. In addition, 97 (2.4%) samples were positive for other vibrios.

Distribution by month and climatic factors

Figure 1 shows the distribution of the numbers of cholera cases by month, together with the monthly mean minimum and maximum temperatures, relative humidity and rainfall. Most cholera cases occurred during the period from May through September, the summer and monsoon months in Delhi. The dry winter months (January to March) were completely free from the disease. The first isolation of *V. cholerae* O1 was on 18 April and the last on 23 November. One case of *V. cholerae* O139 Bengal was recorded in August, whereas 3 cases occurred in October.

Age and sex distribution

Of our 2004 positive samples, age and sex of 1901 cases were available (Table I). Thirty-nine per cent of the cholera cases occurred in children aged less than 5 years, whereas adults (³20 years) contributed to only 30% of the cases. Overall there were significantly more cases in males (57%; 95% Confidence Interval: 55% to 59%) than females (43%; 95% CI: 41% to 45%). In adults of 20 years and more, females and males contributed to almost equal numbers of cases, despite a low female/male population ratio in this age group (Table I). Age and sex distribution of cases did not vary significantly when stratified by months (data not shown).

Hospitalisation rates by age and sex

Based on the census data, the hospitalisation rates for cholera were the highest in children aged less than 5 years, and declined steeply with increasing age (Table II). Males

had significantly higher hospitalisation rates than females aged up to 20 years, whereas the situation reversed in the 20 to 39-year age group. The rates were similar in persons aged over 40 years. Overall, the hospitalisation rates were not different between males and females.

52, 53, 94 in decreasing order of importance) accounted for about 57% of the total cholera cases. In fact, almost one-fourth of cases came from only two PIN code areas (No. 9 and 33). Interestingly, the first cases of cholera occurred in PIN code areas distant from each other.

Table I. Proportion of El Tor cholera cases in different age and sex groups. The figures in brackets are percentages with their 95% confidence intervals

Sex	0-4	5-9	10-14	15-19	20-39	40+	All
Male*	422 (53-57-60)	205 (54-60-65)	87 (57-65-73)	75 (60-69-78)	227 (45-50-55)	64 (45-55-65)	1080 (55-57-59)
Female*	322 (40-43-47)	139 (35-40-46)	46 (27-35-43)	33 (22-31-40)	228 (45-50-55)	53 (36-45-55)	821 (41-43-45)
All**	744 (37-39-41)	344 (16-18-20)	133 (6-7-8)	108 (5-6-7)	455 (22-24-26)	117 (5-6-7)	1901 (100)

* Column percentages ** Row percentages

Table II. Age and sex specific hospitalisation rates of cholera in Delhi, 1995 (per 100,000 population)

Age group (years)	Rates in males	Rates in females	P for difference in rates	Overall rates
0-4	72	59	0.009*	66
5-9	34	26	0.009*	30
10-14	16	10	0.007*	13**
15-19	14	8	0.007*	12**
20-39	12	15	0.02*	13**
40+	6	7	0.7	6
All ages	21	19	0.07	20

* Difference between two sexes significant.
** Overall rates in these age groups are not different from each other (p=0.5). Otherwise, the rates are significantly different between different age groups.
Note: Population from 1991 census.

Geographical distribution

The cases did not occur uniformly in all parts of the city. Certain PIN code areas were heavily involved, while others were almost completely spared. For example, of all the 80 PIN code areas in the city, 10 (9, 33, 41, 42, 62, 19, 44,

Familial cases

By comparing the address of patients and the name of the head of the family (husband or father), we identified 73 (3.8%) families having more than one laboratory-confirmed cases (Table III). Most of the subsequent familial cases were admitted to the hospital on the same day, or virtually all within 2 days of the admission of the index cases. Their age and sex are described in Table IV. About 87% of the index cases and 80% of the subsequent cases were children aged less than 10 years or adult females; less than 5% were adult males in either of the categories. Interestingly, females accounted for significantly more subsequent cases (57%) than index cases (39%). Obviously, more families might have had multiple cases but were not identified because of inaccurate or incomplete addresses.

Resistance to antibiotics

As described in Table V, 260 randomly selected isolates of *V. cholerae* O1 were tested for antimicrobial sensitivity. Of them, 4, 7, 8, 89, 91 and 95% were found resistant to tetracycline, nalidixic acid, chloramphenicol, co-trimoxazole, streptomycin, and furazolidone respectively.

DISCUSSION

Types of *V. cholerae*

The sudden emergence of *V. cholerae* O139 Bengal in late 1992 and its quick dissemination in many countries was

initially considered as the beginning of 8th pandemic of cholera (6,15). It was also feared that it might replace *V. cholerae* O1 biotype El Tor as the latter had replaced *V. cholerae* O1 biotype classical in the 1960s (15). In fact, it did happen in many parts of eastern (l6) and southern (l7) India in the last months of 1992 and the first half of 1993. However, *V. cholerae* O1 biotype El Tor again became the dominant cause of cholera in some of these areas in 1994-1995 (16).

Table III. Distribution of affected families by number of cases

No. of cases per family	No. of families	Total no. of cases
1	1845 (96.2)	1845 (92.1)
2	63 (3.3)	126 (6.3)
3	8 (0.4)	24 (1.2)
4	1 (0.1)	4 (0.2)
5	1 (0.1)	5 (0.2)
All	1918 (100.0)	2004 (100.0)

On the other hand, *V. cholerae* O1 biotype El Tor remained firmly established in Delhi during and after the emergence of *V. cholerae* O139 Bengal (7,8); the latter was an important superimposed problem in 1993 and 1994. Surprisingly, *V. cholerae* O139 Bengal contributed only to 4 cholera cases in 1995, whereas *V. cholerae* O1 biotype El Tor was responsible for 2004 cases. Why *V. cholerae* O139 Bengal has so quickly lost its epidemic potential is not clear.

Geographical distribution

Delhi has always shown a pattern with cases spread over a large community and usually without apparent linkages. On closer examination it was found that they were mostly localised in slums and resettlement and unauthorised colonies (18). These are, however, scattered throughout the city. Analysis of geographical distribution by PIN code areas revealed that certain PIN code areas were heavily involved, while others were almost completely spared. For example, of all the 80 PIN code areas in the city, 10 accounted for about 57% of the total cholera cases. Not all the heavily affected PIN code areas were close to the IDH. Some (No. 9 and partly 33) were very near the hospital, whereas the others (No. 19, 41, 44, 53, 62 and 94) were very far away from the hospital. The early cases in April were found scattered in distant PIN code areas, a pattern consistent with multiple simultaneous introduction of the

Table IV. Cholera cases in multiple-case families by age and sex

Age (years)	Index cases			Subsequent cases		
	Male	Female	Total	Male	Female	Total
0-4	24 (33)	12 (17)	36 (50)	22 (26)	19 (22)	41 (48)
5-9	11 (15)	4 (6)	15 (21)	6 (7)	9 (11)	15 (18)
10-19	5 (7)	1 (1)	6 (8)	5 (6)	7 (8)	12 (14)
20+	3 (4)	11 (15)	14 (19)	4 (5)	9 (11)	13 (15)
Unknown	1 (1)	0	1 (1)	0	4 (5)	4 (5)
All ages	44 (61)	28* (39)	72 (100)	37 (44)	48* (56)	85 (100)

*The difference in proportions between males and females is significant, p=0.03.
Figures in parentheses are percentages of the total numbers for index and subsequent cases separately (for each of the two groups, the last row shows row percentages, the other ones show column percentages)

infection in Delhi, as has been reported from Bangladesh (19). However, spread of the disease from a single focus could have produced the same pattern since the city has a vast network of public transport and all localities are connected by road. Yet, 2 small early focal outbreaks were in wide apart localities (PIN code areas 45 and 62).

Table V. Antimicrobial resistance pattern (n=260), 1995

Antimicrobial	No. of resistant isolates	% resistant isolates
Gentamicin	0	0.0
Tetracycline	10	3.8
Nalidixic acid	17	6.5
Chloramphenicol	20	7.7
Co-trimoxazole	230	88.5
Streptomycin	237	91.2
Furazolidone	248	95.4

Earlier studies considered geographical distribution of cholera in Delhi in terms of the 12 Municipal Zones, each covering thousands of localities (7,20). Consequently, they could not identify small geographic units with high concentration of cholera cases. In contrast, using the 80 PIN code areas allows a more precise description of the most severely affected, but comparatively small geographical areas in Delhi. This should make it possible to focus control measures appropriately.

Climatic factors

One of the remarkable aspects of cholera in Delhi is its seasonality (21,22). Most cases occur between May and September (21-23). The period from December through March remains virtually free from the disease, despite the occurrence of cholera somewhere in India at all times, in areas having different meteorological conditions. Even if the infection is introduced in the Delhi community in the dry winter season, as happened in January 1994, transmission is not sustained (22). The novel strain *V. cholerae* O139 Bengal also had the same seasonality in 1993-1994. Even when many eastern, southern and western parts of India were reeling under massive and explosive outbreaks of *V. cholerae* O139-associated cholera, not a single case was reported from Delhi in the first 3 months of 1993 (7,8). It is almost impossible that the infection was never imported in Delhi during this period. The whole of the population (more than 9 million) was susceptible, since it had no previous exposure to the organism; none of the 64 non-O1 vibrios isolated in 1991 and 1992 epidemic seasons were found to be *V. cholerae* O139 when tested retrospectively (8). Still the organism appeared in April and disappeared in November, along with the El Tor cholera strain (7). It is, therefore, not surprising that the disease followed the same pattern in 1995; it is puzzling that *V. cholerae* O139 Bengal has lost its epidemic potential.

Although the peak of the epidemic was during the rainy season, rainfall alone did not account for this seasonal distribution (Fig. 1). Relative humidity was found to have no relationship with the proportion of cases in different seasons. Indeed, it was nearly as high in the minimum cholera months of December to February as in the maximum ones of the rainy season in July-September (Fig.). In contrast, the mean minimum monthly temperatures showed some relationship to cholera seasonality in Delhi. The infection appeared when the mean minimum temperature was around 20º C. Large numbers of cases appeared once the temperature crossed 25º C. The disease started declining when the mean minimum temperature returned to 20º C, it virtually disappeared when the temperature went below 10º C.

Age distribution

Another feature of cholera in Delhi is the predominance of cases in children (Tables I and II). This has been noted in other endemic areas (24). When *V. cholerae* O1 biotype El Tor was gaining foothold in Delhi in 1960s, about 30% of the cases were recorded in children aged less than 10 years (25). In contrast, this proportion was more than 57% in 1995. Although the immunity against cholera is not solid, it protects many adults from further attacks.

Sex distribution

Higher morbidity in one of the sexes may be a function of differential susceptibility, exposure or treatment-seeking behaviour. The role of women in the care of sick persons and children in the families may be responsible for the higher risk of cholera in adult females than in adult males (19). Why were significantly more male than female infants, children and adolescents hospitalised in these age groups? Even if exposure may be different in adults, it is likely to be similar in young children, especially infants. Nevertheless, 61% of infants' cases in 1995 were males. Are female infants and children less susceptible to cholera than male children? There is no proof of that. Northern

Indian community is considered having a strong bias in favour of males, especially in health-care seeking behaviour. Several of the above factors may be responsible for the observed sex differences in cholera morbidity in different age groups.

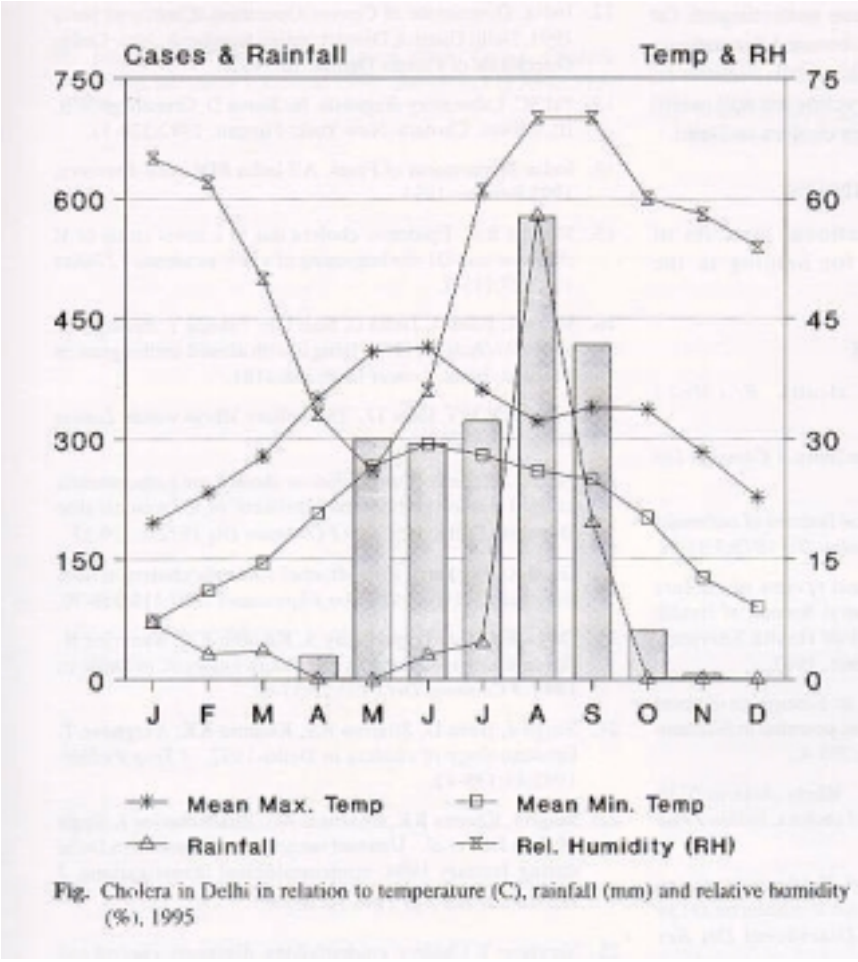

Fig. Cholera in Delhi in relation to temperature (C), rainfall (mm) and relative humidity (%), 1995

Intra-familial infections

Only 3.8% of the affected families had multiple cases in this study (Table III). The literature reports a mean of 7% (range 1-20%) of families with El Tor cholera cases having additional hospitalised and laboratory-confirmed cases (26). Most of these studies were about very small series. The largest of them – based on the hospital-records of 2756 cases of El Tor cholera from Philippines – revealed that 3.6% of households had multiple hospitalised cases (27), a result similar to the one we have found. It is noteworthy that studies on classical cholera revealed that 14% (range 11-16%) households had multiple cases (26). This confirms the different case/infection ratios in the two biotypes.

Almost all subsequent familial cases were admitted on the same day as the admission of the first case, or at the most within two days of it. Since the incubation period of cholera ranges from a few hours to 5 days, it is difficult to say whether subsequent cases got the infection from the index case through person-to-person transmission or were co-primary cases of the index case. However, the data on sex distribution of index and subsequent cases (Table IV) show that females accounted for significantly more subsequent cases (57%) than index cases (39%). This is in favour of many of the subsequent cases not being co-primary cases. Nevertheless, even if we take all the subsequent cases as secondary cases, the secondary attack rate of clinical cholera in the affected families was only 1% (family size was assumed to be 5). This, however, does not mean that infection is not transmitted easily in the affected families. On the contrary, many studies have shown a carrier rate of around 20% in such settings (27-29) which increases further with frequent sampling and prolonged follow-up (30). Probably, a high percentage of family contacts develop asymptomatic or mild infections that do not require hospitalisation.

Antimicrobial resistance

Singh *et al.* have recently reported that in Delhi a very high percentage (64%) of patients having acute watery diarrhoea are prescribed antimicrobials before hospitalisation. They are not only unnecessary but often

harmful (31). The practice may be a major factor in growing resistance of cholera organisms to many antimicrobials, especially the frequently used furazolidone and co-trimoxazole (Table V). Development of antimicrobial resistance is of serious concern, and requires continuous monitoring and calls for judicious use of antimicrobial in clinical practice. The physicians need to understand that the antimicrobials are not indicated for most cholera cases and should only be used for patients with severe dehydration (32). The results, however, showed that nalidixic acid and tetracycline are still useful antimicrobials for treatment of severe cholera in Delhi.

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Time Series Analysis of Patients with Rotavirus Diarrhoea in Pune, India

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ABSTRACT

The effects of seasonality and other temporal patterns on the occurrence of rotavirus diarrhoea were studied among hospitalised cases at Pune, India from July 1992 to June 1996. The well-accepted Box-Jenkins methodology based on modelling was employed for the analysis. This is the first presentation of such analysis for rotavirus diarrhoea. The model suggests strong influence of climatic changes on the incidence of the disease. Further study of weather parameters not only confirms that daily minimum temperature is the principal factor but also reveals that easterly wave, a characteristic feature of tropical weather, is useful in predicting the peak of hospital admissions and the geographical sequence of outbreaks of the disease in tropical India.

Key words: Rotavirus; Diarrhoea; Statistics; Seasonality

INTRODUCTION

Rotaviruses are the most common cause of diarrhoea in children worldwide (1). In India, 20 to 30 percent of the hospitalizations among diarrhoea cases are due to rotaviruses (2-6). Diarrhoea cases of all the age groups were monitored for rotavirus aetiology at the Naidu Infectious Diseases Hospital, Pune, India from July 1992 to June 1996. Faecal specimens, collected from 2,267 diarrhoea patients admitted to the hospital, were examined by an indigenously developed ELISA (7) at the National Institute of Virology, Pune. Preliminary statistical analysis revealed 351 rotavirus-positive cases, of which 266 (76%) occurred in children aged $\leq$5 years. This group was chosen for further investigations and subsequent analysis.

Rotavirus diarrhoea is endemic and has been observed throughout the year in tropical India. Within each year, however, most cases occur in the winter months. So, any outbreak of this disease cannot be regarded as an isolated phenomenon. In such a situation one often speaks of recurrent epidemics, and the elucidation of the underlying factors is of considerable importance (8).

As stated by Cook and co-workers, "Seasonality is an important but poorly understood feature of rotavirus diarrhoea" (9). In the numerous studies of this disease in developed countries and in few studies in developing countries, the seasonality and other temporal patterns are presented in graphical forms. The identification and quantification of the effects of seasonality, using sound statistical methods, are presented in this communication.

MATERIALS AND METHODS

Collection of data

During July 1992-June 1996, faecal specimens were collected from patients admitted to the diarrhoea ward

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of Naidu Infectious Diseases Hospital, Pune, India. Of the total 2,267 specimens, 945 were collected from children aged ≤5 years. Of the 945 specimens, 266 (28%) were positive for group A rotavirus by ELISA. The distribution of these 266 cases over the 48-month observation period forms the basic data set for the analysis. Furthermore, data on various weather parameters of Pune were collected from the Indian Meteorological Department, Pune, for the study period.

From the plot (not shown) of monthly incidence of rotavirus diarrhoea over time it appears to coincide with annual changes of weather.

To identify and estimate the effects of seasonality and other temporal patterns on the incidence of rotavirus diarrhoea, we have used the Box-Jenkins methodology (10). This methodology provides a systematic approach to model selection, using all the information contained in the sample autocorrelation function (ACF) and sample partial autocorrelation function (PACF). It is based on Auto Regressive Integrated Moving Average (ARIMA) models. The methodology consists of 3 iterative procedures of modelling: identification, estimation, and diagnostic checking. To start with, we transformed the data using variance stabilising transformation (11). The series transformed by using square root transformation is depicted in Fig. 1. This series shows less fluctuations, and we have based our subsequent analysis on it.

Notation

A very useful notation to describe the orders of various components in the multiplicative seasonal ARIMA model is given by:

(p, d, q) X (P, D, Q)s. Where

p = Order of the autoregressive (AR) part of the ARIMA model at non-seasonal level.

d = Order of differencing at non-seasonal level.

q = Order of the moving average (MA) part of the ARIMA model at non-seasonal level

and

P = Order of the autoregressive (AR) part of the ARIMA model at seasonal level.

D = Order of differencing at seasonal level.

Q = Order of the autoregressive (AR) part of the ARIMA model at seasonal level.

s = Period of cycle.

The above notation is the same as the one used by MINITAB (PC version of release 8) software, used for the analysis of our data.

RESULTS

Identification of the tentative model

This is done by examining the sample autocorrelation function (ACF) and sample partial autocorrelation function (PACF). For our transformed data, ACF and PACF are displayed in Fig. 2a and 2b respectively. To identify a Box-Jenkins seasonal model, we examined the behaviour of ACF and PACF first at non-seasonal level and then at seasonal level.

1. At non-seasonal level, ACF and PACF both become not significant after lag 1. It follows, therefore, by guidelines given by Bowerman and O'Connell 1991 (12), that we should use AR(1) or MA(1) model at non-seasonal level. Thus, we have tentatively identified two models at non-seasonal levels.

2. At seasonal level, ACF has a spike at lag 12 and thereafter dies off fairly quickly at lag 24 and 36 (Fig. 2a), and PACF becomes very small at lag 24 and 36. Therefore, AR(1) model seems to be suitable at seasonal level. Furthermore, the presence of spikes at near-seasonal level warrants the use of a multiplicative model (13). Thus, we have tentatively identified two seasonal ARIMA models. In the notation used below, SAR stands for seasonal autoregressive.

(1) (1,0,0) X $(1,0,0)_{12}$ (AR(1) X SAR(1))

(2) (0,0,1) X $(1,0,0)_{12}$ (MA(1) X SAR(1))

Estimation of the model parameter

The final estimates of the parameters along with the corresponding t-ratios are shown in the table I.

Diagnostic checking for the two preliminarily selected models

(1) *Examination of the correlograms for the residuals*: Correlograms (ACF and PACF) for the residuals are depicted in Fig. 3a, 3b and Fig. 4a, 4b for the model's AR(1) X SAR(1) and MA(1) X SAR(1) respectively. The sample two-sigma confidence limits for the correlations based on large sample are -0.2887 and

0.2887. None of the autocorrelations of residual series for both the models is significantly different from zero. Hence, both models are adequate according to this criterion.

The following observations are called for here:

(i) The values of Q and their differences for both the models are nearly the same.

Table I. Final point estimate of the model parameters and the associated t-values

Model	Parameter	Estimate	Std. dev	t ratio	p values
(1) AR(1) X SAR(1)	AR 1	0.1604	0.1546	1.04	0.30
	SAR 12	0.6137	0.1466	4.19	<0.001
	Constant	0.6425	0.1464	4.39	<0.001
	Mean	1.9809	0.4514		
(2) MA(1) X SAR(1)	MA 1	-0.1899	0.1508	-1.26	0.207
	SAR 12	0.6048	0.1472	4.11	<0.001
	Constant	0.7852	0.1735	4.53	<0.001
	Mean	1.9868	0.4390		

(2) *The Box-Pierce-L-Jung goodness of fit test:* The modified Box-Pierce (L-Jung, Box) (11) is used in this study. Let k be the number of autocorrelations tested simultaneously by this test. This statistic is evaluated for several choices of k, so that the different sections of the correlograms can be checked for the departure from the goodness of fit hypothesis. For seasonal model, k is s, 2s, etc. The values of statistic Q, computed for the identified models, are shown in table II.

(ii) Neither the values of Q nor their differences suggest any unusual behaviour. Hence, the fit of both models is equally good by this criterion.

(3) *Examination of the t-ratios*: From table I, it is seen that

(i) at non-seasonal level, the constant term is essential, as indicated by the significance of the associated t-ratio,

Table II: Box-Jung statistic

Model	k	Q(k)	p value	D.F.	Q(k2) -Q(k1)	D.F.	p value
AR(1) X SAR(1)	12	12.1	0.27	10	-	-	-
	24	21.2	0.50	22	9.1	12	0.69
	36	28.1	0.75	34	6.9	12	0.86
MA(1) X SAR(1)	12	12.3	0.26	10	-	-	-
	24	21.5	0.49	22	9.2	12	0.68
	36	28.5	0.73	34	7.0	12	0.85

whereas the AR(1) term can be dropped, as the associated t-ratio is not significant for the AR(1) X SAR(1) model.

(ii) at non-seasonal level, the constant term is essential, as indicated by the significance of the associated t-ratio, whereas the MA(1) term can be dropped, as its associated t-ratio is not significant for the MA(l) X SAR(1) model.

(iii) at seasonal level, for both models under consideration, the constant term as well as the AR(1) term are essential, as the associated t-ratios are significant.

Identification of a better model

The above observations suggest that we should consider the parsimonious seasonal autoregression SAR(1) model, which is a purely seasonal model.

Estimation of SAR(1) model

The final estimates of the parameters in the model SAR(1) along with the corresponding t-ratios are shown in the table III.

Diagnostic checking for SAR(1) model

(1) *Examination of correlograms for the residuals*: The ACF and PACF of the SAR(1) model under consideration are depicted in Fig. 5a and 5b respectively. These follow a white noise pattern. Hence, the model is adequate on the basis of this criterion.

(2) *Results of the Jung-Box statistics for the SAR(1) model:* These are presented in table IV.

On the basis of the above values, we accept the hypothesis that the fit of the model is good.

(3) *Examination of the t-ratios:* Both the t-ratios, shown in the table III, are statistically significant. Thus, the constant term and SAR(1) term are essential in the model.

(4) *Stationarity and invertibility check:* The model satisfies the stationarity check. It is enough to check stationarity as it is a purely AR model.

(5) *Checking inference assumptions:*

(i) Normality of errors: The Q-Q plot (14) for the residuals of the model is shown in Fig. 6. It has a straight line appearance. The measure of linearity

Table III. The final estimates and associated t-ratios for SAR(1) model

Parameter	Estimate	Std. dev.	t-ratio	p value
SAR(1)	0.7105	0.1340	5.30	<0.001
Constant	0.5726	0.1451	3.95	<0.001
Mean	1.9777	0.5010	-	-

TABLE IV. L-Jung-Box statistic for SAR(1) model

k	Q(k)	D.F.	p value	Q(k2)-Q(k1)	D.F.	p value
12	12.5	11	0.32	-	-	-
24	20.7	23	0.59	8.2	12	0.76
36	28.6	35	0.76	7.9	12	0.79

for the Q-Q plot (15) is 0.96, which indicates the normality of errors.

(ii) Homoscedasticity of errors: Fig. 7 shows the plot of residuals against time. There are no irregularities. Hence, the assumption of homoscedasticity is tenable.

(iii) Independence of the errors: The value of the Durbin-Watson statistic (11) for the model under consideration is 1.929395. On the basis of this value, we accept the hypothesis of independence of errors. Furthermore, runs test accepts the hypothesis that the errors are random.

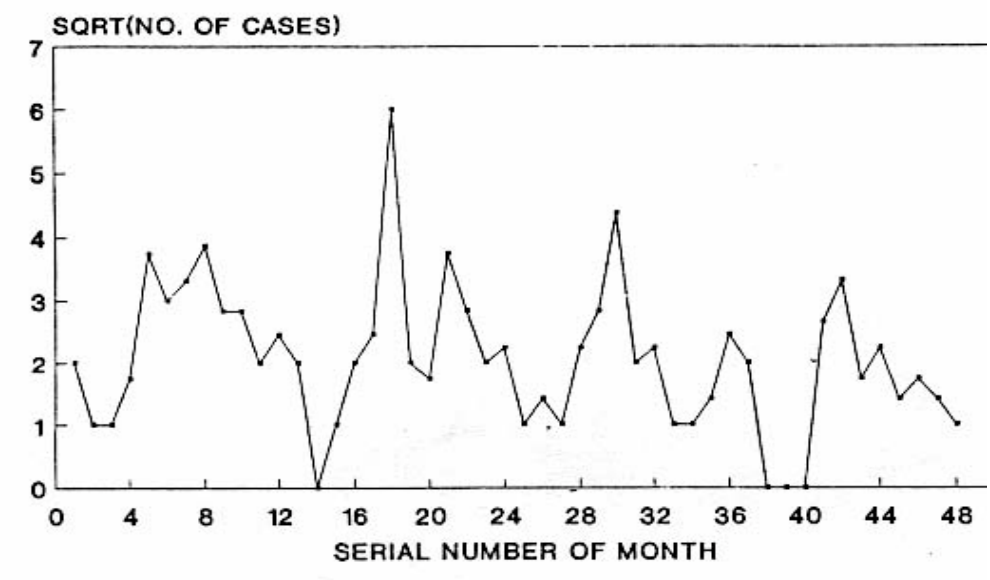

Fig. 1. Time series of transformed rotavirus diarrhoea cases (July 1992-June 1996)

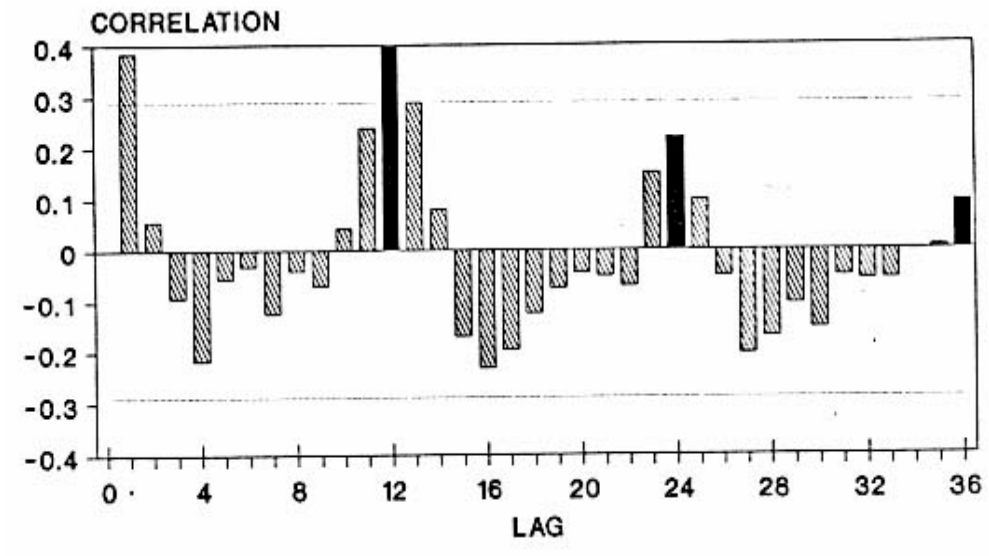

Fig. 2a. Autocorrelation function of transformed data. Values between -0.3 and 0.3 are considered not significant. Lag time is in months. The dark bars represent yearly intervals

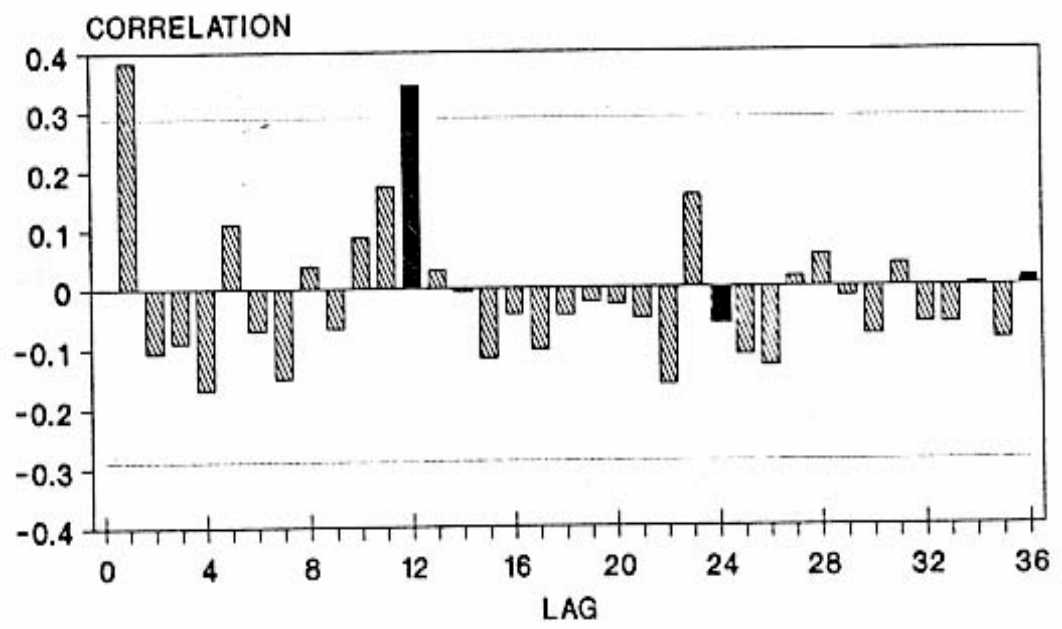

Fig. 2b. Partial autocorrelation function of transformed data. Values between 0.3 and 0.3 are considered not significant. Lag time is in months. The dark bars represent yearly intervals

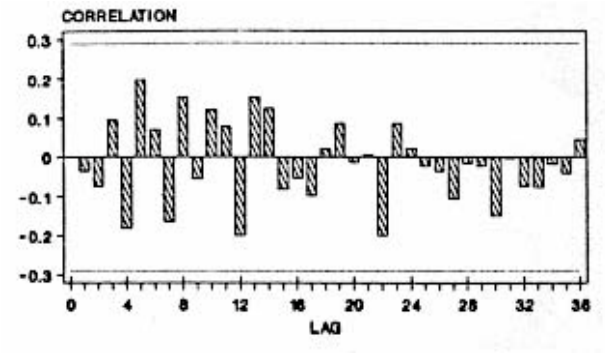

Fig. 3a. Autocorrelation function of residuals for AR(1) X SAR(1) model

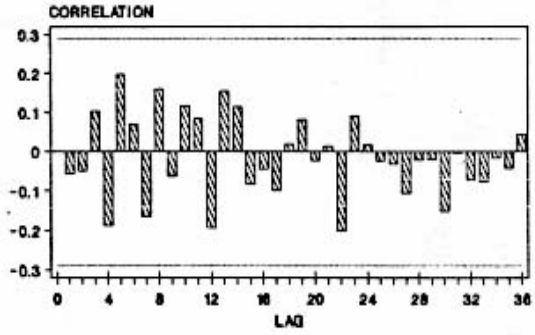

Fig. 4a. Autocorrelation function of residuals for MA(1) X SAR(1) model

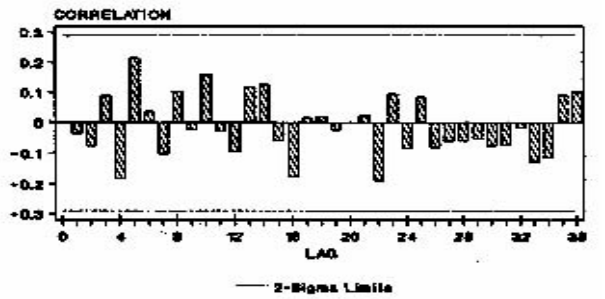

Fig. 3b. Partial autocorrelation function of residuals for AR(1) X SAR(1) model

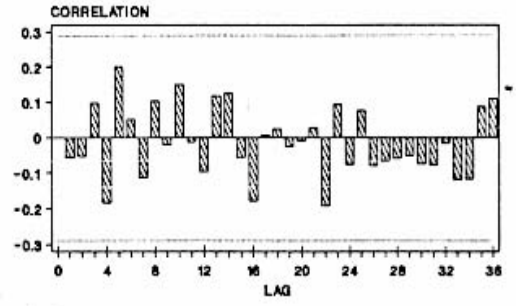

Fig. 4b. Partial autocorrelation function of residuals for MA(1) X SAR(1) model

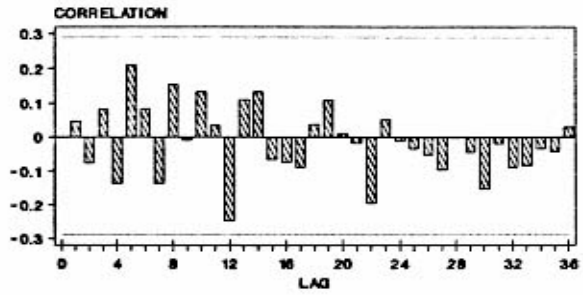

Fig. 5a. Autocorrelation function of residuals for SAR(1) model

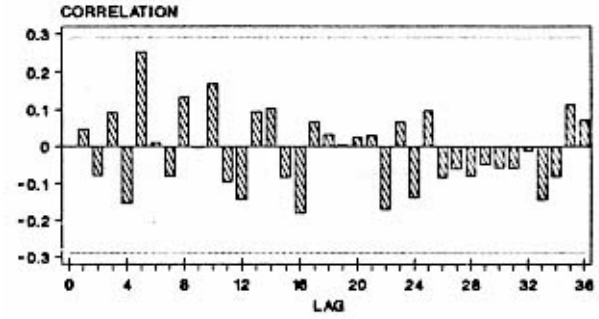

Fig. 5b. Partial autocorrelation function of residuals for SAR(1) model

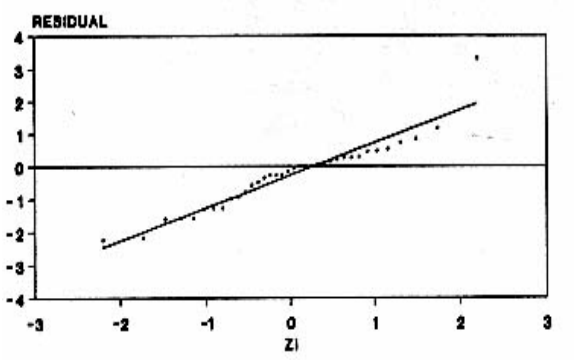

Fig. 6. Q-Q plot for residual of SAR(1) model

(6) *Outlier:* The plot of residuals (Fig.7) shows that the observation corresponding to December 1993 (month 18 in Fig.7) is an outlier. However, we have reasons to believe that it is a genuine observation (see Fig. 9 and related discussion). Hence, it is not dropped from the analysis.

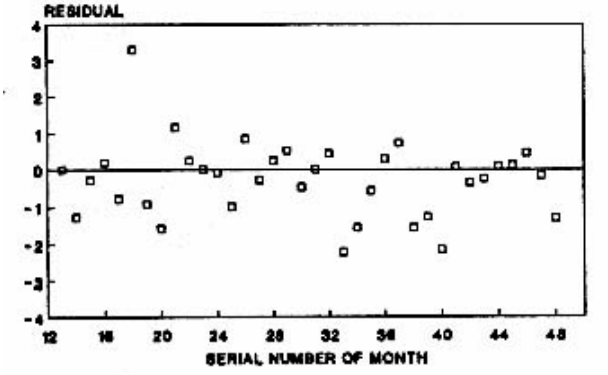

Fig. 7. Residual plot for SAR(1) model, July 1992-June 1996

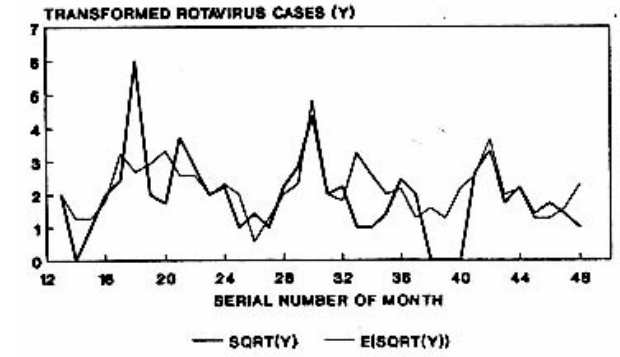

Fig. 8. Time series for observed and estimated transformed rotavirus diarrhoea cases

To describe the seasonality exhibited by rotavirus diarrhoea, the purely seasonal AR(1) model is found to be appropriate. Let,

y_t = The square root of number of cases at time.

δ = Constant term in the model.

Φ = The seasonal autoregressive (SAR) parameter.

s = Period of cycle.

e_t = Error variable at time t.

The identified SAR(1) model can be expressed as

$$y_t = \delta + \Phi y_{t-12} + e_t.$$

Substituting the estimates of the parameters from table III, we have

$$y_t = 0.5726 + 0.7105 y_{t-12} + e_t \quad (1).$$

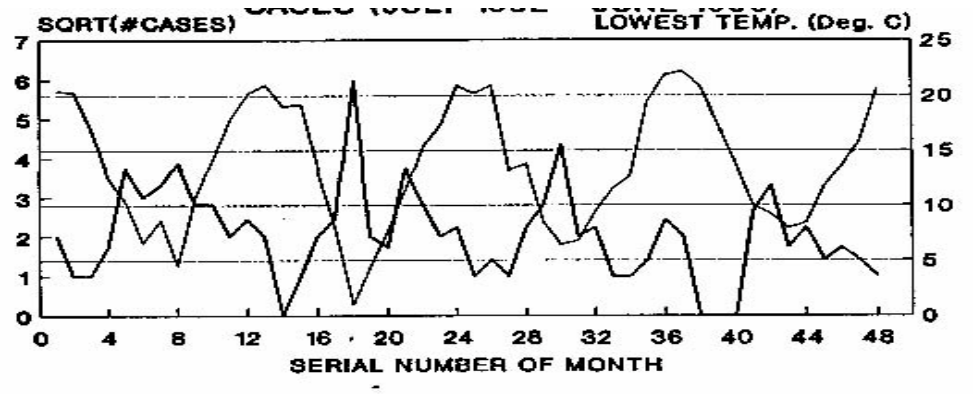

Fig. 9. Time series for lowest temperature and transformed rotavirus diarrhoea cases (July 1992-June 1996)

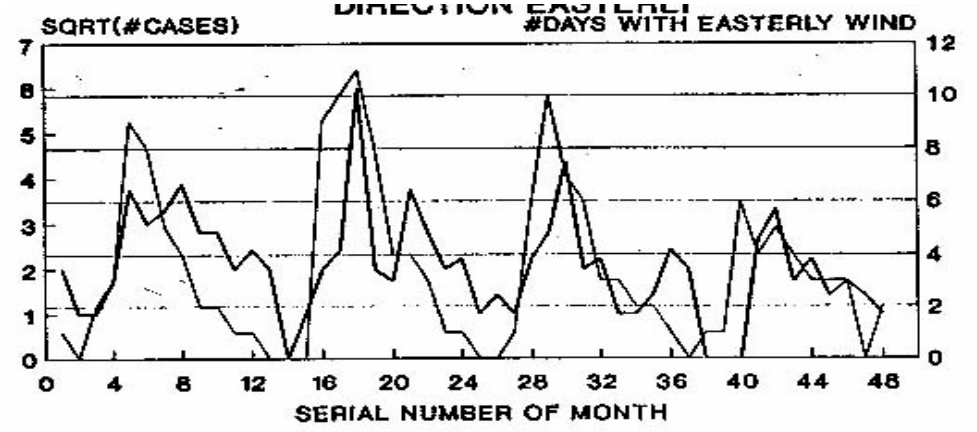

Fig. 10. Time series for number of days with easterly wind and transformed number of rotavirus diarrhoea cases

To note that the use of the BMDP statistical package yielded an equivalent model:

$$y_t = 0.959316 + 0.5434\, y_{t-12} + e_t \quad (2)$$

The models (1) and (2) are equivalent in the sense that the residual sum of squares (SS) for these models is approximately the same. Further, model (2) satisfies all the criteria for model adequacy. The actual values of the residual sum of squares are shown below:

	Residual SS	D.F.	Residual mean SS
Model 1	39.95310	34	1.1751
Model 2	39.33072	34	1.1568

Forecasting: Model (1) was used for forecasting. Fig. 8 presents the actual number and the estimated number of transformed rotavirus cases to get an idea of the closeness of the model. However, the percentage of explained variation is only 33. So, the forecasting power of the model is not high. Yet, this is not a negative feature (16), because this forecasting model is the best that can be developed for the time series under consideration. The capacity of rotaviruses to cause disease outbreaks depends upon complex interaction of a number of factors, and the variations of these diverse factors among the members of the community at risk add to the error variation.

INFLUENCE OF CLIMATIC FACTORS – DISCUSSION

The prominent seasonality observed in rotavirus diarrhoea cases coincides with annual changes in the climate. So, the next logical step is to collect and analyse the meteorological data to separate the influence of different weather parameters. Fig. 9 shows (i) the lowest minimum temperatures during the months, and (ii) the transformed number of rotavirus diarrhoea cases during the 48-month observation period. The comparison of these two clearly shows that the number of diarrhoea cases is inversely related to the temperature. It is interesting that the inverse trend captures even the outlying number of cases corresponding to December 1993 (month 18 in Fig. 9). This clearly suggests that rotavirus cases are more common in cooler months with seasonal peaks matching the lowest minimum temperature. Another interesting fact is revealed by Fig. 10. This plot shows (i) the number of days with easterly wind direction and (ii) the transformed number of rotavirus diarrhoea cases as functions of time. This plot is important to shed light on the seasonality in the tropical setting. A common feature of tropical weather is the easterly wave, which normally forms the convergent flow of trade winds and moves from east to west (17). We observe from the figure that this weather parameter also captures the cyclical incidence of rotavirus diarrhoea. This suggests that the annual rotavirus epidemics may follow a regional sequence from east to west. To note here that in a study of hospitalisations for childhood diarrhoea in the United States (18), the peak of hospitalisations occurs in the winter and repeatedly appears first in the west of the country, and then moves eastward. Since rotavirus is the principal agent of winter diarrhoea, this temporal geographic sequence is likely to be associated with rotavirus. Therefore, it would be interesting to carry out in India a study similar to that in North America (19) to describe the repetitive geographic sequence for seasonal activity of rotavirus diarrhoea. This will help understand the spread of the disease better in the subcontinent.

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Commentary

Time series and (S)ARMA models

A time series is a set of observations x_t, each one being recorded at a specified time t. For example, the time series in the article by Purohit, Kelkar and Simha (1) contains 48 couples of observations, i.e. the month and the number of rotavirus diarrhoea cases for each of 48 successive months. The number of cases is the dependent variable, while time is the explanatory variable. All possible time-related phenomena can be examined by time series analysis. It is widely used in many disciplines, for example in economics and astronomy. It is also applied to medicine. A Medline search without date limit gives 898 hits. Twenty of the most recent references deal with epidemiology, cardiology, neurology, physiology, and pathophysiology.

The purpose of the study of time series is to model the series, this means to express the observations as a function X of time t, X(t) or X_t, that fits the series as well as possible. X_t provides a mathematically appropriate description of the relationship between time and the observations and ascertains that the latter are indeed time-related. In some cases, it can also look for factors that influence the time series, as the climatic factors in the article under consideration, or predict future observations.

Two very important concepts in time series are the *autocorrelation function* and the *partial autocorrelation function*. The correlation between two variables is a value between –1 and 1. A correlation of 1 means high positive correlation: the variables have a great positive influence on each other. A correlation of –1 means highly negatively correlated: when the first variable is small, the other one will be high. Correlation zero means no correlation at all: the two successive variables are independent.

The two correlation functions are calculated in function of the *lag*. The lag is the time between two observations. The autocorrelation function at lag d tells us, stated very intuitively, what the influence is of a value of the time series at a certain moment t on the value d times later, at moment t+d. It is clear that for a normal stationary process, i.e. a process that retains the same statistical properties throughout time, the correlation will become smaller at larger and larger lags. The partial autocorrelation at lag d gives us the correlation between the observation at time t with the observations at times t-1, t-2, …, t-d.

With these basic concepts, we can start building a model.

The first step in the analysis of a time series is to plot the data. In many cases, the graph shows an increasing or decreasing trend. This may suggest the possibility of representing the data as a realisation of the process $X_t = m_t + Y_t$ where m_t is a slowly changing function, increasing or decreasing (the trend component), and Y_t is a stationary process.

A process Y_t is a *stationary* series when all the values vary around the same mean all the time. It is for this kind of processes that the theory of time series can build an adequate model following the Box-Jenkins approach (2). The most important step in this approach is the search for the best possible ARMA process. An ARMA process contains two parts: the autoregressive (AR) part and the moving average (MA) part.

A *moving average* of a sequence of values is a derived sequence of the averages of successive subsequences of a given number of values. It is used in the study of time series to even out short-term fluctuations, and make a trend clearer. So is the 3-term moving average of the hypothetical series 4, 6, 8, 7, 9, 8 equal to (4+6+8)/3 = 6; (6+8+7)/3 = 7; (8+7+9)/3 = 8 and (7+9+8)/3 = 8.

Mathematically, the moving average part of a time series writes the process Y_t as the following sum (in which the q_i's stand for the moving average parameters):

$Y_t = Z_t + q_1 Z_{t-1} + \ldots + q_q Z_{t-q}$ [1] where Z_i is a white noise process, i.e. a set of random fluctuations with mean zero.

Intuitively, a moving average process is in every point a linear combination of the q parameters $q_1, q_2, \ldots, q_q$. The choice of the number of parameters depends on the autocorrelation function. When the autocorrelation function gives high autocorrelations (this means higher than 0.3) until a certain lag j and small autocorrelations for larger lags, the parameter q should then be taken equal to j.

The other part of the ARMA process is the *autoregressive* part. Autoregressive means that the time series at time t is influenced by the preceding observations. An autoregressive process can be written in the following form (in which the F_i's are the autoregressive parameters):

$$Y_t = F_1 Y_{t-1} + F_2 Y_{t-2} \ldots + F_p Y_{t-p} \quad [2]$$

The choice of parameter p is very important. Is the value of a time series at moment t only determined by the value just before t or by several preceding values? The choice of p can be based on the partial autocorrelation function. When the partial autocorrelations are high till lag k, then p should be taken equal to k.

An ARMA process is the combination of a moving average process and an autoregressive process and, combining equations [2] and [1], can be written as:

$$Y_t = [1] + [2] = Z_t + q_1 Z_{t-1} + \ldots$$

$$+ q_q Z_{t-q} + F_1 Y_{t-1} + F_2 Y_{t-2} \ldots + F_p Y_{t-p}$$

A more usual presentation is

$$Y_t - F_1 Y_{t-1} - \ldots - F_p Y_{t-p} = Z_t + q_1 Z_{t-1} + \ldots + q_q Z_{t-q}.$$

The Box-Jenkins approach can be summarised in the following way. Take a look at the data. Remove trend and make the process stationary. Plot the autocorrelation function and the partial autocorrelation function for this process. Decide what to chose for the parameters p and q. Estimate the parameters $F_1, \ldots, F_p, q_1, \ldots, q_q$ and the ARMA model is found.

Seasonal series are characterised by a strong serial correlation at the seasonal lag (and possibly multiples thereof). For example, the correlation function in figure 2a of the paper by Purohit and co-workers strongly suggests a seasonal series with seasons of 12 months. Seasonal ARMA models, used in this article, are a variation on the explained ARMA model and allow for randomness in the seasonal pattern from one cycle to the next.

The method the authors use to model their data-set can be explained as follows. The first step is plotting the data and transforming them to eliminate high variability in the time series. In this case, the square root of the number of diarrhoea cases is taken. To choose the number of parameters in the MA part, we take a look at the autocorrelation function. The parameter q, i.e. the number of parameters q in the moving average part of the model, is the last lag with a significant autocorrelation. For the non-seasonal part, we look at lag 1, 2, 3, etc., for the seasonal part at lag 12, 24, 36,… . The choice of parameter p in the autoregressive part is made in the same way, but using the partial autocorrelation function instead of the autocorrelation function. So, instead of a simple ARMA (p,q) model, we get a combination of an ARMA (p,q) at lag 1 model and an ARMA (P,Q) at lag 12 model, the latter sometimes being called a SARMA model because of the seasonality.

Several choices for a model are made and the next step is to test the adequacy of the models, to decide which model is the best. The authors calculate the Box-Pierce-Jung statistic. For this test, the null hypothesis is that the model is a good model. If the probability of the null hypothesis (the p value) is small, the model has to be rejected, but if the p value is larger than 0.05, the model can be accepted as a good model.

When different models have large p values, there are still the residuals to examine. The residuals are the differences between the real observations and the model. It is easy to understand that the smaller the residuals are, the better the model is. In their article, the authors finally decide to take an AR(1) x SAR(1) model. This means that the moving average component plays a negligible role, so that it is not retained in the model, and that the AR and SAR parameters are limited to, respectively, one month and one year after the surge in the numbers of observation. Though it has a low predictive value, it appropriately represents the seasonality of rotavirus diarrhoea in Pune: the epidemics occur at an yearly interval and last about two months.

Time series are not easy to explain in non-mathematical terms. Standard introductory books on medical statistics (3,4,5) do not mention the subject. Armitage and Berry (6) only discuss it briefly. The book by Brockwell and Davis (7) is a good introduction but requires a more than average mathematical background. Still, time series analysis can be a powerful tool in medical research. It is one of the areas where close collaboration between medical researchers and statisticians can be very rewarding.

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SHORT REPORT

Detection of *tdh* and *trh* Genes in a Urea-hydrolysing Environmental Isolate of *Vibrio parahaemolyticus* from the Andamans

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ABSTRACT

Co-existence of *trh* gene and urea-hydrolysing property in one of 44 marine water isolates of *Vibrio parahaemolyticus* correlates strongly with both genotypic and phenotypic characteristics of the bacterium. Thus, urease-producing phenotype can be considered a marker of virulence for the production of thermostable direct haemolysin-related haemolysin (TRH) (i.e. possession of *trh* gene). The same isolate also possessed the *tdh* gene. An environmental isolate possessing all the characteristics of a pathogenic *V. parahaemolyticus* in this marine environment suggest that there is a likelihood of the occurrence of clinical cases of gastroenteritis caused by *V. parahaemolyticus* in the Andamans.

Key words : *Vibrio parahaemolyticus*; Gene, Bacterial; Urease; Gastroenteritis; Virulence

INTRODUCTION

The Kanagawa phenomenon (KP), a b-type haemolysis on Wagatsuma agar medium, is a common phenotypic characteristic of clinical isolates of *Vibrio parahaemolyticus*. It is considered a marker for virulence (1). This haemolysis has been related to the production of the thermostable direct haemolysin (TDH) by *V. parahaemolyticus* (2). Another thermostable haemolysin, known as TDH-related haemolysin (TRH), has been described in KP-negative *V. parahaemolyticus* strains and is known to play an important role in the causation of diarrhoea (3). Urease production, which is not commonly associated with strains of *V. parahaemolyticus*, has been reported in 7.5% of clinical isolates from Thailand, and a majority of these strains showed the presence of *trh* and *tdh* genes (4). The *trh* and *tdh* genes are now considered to be important virulence genes that play a role in the pathogenicity of *V. parahaemolyticus*. Presence of these characteristics has not been reported among environmental isolates of *V. parahaemolyticus* till now. We report the isolation from marine waters of the Andaman Islands of a *V. parahaemolyticus* strain that had all the above-mentioned characteristics.

MATERIALS AND METHODS

Study area

The Andaman and Nicobar Islands, an archipelago of more than 500 islands, islets and rocks, stretch as a chain

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in north-south direction. They lie between longitude 92º to 94º East and latitude 6º to 14º North in the Bay of Bengal, about 1200 km east of the Indian subcontinent but only 100-250 km west from Thailand, Myanmar, and Indonesia. Port Blair, the capital town, is connected with the Indian mainland by air and sea routes and with other islands by regular ferry services. Fishing and tourism are important industries of the islands, and consumption of seafood is high.

Bacterial strains

From August 1996 to July 1997, we isolated 44 strains of marine *V. parahaemolyticus* from five different coastal sites around Port Blair. The strains were biochemically characterised as *V. parahaemolyticus* using standard bacteriological procedures (5). In brief, suspected strains were identified by performing a battery of tests, including the detection of oxidase, catalase, motility, lysine decarboxylation, fermentation of glucose and mannitol, neither H_2S nor visible gas production in Triple Sugar Iron agar (Hi-media, India), production of indole, and salt tolerance at 3%, 6%, 8% and 10% concentration of NaCl. Urea agar (Hi-media, India) was used for the detection of urease production.

A TDH-positive (VP-15) strain and a TDH-negative (VP-3) strain of *V. parahaemolyticus* obtained from the National Institute of Cholera and Enteric Diseases, Calcutta were included as controls.

Kanagawa phenomenon (KP)

KP was tested as described earlier (6). All the strains were examined by spotting overnight culture in tryptic soy broth (Difco, USA) supplemented with 3% NaCl on Wagatsuma agar (Eiken Ltd., Japan) containing a freshly collected 20% suspension of washed human blood group O erythrocytes. Results were recorded after 24 hours of incubation at 37ºC. A clear zone of haemolysis around the spot on Wagatsuma blood agar plate was considered a positive result.

Haemolysin production

V. parahaemolyticus strains were inoculated into tryptic soy broth with 3% NaCl and incubated overnight at 37º C in a shaking bath. One aliquot of culture broth was directly spotted onto the Wagatsuma blood agar and other was centrifuged with a Beckman cold centrifuge (USA) at 10º C for 10 minutes at 10,000 rpm. The supernatant was again divided into two aliquots; one was tested for KP and other was heat-treated at 60º C for 10 minutes followed by test for KP. Plates showing distinct haemolysis were kept at 4º C and examined daily.

Polymerase Chain Reaction (PCR) assay

A PCR assay was used for the detection of the *tdh* and *trh* genes of *V. parahaemolyticus*. Presence of a 72-bp fragment of *tdh* and a 249 -bp fragment of *trh* amplicon was tested by PCR using the primer pairs 5'-CCA TCT GTC CCT TTT CCT GC-3' (sense); 5'-CCA AAT ACA TTT TAC TTG G-3' (antisense) and 5'-GGC TCA AAA TGG TTA AGC G-3' (sense); 5'-CAT TTC CGC TCT CAT ATG C-3' (antisense) respectively (7).

The following were added to each 100 mL of Mg-free 10x amplification buffer (500 mM KCI, 100 mM Tris HCl [pH 9.0], 0.1% Triton-X100); 8 mL of 25 mM $MgC1_2$; 2 mL each of 2 mM dATP, dTTP, dGTP and dCTP; 50 pmol each of the primers and 5 U of Taq DNA polymerase (Takarashuzo, Otsu, Japan). PCR was carried out in 0.2 mL microcentrifuge tubes, with 19.7 mL of the PCR mixture described above and 5.3 mL of Luria broth (Difco, USA) culture of the test strains heated at 94º C for 5 minutes. The solution was overlaid with a drop of sterile mineral oil (Sigma Chemicals, USA), and PCR was performed in an automated thermocycler (Biometra, Göttingen, Germany). PCR amplification was performed for 30 cycles, and the cycling conditions were as follows: denaturation at 94º C, annealing at 50º C and extension at 72º C, each for 1 minutes and 30 seconds. A reagent blank (containing all the components of the reaction mixture and water instead of broth containing template DNA), and VP36 (*tdh* and *trh* positive *V. parahaemolyticus*) were run as controls. Electrophoresis of the amplified products was done on 2% agarose gel with ethidium bromide staining. A 1-kb molecular ladder (Gibco BRL, Gaithensburg, MD, USA) was run with each gel.

RESULTS

All 44 isolates produced clear haemolysis on Wagatsuma agar, i.e. were KP-positive. Yet, the haemolytic activity was temperature-sensitive: it disappeared after exposure of the samples to 60º C for 10 minutes, while enhancement of the activity was observed when plates were kept at 4º C for more than 24 hours. Only one of the strains revealed the presence of both *tdh* and *trh* genes by PCR analysis; it also showed urease activity. Control strains used in this study showed results according to their known characteristics.

DISCUSSION

V. parahaemolyticus-associated gastroenteritis is a major public health problem specially in areas where seafood is commonly consumed, but many strains are not pathogenic (8). Clinical strains generally do produce a typical b-haemolysis on Wagatsuma agar, but very few environmental strains do so (1,2). Thus, environmental strains are considered mostly non-pathogenic. Recent research in *V. parahaemolyticus* demonstrates a number of haemolysins, both heat-stable (TRH I, TRH II, etc.) (9) and heat-labile (10,11). Virulence of *V. parahaemolyticus* is mostly associated with thermostable haemolysin(s). In our observation, all the environmental strains produced very clear b-haemolysis, which contrasts with the presently held view that only 1% environmental strains possess this property (1). The emergence of higher rate of KP-positive environmental strains has also been reported from different parts of India and elsewhere (12,13). The haemolysis on Wagatsuma agar is due to production of TDH, and, thus, TDH has long been considered a major virulence factor (14). However, haemolysin produced by our KP-positive isolates is thermolabile and *tdh* gene-negative (15). This observation needs further investigation.

Recently, the proportion of the *trh* gene among clinical strains has been increasing, and a strong correlation has been established between the presence of *trh* gene and urease production (4,16). Urea hydrolysis is not a common biochemical criterion for the identification of *V. parahaemolyticus*, but urease-producing isolates of *V. parahaemolyticus* have been reported since 1979 from different parts of the world, both from clinical cases (17) and environmental sources (18). The unique observation of our study is that one of our marine water isolates possesses all the virulence characteristics: presence of *tdh* and *trh* genes and hydrolysis of urea, which have recently been reported among Thai isolates from clinical cases (4). This demonstrates that urease production and possession of *trh* gene are not only restricted to Thai clinical isolates but are also present in an isolate from Andaman coastal sea water. It indicates the possibility of transmission of virulent *V. parahaemolyticus* from one coast to another through water. We agree with the earlier observation of Suthienkul *et al*. (4) that urea hydrolysis may be considered a phenotypic marker for the *trh* gene in *V. parahaemolyticus* isolates. This phenotypic property may be used for initial selection of potentially virulent *V. parahaemolyticus*. Though we have not been able to detect any clinical case due to *V. parahaemolyticus* during the last five years of surveillance, the presence of at least one isolate having all the characteristics of a pathogenic *V. parahaemolyticus* in the marine environment does not rule out the likelihood of occurrence of clinical cases of gastroenteritis caused by this bacterium in the Andaman Islands in near future.

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Emerging and Re-emerging Infectious Diseases

Perinatal Transmission of Hepatitis B in Rural Bangladesh

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Objective: Estimate the relative importance of perinatal transmission of hepatitis B in Bangladesh.

Methodology: Paired-serum samples (330 mothers and their 334 infants) were tested for hepatitis B markers in a cross-sectional study. Infants were aged 2-8 months (~40 per age month), and cord blood was drawn from 33 deliveries. Laboratory personnel were kept blind on any information regarding the individual. The study evaluated hepatitis B core (HBcAg), surface antigen (HBsAg) and e-antigen (HBeAg) using a commercial ELISA test kit. All samples were screened for HBcAg and those testing positive were further tested for HBsAg. Those positive for HBsAg were, in turn, tested for HBeAg.

Results: The mothers were young and of low parity, with a mean (SD) height of 150 (5.4) cm. The mean birth weight of 33 infants delivered at the hospital was 2.5 (0.46) kg. In maternal samples, 107 (32.4%) were positive for HBcAg, 18 (5.4%) for HBsAg, and 4 (1.2%) for HBeAg. In infant samples, 35 (10.5%) were positive for HBcAg, 1 (0.3%) for HBsAg, and none for HBeAg. Of the 35 HBcAg-positive infants, only 1 was an offspring from a HBcAg-negative mother, and was a 7-month old girl who was otherwise HBsAg-negative. Of the 18 HBsAg-positive mothers, 4 (22%) were HBeAg-positive. All 14 children of the mothers who were HBeAg-negative were negative for HBsAg. Only one of four (25%) of the children of the HBeAg-positive mothers was HBsAg carrier (8 months old), and in three children, transmission did not occur (two 8 months old, one 6 months old).

Conclusion: Hepatitis B is prevalent in rural Bangladesh. Perinatal transmission mode is relatively low. HBsAg-positive and negative for e-antigen mothers do not infect their babies. The low transmissibility of surface antigen to infants reported in this study contrasts with the published reports from other developing countries.

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Multiresistant Salmonella Infections: An Emerging Health Problem in Bangladesh

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Objective: Determine the prevalence and importance of multiresistant Salmonella isolates in Dhaka, Bangladesh.

Methodology: The present status of multiresistant Salmonella infections was studied by analyzing cases who submitted faecal samples for culture to ICDDR,B hospital in Dhaka during 1989-1995.

Results: In total, 4,044 Salmonella strains were isolated from 120,489 (3.36%) faecal samples during 1989-1995. Of the 499 salmonellae isolated in 1989, group C (33%) was the most common isolate, followed by S. typhi (21.2%), and group B (20.8%). In 1992, the isolation rate of Salmonella group B increased significantly to 57% ($p<0.01$) of the 628 Salmonella isolates, which increased further to 65% of the 977 Salmonella isolates in 1995. The isolation rates of S. typhi and all other serogroups decreased significantly in 1995 compared to 1989. Of the 82 Salmonella group B isolates tested, 45 (55%) were S. typhimurium and 35 (43%) S. gloucester. Both the serotypes were resistant to ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, and tetracycline which are mediated by a 157-kb conjugative plasmid.

Conclusion: The findings of the study suggest that multiresistant S. gloucester, a rare serotype, and S. typhimurium were responsible for Salmonella-associated diarrhoea in Bangladesh.

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Sero-epidemiological Study of Dengue and Dengue Haemorrhagic Fevers in a Metropolitan City of Bangladesh

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Objective: Identify the proportion of dengue and dengue haemorrhagic fevers (DHF) in children attending the Chittagong Medical College and Hospital (CMCH).

Methodology: The Ministry of Health and Family Welfare, Government of Bangladesh and the World Health Organization (WHO) jointly sponsored a hospital-based descriptive cross-sectional survey which identified dengue and DHF serologically. At the paediatrics departments (both out- and in-patients) of the CMCH during September 1996-June 1997, 255 cases were included consecutively following inclusion criteria, i.e.: febrile illness for 72 hours, 1-15 years of age, no focal clinical sign, and no evidence of any other infections detected by the available routine tests. Paired serum samples were taken from each case - the first sample on admission or at attendance, and the second sample after 7 days. The serum samples were processed and preserved for bulk transportation to the Virology Laboratory of the Institute of Epidemiology, Disease Control and Research in Dhaka. A specified data collection form was used for each subject. For serology, haemagglutination inhibition (HI) test was used following the Clarke and Casals Technique (1958) and interpreted according to the WHO criteria. Data were analyzed using the EPINFO 6 software.

Results: 255 were finally included for the study yielding same numbers of paired samples for HI test. The total number of males was 155 (60.7%) and of females 100 (39.3%), ratio being 1.5 and the mean age 7.2 years. Thirty-five (13.7%) cases were found to be positive for dengue, of which 71.4% were males and 28.5% females (ratio 2.5); 14.3% were of primary, 37.1% of secondary, and 48.6% of mixed (primary/secondary) infections. Dengue virus subtypes alone or in combination were: D2 2.9%, D3 47.7%, D4 28.6%, D2+D3 2.9%, D2+D4 11.4%, D3+D4 8.6%, and no D1. The 5-9-year age group was most affected with 57.1% frequency, followed by 1-4 and 10+ years age group. Seasonal occurrences of the positive cases were: premonsoon 28.5%, monsoon 25.7%, and postmonsoon 45.7%.

Conclusion: Contrary to the common notion, dengue is present in Bangladesh with high male preponderance; higher frequency relates to monsoon; secondary and mixed types and all subtypes of virus except D1 predominate. Present situation is possibly an alarming harbinger of future catastrophe.

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Community-based TB Control Programme in Bangladesh: A Grassroots-level Experience of BRAC

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Objective: Review the experience of BRAC in community-based tuberculosis control programme in Bangladesh. Tuberculosis is one of the leading killers in Bangladesh accounting for nearly 80,000 deaths and 150,000 additional cases each year. For TB diagnosis and for making treatment available and accessible at the village level, BRAC initiated a community-based TB control programme in 1984 in one thana (phase one). Shasthya Shebikas (voluntary female health workers) were the nucleus of the programme, who were involved in symptomatic identification and ensuring directly observed treatment. In the phase two (1992-1994), the programme was expanded to include nine more thanas, and in the phase three (1995), another eight thanas were included.

Methodology: All data from 1992 to 1995 were analyzed. Analyses in phase two (12-month therapy) and phase three (8-month therapy) were done separately to find out the treatment outcomes. In addition, a cross-sectional survey of tuberculosis was conducted in more than 9,000 randomly selected households in two phases—two intervention or programme thanas and one non-programme thana. Follow-up analysis of all the treated patients in the programme thanas was also done.

Results: In phase two analysis, 90% of the identified 3,886 cases accepted the 12-month regimen treatment. In phase three, all of the 1,741 identified cases accepted the 8-month regimen treatment. The cure rate was 81% and 85.9% in phase two and phase three respectively. The relapse rate two or more years after the completion of treatment was found to be higher than the earlier rate. The drop-out rate was found to be 3.1%. In the cross-sectional survey, the prevalence of tuberculosis was found to be half in the BRAC intervention area (0.07 vs.15 per 100).

Conclusion: The community-based tuberculosis control programme of BRAC, in collaboration with the National Tuberculosis Programme, achieved 85% cure rate and high case-detection rate. The prevalence survey suggested that at least half of the existing cases have been detected by the programme.

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Early Termination of a Randomized Controlled Trial for Evaluating Alternate Therapeutic Regimens for Uncomplicated Malaria in a Thana Health Complex of Bangladesh

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Objective: Compare the efficacy of alternate therapeutic regimens for uncomplicated malaria recommended for hyper-endemic malarious zones of Bangladesh.

Methodology: The Ministry of Health and Family Welfare, Government of Bangladesh and the World Health Organization jointly sponsored a Thana Health Complex (THC)-based randomized controlled trial for comparing 1st, 2nd and 3rd line regimens for uncomplicated malaria. The regimens were chloroquine (CQ) for 3 days, oral quinine sulphate for 3 days followed by single-dose sulphadoxine/pyrimethamine (Q3+SP), and oral quinine for 7 days (07). The sample size included 400 patients, and randomization was done by lottery. Patients aged over 12 years and of both sexes with fever or history of (H/O) fever over the last 48 hours, blood slide showing asexual P. falciparum parasite count between 500-250,000/cmm, with no severe manifestations, no H/O antimalarials over the last 7 days and having no concomitant febrile illness, were included in the study. Patients agreeing with written consent to stay in hospital for 8 days and allowing daily blood slide examination were included and followed up on day 14, 21, and 28. Patients failing to attend for follow-up were visited in their homes. Drug administration was supervised. Clinical and parasitological responses were recorded on a pre-designed scale. The results were analyzed, using the EPI INFO 6 software.

Results: Complete data on 212 patients were available for a mid-term evaluation, of which 8 patients were lost to follow-up. A sensitive clinical response was observed in 22 (17.32%) of the 81 patients in the CQ group, 59 (77.63%) of the 76 patients in the Q3+SP group, and 47 (100%) of the 47 patients in the Q7 group. An early treatment failure was observed only in the CQ group (45.67%), and all the failures with Q3+SP were due to late treatment. The parasitological response was almost similar except that two patients in the Q7 group had asymptomatic parasitaemia on day 28. The groups were comparable in respect of all other variables.

Conclusion: The highly significant difference in sensitivity led to the termination of the trial. If similar sensitivity pattern is observed in other parts of the country, modification of national recommendations would be required. Mefloquine may be considered the second choice of treatment if not the first.

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Molecular Analysis of Toxigenic Vibrio cholerae Strains Isolated in Bangladesh During 1961-1996: Relationship Between Continual Emergence of New Toxigenic Clones and Epidemics of Cholera

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Objective: Analyze virulence-associated genes, and study clonal relationships among toxigenic Vibrio cholerae strains isolated in Bangladesh during 1961-1996 to establish whether seasonal epidemics are caused by repeated emergence of the same strains or by diverse clones of toxigenic V. cholerae.

Methodology: Three hundred seventy-eight V. cholerae isolates obtained from cholera patients or from surface water were included in the study. Molecular analysis of virulence-associated gene clusters, including the CTX genetic element, tcpA, tcpI, and ToxR was performed using specific probes or PCR assays. Comparative analysis of serotype-specific rfb gene clusters in selected V. cholerae O1 and O139 strains was performed by multiple PCR assays using primers corresponding to defined regions of the rfb genes. Clonal relationships among strains were studied by computer-assisted numerical analysis of restriction fragment length polymorphisms in conserved rRNA genes. Induction and propagation of the lysogenic bacteriophage-encoding cholera toxin (CTX?) were studied both in animal models and under laboratory conditions.

Results: Analysis of toxigenic V. cholerae strains isolated during 1961-1996 revealed clonal diversity among the strains isolated during different epidemics. This study demonstrated the transient appearance and disappearance of more than 6 different clones among classical vibrios, at least 5 clones of El Tor vibrios, and 3 different clones of V. cholerae O139. Different clones of strains belonged to different ribotypes and often to different CTX genotypes resulting from differences in the copy number of CTX element and variations in the integration site of CTX in the chromosome. Studies on the induction of lysogenic CTX? revealed that 37.95% of the strains tested could be induced with mitomycin-C to produce infectious extracellular CTX? particles which infected recipient strains under conditions conducive to the expression of tcp genes.

Conclusion: There has been a continual emergence of new clones of toxigenic V. cholerae replacing the existing clones, possibly through the horizontal transfer of virulence-associated genes, and natural selection involving unidentified environmental factors and immunity of the host population. In view of the recent discovery of lysogenic conversion by CTXF as a possible mechanism of origination of new toxigenic clones, it appears that the continual emergence of new toxigenic clones and their selective enrichment during cholera outbreaks constitute an essential component of the natural eco-system for the evolution of epidemic V. cholerae strains and the genetic elements that mediate the transfer of virulence genes.

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Contrasting Findings of Acute Respiratory Infection between Urban and Rural Children: Recent Observations from an Ongoing Study

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Objective: Compare the clinico-epidemiological and microbiological findings on acute respiratory infection (ARI) between the urban and rural Bangladeshi children aged less than five years.

Methodology: The study, initiated in 1994, was conducted on the basis of a series of cross-sectional observations on the children aged less than five years with the complaints of ARI, attending the outpatient departments (OPDs) of selected urban hospitals and rural thana health complexes (THCs). The children were clinically examined, and epidemiological data were collected from the children's family. Naso-pharyngeal swabs were streaked onto enriched chocolate agar and were cultured bacteriologically. Data were analyzed using the SPSS/PC+ software.

Results: Although 546 children have been studied so far since 1994, recent cross-sectional data on 83 children (38 from urban and 45 from rural OPDs) are reported here. As per WHO's classification of ARI, pneumonia was predominantly prevalent (44%, 36/83), followed by non-pneumonic (41%, 34/63) cases. Infants (aged less than one year) from rural OPDs suffered more than their urban counterparts (p=0.01). Moreover, the rural subjects suffered from pneumonia (p=0.003) more than the urban ones (p=0.24). The male and female subjects differed in their clinical types of ARI (p=0.002), while more (92%, 11/12) girls suffered from severe pneumonia than the boys (8%, 1/12), and more boys (69% 25 of 36) suffered from pneumonia than the girls (31%, 11/36). Haemophilus influenzae was predominantly isolated (63%, 52/83) over other bacteria (37%, 31/83). H. influenzae was isolated from pneumonia and severe pneumonia cases more from the rural subjects (61%, 17/28) than their urban counterparts (58%, 14/24). In contrast, serotype-specific H. influenzae was more significantly associated with the pneumonia cases from the urban (p=0.000) than the rural (p=0.16) areas. Over 95% of the subjects had cough, and over 63% had difficult breathing. Chest indrawing, wheeze, and crepitation were significantly associated with the isolation of H. influenzae more among the urban than rural subjects (p=0.003 and 0.007 respectively).

Conclusion: The clinical and bacteriological findings on ARI cases among the children aged less than five years contrast between the urban and rural areas. A more detailed study is underway.

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Immunoblot Analysis as a Diagnostic Tool for Detection of Visceral Leishmaniasis in Bangladesh

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Objective: Develop a specific diagnostic test for visceral leishmaniasis in Bangladesh.

Methodology: Sera of 32 confirmed visceral leishmaniasis (VL) patients, obtained from different hospitals in Bangladesh during November 1996-April 1997, were studied by the immunoblot technique with antigen prepared from Leishmania donovani. Controls included sera of 34 healthy individuals from both endemic and non-endemic regions, 25 patients with non-leishmanial infections, and one individual treated for visceral leishmaniasis. Direct agglutination test (DAT) was performed on all sera.

Results: Sera of the VL patients showed heterogeneity of polypeptide recognition and identified many polypeptides with relative molecular mass ranging from 16 to >106 kD. The 56-64-kD band was recognized by all sera, while the 106, 78, 76 and 66-kD polypeptide bands were identified by 91%, 91%, 97%, and 97% of the sera from the VL patients respectively. Three of these polypeptides and the 56-64 kD polypeptide were recognized by 97% of the sera from the VL patients. The 76-kD polypeptide band was not recognized by sera of only two patients, of whom one had been treated for VL. The recognition of the 56-64-kD band had a sensitivity and specificity of 100% and 90% respectively and that of the 76-kD band has a sensitivity and specificity of 97% and 98%. For both VL patients and the treated individual, DAT was positive at high titre (1:102400). The sera of patients with non-leishmanial infection identified one or two of the five specific polypeptides, but in no case more than two.

Conclusion: Immunoblot analysis can be a valuable tool in specific diagnosis of active visceral leishmaniasis in Bangladesh. The recognition of the 56-64-kD band, in addition to any three bands, may be considered diagnostic of VL. Additionally, further studies can confirm if this technique can differentiate active infection from treated infections unlike DAT, which is currently used in Bangladesh.

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Miscellaneous Health-related Topics

Reported Morbid Symptoms and Conditions of Pregnant, Intrapartum and Postpartum Women: Experience from Three Villages

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Objective: Investigate reported morbid symptoms and conditions of antepartum, intrapartum and postpartum women.

Methodology: In early 1996, a cross-sectional survey was conducted in three villages outside ICDDR,B Matlab intervention and comparison areas. All married women aged 14-49 years were identified, and 208 women were found to be either pregnant or within 12 weeks of postpartum period. One hundred and fifty-seven (76%) of these women were interviewed for morbid symptoms and conditions they had during the survey, and were also asked to recall symptoms and conditions they suffered during their antepartum, intrapartum or postpartum periods.

Results: During the survey, 127 (81%) women reported at least one morbid symptom or condition, while 85 (54.1%) women reported symptoms, indicative of anaemia, 36 (22%) leukorrhoea, 34 (21.7%) urinary problems, and 33 (21%) genital prolapse. In addition, 67 (43%) women reported postpartum haemorrhage, 44 (28%) prolonged, obstructed or difficult labour, 27 (17.2%) perineal tear, 19 (12 %) breast problems, and 16 (10%) postpartum fever. Women of older age and higher parity were more likely to report at least one morbid symptom or condition and also were more likely to report symptoms, indicative of anaemia ($p<.05$).

Conclusion: Pregnant, intrapartum and postpartum women in rural Bangladesh bear significant burden of morbid symptoms and conditions, especially with increasing age and parity. Though not validated by physical examinations or laboratory tests, the data suggest a true account of women's perceptions of their own illnesses which are of crucial importance since women, in most situations, use health services according to their perceptions of sufferings. To improve the rural women's health, health policy-makers need to be aware of women's health problems as women view them.

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Efficacy and Safety of Ciprofloxacin Suspension in the Treatment of Childhood Shigellosis

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Objective: Compare the clinical and bacteriologic efficacy and toxicity of ciprofloxacin and amdinocillin pivoxyl in the treatment of shigellosis in children.

Methodology: This randomized, double-blind, controlled clinical trial was conducted at the Dhaka Hospital of ICDDR,B from August 1995 to March 1997. Children aged 2-15 years with Shigella-associated dysentery of £72 hours were eligible, provided their parents gave a written consent. Patients stayed in the hospital for 6 days and returned for follow-up evaluation 7 and 30 days after discharge. Assessment of joint symptoms and function was made daily and at follow-ups. Safety of the study drugs was evaluated in a total of 141 children enrolled in the study (70 in the ciprofloxacin group and 71 in the amdinocillin pivoxyl group), of whom the clinical and bacteriologic efficacy could be evaluated in 120 children (60 children in each treatment group). Patients received either ciprofloxacin suspension, 10 mg/kg 12 hourly or amdinocillin pivoxyl tablets, 15-20 mg/kg 8 hourly for 5 days. Therapy was clinically effective, if a patient did not have frank dysentery on day 3; and had no bloody-mucoid stools on day 5, had £1 watery stool, £6 total stools, and was afebrile; and bacteriologically successful, or if Shigella could not be isolated from faecal samples on day 3 and thereafter. The rates of clinical and bacteriologic cure, and the rates and types of the adverse events in the two treatment groups were compared using the standard statistical tests.

Results: Therapy was clinically successful in 48/60 (80%) and 39/60 (65%) patients (difference 15%; 95% CI -0.7- 30.8), and bacteriologically successful in 60 (100%) and 54 (90%) patients (difference 10%; 95% CI 2.4 -17.6%) who had received ciprofloxacin and amdinocillin respectively. Joint pain after initiation of therapy occurred in 13/70 (18.6%) and 16/71 (22.5%) patients who had received ciprofloxacin and amdinocillin respectively (p>0.2), and no patient had signs of arthritis.

Conclusion: Ciprofloxacin is an effective and safe drug for use in multiply-resistant childhood shigellosis.

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Health Conditions of Pregnant Women and Perinatal Mortality in a Slum of Dhaka City, Bangladesh

Abdullah Al Mamun[1] and Therese Juncker[2]

Objective: Examine the association between perinatal mortality with health conditions, and treatment-seeking behaviour of pregnant women in a slum population of Dhaka city in Bangladesh.

Methodology: A cohort of 2,007 pregnant women with a community setting was studied between June 1994 and January 1997. Women who, according to their last menstruation date, had less than 21 weeks of amenorrhoea, were considered for the study. The women were recruited from two antenatal clinics run by Radda Barnen, a large organization established in Mirpur, and from house-to-house survey in the catchment area of Radda Barnen. Those who signed a consent form were interviewed. The women were followed from 21 weeks of gestation to pregnancy termination. Information was collected on height, weight, mid-upper arm circumference (MUAC), haemoglobin, blood pressure, incidence of illnesses, and their treatments. Socioeconomic information was collected during the recruitment of the women, and information on the type of pregnancy termination was collected within 3 days of termination. Stillbirth or mortality of the newborns within 3 days of delivery was taken as the indicator of perinatal mortality.

Results: Perinatal mortality was 60.3 per 1,000 livebirths (stillbirth ratio was 40.5 and 0-3-day mortality was 18.8 per 1,000 live-births). The logistic regression analysis showed that eclampsia, history of blood loss during pregnancy, hypertension, haemorrhage, and other infections were significant risk factors for perinatal mortality. Intrauterine device used within the last two years preceding the present conception was also a significant risk factor for perinatal mortality. Body mass index or heights were not associated with perinatal mortality. Perinatal mortality was negatively associated with the number of visits to medical professionals during pregnancy. The educated women had significantly lower perinatal mortality than the uneducated ones, after controlling for demographic, biomedical and economic factors.

Conclusion: Access to medical care during pregnancy can substantially reduce perinatal mortality among the poor women in the urban slums.

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Determinants of Safe Delivery Practices in Rural Bangladesh

M Mafizur Rahman, Barkat-e-Khuda, Thomas T Kane, Masud Reza, and ABM Khorshed A Mozumder

Objective: Investigate the demographic, socioeconomic and cultural factors associated with safe delivery practices in rural Bangladesh.

Methodology: Data were drawn from a cross-sectional survey conducted in six rural subdistricts of Bangladesh in 1994. A sample of 10,368 currently married women of reproductive age was selected, following the multistage random-sampling procedures, they were interviewed using a structured questionnaire to elicit information on various demographic, socioeconomic, cultural and selected programmatic variables, including public health issues, such as maternal health care and delivery practices. In analyzing data, descriptive statistics and multivariate regression methods were employed. In this analysis, the term "safe delivery" refers to a delivery conducted by a qualified person, such as registered physician, paramedic, nurse, or trained traditional birth attendant.

Results: The results of the study showed that almost all deliveries (99%) took place at homes of the women, and most of them (93%) were conducted by the untrained traditional birth attendants, relatives, or neighbours in unsafe and unhygienic conditions. Only 7% of the deliveries (referred to here as "safe deliveries") were conducted by the qualified persons, such as registered physicians, nurses, paramedics, and traditional birth attendants. The multivariate regression results showed that the younger women and the women with lower parity were significantly more likely to have safe deliveries. Education and exposure to radio and television were significantly associated with the safe delivery practices. The women with five or more years of education, and those who possessed radio or television, were also more likely to have safe delivery.

Conclusion: The results of the study suggest that information, education, and communication (IEC) activities should be strengthened to educate the community, particularly the uneducated and older women on the importance of safe delivery. Pregnant women need greater access to trained providers and facilities providing emergency obstetric care.

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Teenage Pregnancy and its Consequences: Evidence from Rural Bangladesh

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Objective: Investigate the factors regarding health of mother and children associated with teenage pregnancy and the consequences of pregnancy for these women who gave birth by their teenage.

Methodology: Data for this study came from a longitudinal Sample Registration System (SRS) in two rural sub-districts under the ICDDR,B Operations Research Project, for a two-year period covering January 1995 to December 1996. A total of 6,508 married women of reproductive age (15-49 years) categorized in two groups: those who had their first birth under 20 years of age and those 20 years and above. Children belonging to these two groups of women were also covered in the study. Social and economic status, education, contraceptive practice, and healthcare-seeking behaviour of these women and their family were observed. Both bivariate and multivariate analyses were carried out by considering women who got married at an early or later age as the dependent variable.

Results: Preliminary findings indicate that the poor and economically disadvantaged women had their first child earlier compared to the economically advantaged groups. The median age of the women who had their first birth in their teens was 17 years, and for age 20 years and above it was 22. Women who started childbearing early tend to have more children than those who started late. Women who postponed motherhood until after the ten years were more likely to have fewer children and stayed in school longer. Fifty-seven percent of the women who started their child birth before the age of 20 years had experienced at least one child death or pregnancy wastage compared to those who had started child birth at the age of 20 years or later, and 41% had the same experience. Although knowledge of contraceptives was higher among the younger group of women, its practice was higher among the older groups. Delivery attended by trained prsonnel in both groups of women has substantially increased in the study area compared to the national average, but intention to go to the service facilities for delivery did not change neither of the group. More than 90% of the deliveries still occurred at home, irrespective of age.

Conclusion: This study reveals that most women spend, on an average, 10-21 years of their lives carrying for younger children. Teen-aged mother and her children face increased health risks as well as limited social and economic options when compared with older mothers and their children. Young women should be encouraged to go to school and continue their education, and should be targeted for contraceptive use to delay their first pregnancy until the age of 20 years. Efforts should be made to enforce the legal age of marriage.

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Target-Specific Home-based Motivation: Test Case with Family Planning

Subrata Routh, Shamim Ara Jahan, and Aye Aye Thwin

Objective: Develop systematic approach toward targeting of non-users of family planning (FP) methods and provide motivation at their homes, and examine the effects on acceptance of modern FP method.

Methodology: Motivation of non-users of FP method through targeted home visits was carried out as a component of the operations research intervention on Alternative Strategies for Delivery of MCH-FP Services (ASDS) tested in two sites of Dhaka city during January 1996-May 1997. This strategy was experimented to affect focused outreach activities, in place of door-to-door delivery of services, to attain maximum effectiveness within fund constraints. To assess the effects of the approach and acquire insights of the providers' and clients' perspectives, an evaluation was conducted during March-May 1997. The service records of the fieldworkers on the non-users of modern FP methods among slum and non-slum households were analyzed; 40 observations of fieldworker-women encounters were made, 48 in-depth interviews of the target clients, and in-depth interviews with the seven fieldworkers of the intervention sites were conducted.

Results: Despite a major change in the two-decade-old conventional service-delivery system relating to withdrawal of home distribution of contraceptives, target-specific home-based motivation resulted in the high acceptance of modern FP methods among the non-users. A third of the non-slum and a fourth of the slum non-users in one intervention area (at Hazaribagh), and little more than a fourth of both non-slum and slum non-users in another (at Gandaria) became acceptors of modern FP methods. The systematic approach of addressing the non-users developed in participatory workshops with the fieldworkers was found effective by both fieldworkers and target clients. The changed role of the fieldworkers, from FP commodity suppliers to case-workers, resulted in more time allocated for motivation, and more effective need-based motivation and counselling.

Conclusion: Focused home visits for providing motivation to the target population led to higher effectiveness of programmes with fewer fieldworkers, hence with reduced costs. Although target-specific home-based motivation was applied for FP in this study, this approach may be adapted for other health programmes too.

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Determinants of Infant and Child Mortality in Rural Bangladesh

ABM Khorshed A Mozumder, Barkat-e-Khuda, Thomas T Kane, and Kenneth Hill

Objective: Examine the trends in and covariates of infant and child mortality in several rural areas of Bangladesh.

Methodology: Data on a cohort of 21,268 children born between 1983 and 1991 in three rural project sites were obtained from the longitudinal Sample Registration System (SRS) of the MCH-FP Extension Project (Rural) of ICDDR,B—now called Operations Research Project (ORP). The analysis followed the model specified in the extended analysis of the 1993-94 Bangladesh Demographic and Health Survey (BDHS) and was divided into three components: neonatal mortality, postneonatal mortality, and mortality between 12 and 23 months. Estimates of mortality differentials by sociodemographic characteristics were derived, using a life-table technique. Multivariate logistic regression procedures were also applied separately to include fixed and temporal characteristics of the newborn cohort. The mortality estimates were compared with those of the national-level (BDHS) extended analysis.

Results: Reduction in the rates of mortality of children aged less than five years was slightly more rapid at the ORP sites than in the country as a whole. Childhood mortality has been declining in the ORP areas since 1983, compared to the national average. Part of the decline can be attributed to increasing educational levels among parents, and changes in the length of birth interval associated with fertility decline.

Conclusion: The results of the study confirm the findings of other research work, showing that longer preceding birth intervals play a significant role in reducing child mortality. Of course, provision of primary health care services are associated with reduced risk. The data from the SRS in the ORP sites show a significant relationship between childhood immunization and reduced child mortality. Access to tubewell water was also associated with reduced mortality risk for young children.

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An Assessment of Injecting Drug Users in Dhaka City: Need of Intervention for a Vulnerable Group

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Objective: Estimate the size, sociodemographic characteristics, risk perception, and behaviours of the injecting drug users (IDUs) as part of designing an appropriate risk-reduction intervention directed toward the IDUs in Dhaka city.

Methodology: A street-based IUD was defined as one who injected drug during the past six months. Extensive ethnographic field observations were made to identify locations of drug use in 15 thanas of Dhaka city. Identified IDUs were recruited as guides. For quantitative data collection, structured questionnaires were supplied to 234 IDUs. For qualitative data collection, in-depth interviews and focus group discussions were conducted. For clinical examination of biological markers, blood and saliva samples were collected.

Results: Tidigesic was the most common injectable drug used. About 40% of the IDUs lived on footpath, slums, or bus/rail stations, and 37% were rickshaw-pullers and transport workers. Sixty-three percent were in jails. About 45% and 24% started using drugs when they were aged 16-20 years or below respectively. Eighty-two percent shared their needles/syringes, and 48% shared with more than ten persons. Fifty-two percent shared with their sexual partners. Seventy-eight percent took injection 1,000 times or more during their life time. The average frequency of injection was three times a day. Only 15% used their own syringes/needles. Sixty percent heard about HIV/AIDS, and one-fourth knew that needle-sharing might transmit HIV/AIDS. Friends and radio/TV were the common source of information regarding HIV/AIDS. Peers were mentioned as good choice for the source of information .

Conclusion: The prevalence of needle-sharing among the IDUs in Dhaka city is very high. Knowledge on danger of HIV/AIDS and other health hazards due to needle-sharing is low. As happened in Thailand or northeastern states of India, the IDUs may initiate the HIV epidemic here. There is a need of an intervention to make information on needle/syringe exchange and counselling facility accessible to the IDUs. There is also a need of an environment which would facilitate intervention on needle exchange or the introduction of drug substitution.

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Waning of Maternal Measles Antibody in the Offsprings

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Objective: Assess the decline of measles antibody, passively acquired from the mother, in infants from birth to nine months of age, and determine the age at which maternal measles antibody declines markedly, making infants susceptible to measles.

Methodology: The study was conducted in the departments of Paediatrics and Obstetrics and Gynaecology, Institute of Postgraduate Medicine and Research, Dhaka, during September 1992-December 1993. Children were followed up in the Paediatrics Outpatient Department up to 9 months. Blood samples from 120 full-term infants were collected at birth, at 3, 6, and 9 months of age. Samples of the mothers' blood were collected within 7 days of delivery. Neasles antibody levels were detected using the ELISA IgG-antibody kit.

Results: Eighty-nine percent of the blood samples of the mothers were positive for measles IgG antibody, and were associated with their weights. In infants, this was positive in 91% at birth, and was associated with birth length. The antibody levels were positive in 64% of the infants at 3 months, 21% at 6 months, and only 17% at nine months. This decline with age was highly significant, and was more marked after three months of age. In males, the decline was more marked between 3 and 6 months of age, while in females, it was more marked between 6 and 9 months. Three infants developed measles before they were aged 9 months.

Conclusion: The maximum decline in measles antibody occurred in infants during 3-6 months of age. Maternal measles antibody was extremely low at 9 months of age. The study recommends a vaccine trial infants at 6 months of age with the standard measles vaccine in Bangladesh.

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Aetiology and Pathogenesis of Chronic Diarrhoeal Illness in Adults

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Objective: Study the aetiology of chronic diarrhoea and understand the underlying pathology leading to the disease.

Methodology: Chronic diarrhoea was defined as duration of diarrhoeal illness for approximately three weeks or longer. Nine fatal cases fulfilled the criterion and constituted the study population. Autopsy was carried out on all of them. Relevant clinical, laboratory and pathologic findings were demonstrated to understand the pathogenesis of the disease.

Results: Intestinal amoebiasis, pancolitis with mucosal necrosis and variable degree of ulceration leading to serositis or colonic perforations ranked top, being present in 3 (33.3%) cases. Coinfection with Shigella spp., malaria, and disseminated tuberculosis were found. Disseminated tuberculosis secondary to pulmonary tuberculosis with tuberculous ulcerations of the small bowel, or small bowel and ascending colon ranked second, being present in 2 (22.2%) cases. Severe cytomegalovirus (CMV)-associated ileitis and Shigella-associated colitis were noted in these cases. Severe malnutrition was a common concomitant illness in all cases. Infections, suggestive of depressed cell-mediated immunity and/or pathologic findings of lymphoid atrophy, were present in 4 (44.4%) cases. One case with severe malnutrition and lymphoid atrophy had hyperinfection with Strongyloides stercoralis with evidence of auto-infection. Other rare causes included diabetes mellitus with pancytopenia and ileal ulcerations and shigellosis in one, combined congenital generalized lymphangiectasia with entire gastrointestinal (GI) tract involvement and secondary systemic amyloidosis with extensive GI tract involvement in one, and immunoproliferative small and large bowel disease in one. This postmortem study failed to enrol the cases of ulcerative colitis who got well with either medical or surgical management.

Conclusion: Early management of potentially treatable infectious diseases leading to chronic diarrhoeal illness and concurrent nutritional support should be tried. Strongyloidiasis in malnourished patients should be treated

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Typhoid Ileal Perforation: Experience with 64 Cases

Anisur Rahman

Objective: Survey patients with typhoid ileal perforation admitted to a general hospital.

Methodology: Sixty-four patients (51 males and 13 females of average age 27.36 years) with perforated ileum managed operatively were studied prospectively for over one year.

Results: Fever was not always present at admission. Clinical features were consistent with bacterial peritonitis with paralytic ileus associated with hypovolaemic and/or septic shock. A detail history, clinical examination, and a plain X-ray of the abdomen were the mainstay for diagnosis. During laparotomy, various procedures were followed for dealing with the perforations. Patients treated by wedge resection of the perforation, followed by repair, seem to have the best prognosis. There were 18 deaths in this study. Postoperative complications included wound infection, wound dehiscence, anastomotic leakage, septicaemia, bedsores, and respiratory problems.

Conclusion: Compared to other studies, the present one indicates an improvement in the prognosis of the above condition.

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Need for HIV/AIDS Prevention Programme for Bangladeshi Migrants

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Objective: Assess the prevalence of HIV-related risk factors among Bangladeshi migrants returned from abroad, and also assess the present risk behaviour of departing migrants, as well as possible risk behaviour when migrants reach the country of destination.

Methodology: This study was conducted on people who go abroad (both first time migrants and returnees), to see the kind of sexual practice they have in the country who are not yet labelled as high-risk population. A behavioural study was conducted with a sample of 125 people through a questionnaire survey and in-depth interview. The questionnaire survey was done at the clinics where the leaving migrants came for their medical check-ups during November 1997. Data were analyzed, using statistical package, and qualitative methods were followed to perform the case studies that gave insights into the behavioural pattern.

Results: The study revealed that a significant proportion of the migrants had pre- and extramarital sexual activities, including multiple sex with both females and males before leaving the country. The use of condom was insignificant among those who ever had sex, and this was for the purpose of family planning only. Less than 5% of the study population had knowledge and perception of risk and prevention methods on sexually transmitted diseases (STDs) and HIV/AIDS. The result of the study suggests that high-risk behaviour exists among the population planning to work outside the country.

Conclusion: All research organizations, clinical and behavioural support services should make a collaborative effort to develop programmes to prevent HIV epidemic before a disaster takes place. Based on the results of the study, the following interventions for the people leaving the country are suggested: (a) development of a thorough pre-departure programme (in-country); (b) development of a thorough post-departure programme (in countries of destination); and (c) development of an integrated programme for possible and potential migrants and their families.

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Neurologic Manifestations of Childhood Shigellosis

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Objective: Review the neurologic manifestations of shigellosis in children.

Methodology: Eight hundred sixty-three consecutive patients with shigellosis, admitted to the treatment centre of ICDDR,B during a one-year period, were prospectively studied. Patients were divided into four groups based on history and findings of physical examinations: conscious; unconscious; seizure witnessed in hospital; and seizure by history but not witnessed.

Results: Of the 71 patients aged about 15 years, 14% were unconscious either on admission or during hospitalization; none had seizures. Seven hundred ninety-two patients were aged less than 15 years; 9% were unconscious, 5% had a seizure witnessed, and 3% had a seizure by history. Patients aged less than 15 years, who had a seizure witnessed, had a significantly higher median weight-for-age (67% of NCHS median vs. 57%); higher mean temperature (38.7ºC vs. 37.9ºC); lower mean sodium (126 mmol/L vs. 129 mmol/L); and were more often bactaeremic (24% vs. 7%) and hypoglycaemic (blood glucose <2.2 mmol/L; 24% vs. 2%) than conscious patients. When the analysis was restricted to patients aged less than five years and to those infected with Shigella flexneri (who accounted for 64% of all patients with shigellosis), the findings were similar. Shigella was not significantly associated with any of the 4 neurologic categories. In the multiple regression analysis of patients aged less than 15 years, factors independently associated with unconsciousness were: shock, elevated admission temperature, elevated immature and total leukocyte counts, and weight-for-age less than 60% of the median; for witnessed seizure, factors independently associated were: shock, weight-for-age less than 60% of the median, and elevated immature leukocyte count. Forty-eight percent of the 73 unconscious patients aged less than 15 years died in the hospital compared to 29% of the 41 patients who had a seizure witnessed ($p=0.081$), 6% of the conscious patients ($p<0.001$), and none of the 24 patients who had a seizure by history ($p<0.001$).

Conclusion: Both diminished consciousness and seizure were associated with a poor outcome in children with shigellosis. Prompt reduction of fever and correction of metabolic alterations may reduce the incidence of these potentially lethal complications and deaths.

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Molecular Epidemiology and Antimicrobial Susceptibility of Neisseria gonorrhoeae Isolated from Commercial Sex Workers in Dhaka City

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Objective: Analyze epidemiological data on gonococcal infection among the commercial sex workers in Dhaka city, antimicrobial susceptibility testing for Neisseria gonorrhoeae, and plasmid profile of isolates.

Methodology: Endocervical swab samples from 224 commercial sex workers (CSWs) were cultured for N. gonorrhoeae. The isolates were identified by the standard microbiological method and by PCR based on primers which amplify a 390-bp region of the cryptic plasmid of N. gonorrhoeae. Susceptibility to and minimum inhibitory concentration of penicillin, tetracycline, ciprofloxacin, cefuroxime, ceftriaxone, and spectinomycin were determined by the agar dilution and disc diffusion method. The total plasmid was extracted from the isolates, and the plasmid profiles were analyzed.

Results: N. gonorrhoeae was isolated from 94 (41.96%) of the 224 CSWs. Of the isolates, 65.96% were resistant and 34.04% were moderately susceptible to penicillin; 60.64% were resistant and 38.3% were moderately susceptible to tetracycline; 11.75% were resistant and 26.6% had reduced susceptibility to ciprofloxacin; 1.18% were resistant and 11.7% had reduced susceptibility to cefuroxime, and 1% were resistant to ceftriaxone. All isolates were sensitive to spectinomycin. Plasmid profile analysis showed that (32) 34.04% of the strains contained antibiotic-resistant plasmid. All strains contained 2.6 MDa cryptic plasmid. Thirty-eight (40.4%) isolates contained 24.5 MDa conjugative plasmid. Twenty-two isolates were penicillinase-producing N. gonorrhoeae (PPNG), and all of them contained 3.2 MDa ß-lactamase-producing plasmid of African type. Ten isolates were tetracycline-resistant N. gonorrhoeae (TRNG) and contained 25.2 MDa TRNG plasmid.

Conclusion: High level of resistance to ciprofloxacin may limit the usefulness of this agent for the primary treatment of gonorrhoea in Bangladesh.

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Effect of Vegetarian Diet on Rheumatoid Arthritis, Seronegative Spondarthropathies, and Fibromyalgia

Mohammed Yakub Ali, Muhd. Mostafizur Rahman, SM Keramat Ali, and AKM Yunus Halim

Objective: Study the effect of vegetarian diet along with routine drug treatment on rheumatism.

Methodology: Initially, 90 patients were recruited, 30 patients each with rheumatoid arthritis (RA), fibromyalgia (FMS), and seronegative spondarthropathies (SNSAP) having no other medical problems. In each of these three groups, 15 patients (cases) were randomly provided prescribed vegetarian diet of 2,253 kcal (consisting of low aromatic aminoacids) along with indomethacin/ibuprofen, and the rest 15 patients (control) received similar medications with normal diet. All patients had clinical examinations (joint swelling/tender point count with tenderness indices, duration of morning stiffness/fatigue, sleep disturbances, 1-10 cm visual analogue scale) before and after dietary intervention for one month. Tests for Hb%, ESR, total WBC, platelet, RF tit, C-reactive protein, IgG and IgM levels were also performed.

Results: All cases in RA and FMS showed significant improvement in all clinical and laboratory parameters ($p<0.05$) compared to control as well as their baseline levels and mean difference. However, in SNSAP, the significant improvement was observed in both case and control groups compared to their respective baseline levels except 1 g.

Conclusion: A vegetarian dietary intervention with indomethacin/ibuprofen for one month relieved the sufferings of patients with RA and FMS, indicating that diet selection is essential for the management of these chronic conditions. Thus, dietary intervention may be the main regimen for the management of FMS where pain-relieving drug does not have any beneficial effect. The role of diet in all types of rheumatic disorders needs further study.

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Arsenic in Drinking Water: An Emerging Environmental Health Challenge

Bilqis A Hoque[1], Shafiul A Ahmed[1], Uttam K Chowdhury[1], JTA Chowdhury[1], G Morshed[1], Donald M Maynard[2], and Seth H Frisbie[2]

Objective: Determine the nature of arsenic contamination in drinking water in Bangladesh.

Methodology: This preliminary study was conducted during July-August 1997. A cross-sectional survey was carried out to collect information at the household level (observational study design). The performance of field kits used by other agencies available to us was compared with a selected laboratory technique. Five hundred and seventy tubewells and their users were studied. Tubewell water samples collected from almost the whole country, except some areas in Chittagong and Sylhet, were analyzed for arsenic and ferrous iron contents. Users of these tubewells were interviewed and their statements reviewed, and the methods commonly used by other agencies to measure arsenic contamination were compared.

Results: About 61% of the tubewells were found to contain arsenic in excess of the WHO-recommended value of 0.01 mg/L. The arsenic concentration varied from 0 mg/L to approximately 1.0 mg/L, and the ferrous iron content varied from 0 mg/L to 41 mg/L. The association between arsenic and iron and the depth of handpumps was found to vary with the hydrogeological conditions. The study revealed that only 4% of the respondents were aware of the arsenic contamination in the used tubewells. Unclear messages related to water treatment were as well found to be disseminated at the field level.

Conclusion: The environmental health challenge relating to arsenic contamination is massive and complex, and needs to be addressed appropriately. Findings of the research and development activities should be coordinated appropriately.

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Economic Evaluation of MCH-FP Clinic-based Syphilis Screening in Rural Bangladesh

M Mahmud Khan[1], Sarah Hawkes[2], and Disha Ali[1]

Objective: Examine the costs and benefits of screening women of reproductive age visiting the MCH-FP centres, to identify the syphilis cases for treatment and estimate the cost-effectiveness of alternative screening strategies at different levels of syphilis prevalence.

Methodology: A field study was carried out to estimate the population-based rates of reproductive tract infections (RTIs), including STIs, in men and women in Matlab. The prevalence rate of syphilis was found to be about 0.8%, indicating a low prevalence of syphilis in rural Bangladesh. Based on the finding of the study, costs of screening and treatment of syphilis have been estimated assuming that the prevalence rate should be less than 3% in most rural communities. For costing the medical interventions, the market price of the laboratory tests and drugs was used. The study estimated the direct medical costs associated with screening and treatment of syphilis, excluding all direct non-medical and other indirect costs.

Results: If the prevalence rate of syphilis remains less than 6% in the population, screening with RPR, followed by TPHA, will be more cost-effective than performing RPR only. At the higher prevalence rates, RPR alone should be used for screening population for syphilis. The benefit-cost ratio of syphilis screening with treatment (treating both woman and her husband) was found to be about 4.5 for Bangladesh. Although the ratio was significantly higher than one, it was lower than the ratio obtained for the control of diarrhoeal diseases, childhood immunizations, and many other preventive interventions in the developing countries.

Conclusion: With the increased use of the MCH-FP centres, the unit cost of RPR and TPHA should fall significantly, which will further improve the benefit-cost ratio. Moreover, the reduced costs will make screening with RPR, followed by TPHA which is cost-effective at a much higher cut-off prevalence. In this study, the indirect benefit of curing syphilis, especially in preventing HIV infection, has not been considered. The prevalence of HIV in this population is not known, but if we assume the prevalence rate to be 1%, the probability of preventing an HIV infection through treatment of syphilis becomes so low that we can safely ignore this additional benefit at this low level of HIV and syphilis prevalence. However, at the higher prevalence rates, the benefits may become significant and should be taken into account.

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Role of Nitric Oxide in the Pathogenesis of Shigellosis and Cholera in Children

Sufia Islam[1], GJ Fuchs[1], AK Chowdhury[1], A Rahman[1], M Miller[2], and GH Rabbani[1]

Objective: Evaluate the role of nitric oxide in the pathogenesis of shigellosis and cholera in children.

Methodology: Concentrations of nitrite, a stable metabolite of nitric oxide (by Griess reaction) in urine and serum samples of 24 patients aged 1-5 years (10 shigellosis, 14 cholera) were determined. Tests were done on admission to hospital with acute diarrhoea and repeated at early convalescence after 3-7 days of specific antimicrobial therapy.

Results: In children with shigellosis, urinary nitrite excretion (nM/mg creatinine) was significantly increased during acute illness compared to early convalescence, median (range): 7061 (1046-18264) vs. 4316 (2369-12428, $p<0.05$). Concentration of nitric oxide in serum (mmol/L) also significantly ($p<0.05$) increased during acute illness compared to convalescence values: 206 (159-214) vs. 104 (102-273). Similarly, in children with cholera, both urinary and serum nitrite excretions were significantly ($p<0.05$) elevated during acute illness compared to convalescence: 5034±1345 vs. 2178±404 (urine); 450±89 vs. 201±56 (serum).

Conclusion: These results indicate that production of nitric oxide is increased both in acute shigellosis and cholera, more markedly in the former infection because of colonic inflammation. Urinary nitrite excretion can be a useful marker of severity of these infections.

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Poster Presentation

Copper, Iron and Manganese Status in Severely Malnourished Hospitalized Children

M Salim Shakur[1], Nasreen Bano[1], and SA Tarafder[2]

Objective: Determine the status of few essential trace elements, like copper, manganese, and iron in patients with severe protein-energy malnutrition (PEM) by estimating their concentration in serum and hair, and find their relationship with various anthropometric measurements.

Methodology: This case-control study was carried out in the Nutrition Ward of the Dhaka Shishu (Children) Hospital. Forty severely malnourished children (weight-for-age <60% of NCHS median) aged 6-60 months were taken as active cases. Forty-six relatively well-nourished children (weight-for-age >90% of NCHS median) of the same age group were taken as control. Serum and corresponding hair copper, manganese, and iron contents were estimated by flame atomic absorption spectrophotometry, and the results were expressed as particle per million (ppm).

Results: Both serum and hair copper contents were found low in PEM (0.80±0.38 ppm vs.1.98±1.16 ppm, $p<0.05$ and 13.44±13.09 ppm vs.14.52±10.58 ppm, $p>0.05$). However, serum and hair iron contents were found higher in PEM (1.54±1.38 vs. 0.85±0.53 ppm, $p<0.05$ and 57.93±53.85 ppm vs. 20.88±11.38 ppm, $p<0.05$ respectively). Hair manganese content was also found higher in PEM (7.22±4.62 ppm vs. 6.03±3.39 ppm, $p>0.05$), while serum manganese was not recordable. Serum copper content maintained positive correlationship with weight-for-age ($r=0.4403$, $p<0.001$), while serum iron content in PEM maintained negative relationship with age in month (r-0.3572, $p=0.012$). Hair iron content had negative relationship with weight-for-age (r-0.3979, $p=0.001$), and hair iron content had positive relationship with hair manganese content ($r=0.2794$, $p=0.020$)

Conclusion: Considering the low copper status in PEM from the above results, copper supplement may be beneficial in severe PEM. However, iron supplement in severe PEM may aggravate clinical condition as there may be already iron overload in these groups of patients contributing more to existing oxidative stress of PEM.

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Effect of Zinc Supplement on Children Suffering from Feeding Refusal with Failure to Thrive

M Salim Shakur[1], Nasreen Bano[1], and SA Tarafder[2]

Objective: Evaluate the effect of oral zinc supplement on feeding refusal with failure to thrive children.

Methodology: A randomized prospective case-control trial was conducted on 40 children aged 36-72 months with complaints by parents of feeding refusal and failing to thrive. Children having weight-for-age between 60 and 89% of NCHS median and not gaining weight satisfactorily (weight-for-age remaining static or decreasing) for the last three months, but otherwise active and having satisfactory development and having no acute or chronic clinical disorder, were included in the study. Twenty children in Group A received oral zinc acetate (2 mg elemental zinc per kg) and oral multivitamin syrup while 20 children in Group B received oral multivitamin syrup only for one month, and both the groups were followed up biweekly. The groups were comparable with respect to initial characteristics, including nutrition (weight-for-age), zinc status (serum and hair zinc concentration of 141 mgm/dl±54.70, 184 mgm/g±48.60 and 152 mgm/dl±39.20, 192 mgm/g±82.54 in Group A and Group B respectively). Body composition, in the form of fat-free body mass (FFBM), fat mass (FM), total body water (TBW), and body mass index (BMI) were also measured before and after the completion of trial using a bioelectric impedance analyzer (BIA).

Results: At the end of the trial, a significant number of patients in Group A had improved appetite (60% vs. 15%, OR 8, 95% CI OR, (1.752,36.488, $p<.01$). The increased appetite was associated with significant increased weight gain (>5% increase in weight) in group A ($p<0.01$). The increased weight gain was associated with increase in FFBM, FM, and BMI in Group A.

Conclusion: Low-dose zinc supplement may help improve appetite in non-specific feeding-refusal children without any adverse effect on body composition even though the children are biochemically not zinc-deficient. However, appetite stimulation effect of zinc and its rational use to feeding-refusal children with failure to thrive merits further study.

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Pre-lacteal Feeding Practices in Matlab, Bangladesh

Sabah Tarannum[1], SM Ziauddin Hyder[1], AMR Choudhury[1], and Abbas Bhuiya[2]

Objective: Analyze the nature and extent of the knowledge of mothers on pre-lacteal feeding practices in a rural community of Bangladesh.

Methodology: The study was based on the analysis of data collected under the BRAC-ICDDR,B Joint Research Project in Matlab during April-August 1995. Data on the feeding practices were obtained by interviewing the mothers of 473 children aged less than two years. Households were categorized into two groups: poor and not poor, based on their socioeconomic conditions. Data were analyzed using bivariate and multivariate tools.

Results: Results of the study showed that only 7% of the infants were given breastmilk as first feeding. Honey, mustard oil, and water with sugar were found to be the most frequent types of pre-lacteal liquid given. About 13% of the infants did not receive any liquid within 24 hours of birth. In most cases, grand mothers (47%) initiated the feeding followed by traditional birth attendant (25%). Although about 30% of the respondents knew the importance of breast-feeding, they did not have accurate knowledge on how and when to initiate it. The findings of the study confirm that exclusive breast-feeding is still non-existent in rural Bangladesh, and in most cases, non-beneficial liquid is given to newborns in an unhygienic way.

Conclusion: It is necessary to create awareness about the child-feeding practices, especially about the colostrum and breast-feeding, among the mothers, grand mothers, and midwives who are mainly concerned to reduce early infections and promote better nutrition. The existing nutrition education efforts should emphasize on the importance of exclusive breast-feeding and harmful effects of the pre-lacteal liquids.

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Study on Food-refusal, Morbidity and Nutritional Status of Children of the Middle and Upper Socioeconomic Class in Dhaka City

M Asirul Hoque[1], Quazi Salamatullah[2], Mamunar Rashid[3], and Maqbul H Bhuiyan[4]

Objective: Identify the food-refusal and morbidity among the children of urban middle and upper socioeconomic class, assess 24-hour energy intake in selected morbidity in food-refusal and non-refusal group of children, and also assess their nutritional status.

Methodology: This was a descriptive type of study, and the study design was a non-experimental one with static group comparison between two groups: (a) a study group where event of food-refusal was present, and (b) a comparison group where event of food-refusal was absent. Four hundred children were included in the study which fit in the timeframe of data collection. As the study was qualitative in nature, the sample size was not determined, and was detected arbitrarily. The method of systematic random sampling was used for the collection of data from three organizations of Dhaka city.

Results: Diarrhoea, acute respiratory infection, and fever were common in both study and comparison groups. In presence of diarrhoea, acute respiratory infection, and fever, the majority of children took energy <RDA in both the groups, but the refusal group was affected more in the majority of cases. In presence of diarrhoea in the upper middle class, 96.3% of the study and 50% of the comparison group children took energy <RDA, and in the upper class, more than 90% of the children from both the groups took energy <RDA. In presence of diarrhoea, in the upper middle class study group, 22.2% of the children were short and 14.8% of the children were thin, and in the upper class study group, 8.0% of the children were short and 22.0% of the children were thin. In the comparison group in both the classes, children were not short or thin.

Conclusion: In presence and absence of food-refusal, the majority of the children having morbidity consumed energy less than RDA. Increased energy and nutrient intake during illness may play a strong role in the prevention of malnutrition.

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Anaemia in Pregnancy: A Rural Community Perspective

SM Ziauddin Hyder

Objective: Investigate the prevalence of anaemia and the associated factors among pregnant women in two rural areas of Bangladesh.

Methodology: Data were collected in March 1997 from 90 pregnant women residing in 6 villages of Saturia and another 6 villages of Fulpur thana in Manikganj and Mymensingh districts respectively. All the identified pregnant women in the 12 villages who were willing to participate were included in the study. The women were interviewed to collect their socioeconomic information. Fingerprick blood samples were taken, and haemoglobin concentration was measured in the field using a portable HemoCue photometer system. The system uses cyanomethaemoglobin method to assess haemoglobin concentration. The WHO criteria (haemoglobin concentration <110 g/L) were used for defining anaemia during pregnancy.

Results: The results of the study showed that 54% of the women had anaemia according to the WHO criteria. Area of residence, literacy, iron tablet intake, and the duration of BRAC membership were significantly associated with the prevalence of anaemia ($p<0.05$). The women who lived in Fulpur had higher prevalence of anaemia (63%) compared to those who lived in Saturia (32%); the illiterate women had higher prevalence (60%) than the literate women (23%); the women who reported to take iron tablet had lower prevalence (36%) than the women who did not (60%); and lastly, the women who were associated with BRAC for more than one year had the lower prevalence of anaemia (27%) compared to the women who were involved with BRAC for less than one year (74%).

Conclusion: A very high proportion of the pregnant women is anaemic in the rural communities of Bangladesh. The BRAC's rural development programmes, in addition to its ongoing income-generating, food production and essential health care-delivery activities, should find ways to increase the effectiveness of the existing iron supplementation programme through increasing its compliance and coverage.

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Iron Supplement and its Predictors among Newly-married Girls and Women in Rural Bangladesh

Thomas T Schaetzel and Mohammad Shahjahan

Objective: Determine the level and predictors of iron supplement among the primaparous, newly-married girls and women in rural Bangladesh.

Methodology: A baseline survey relating to nutrition and pregnancy was administered to 2,259 newly-married women and girls in December 1996 for ongoing monitoring of the pilot intervention among the newly-married couples (NMC) of the Bangladesh Integrated Nutrition Project (BINP). All available girls and women married four months or less were interviewed in three rural thanas: one thana implementing the BINP NMC strategy, one thana implementing the standard BINP strategy, and a non-BINP thana. The first of a series of follow-up surveys was administered in March 1997 to a subset of the baseline group, and iron supplementation behaviour was analyzed among the 321 women pregnant at follow-up. Survey datasets were linked electronically using shared identifiers, and predictors of supplement were determined through logistic regression model-building procedures.

Results: 25.5% of the pregnant women in the sample were/had been taking medicine during pregnancy, and 63.4% of this group (52 girls and women) reported taking iron/folate tablets. 61.5% of those who had taken iron/folate reported a twice daily dose, and 67.3% were still taking iron at the time of the survey. 40.2% of those still taking iron had been taking it for 3 months or longer; 88.2% of those who had discontinued supplementation did so within two or fewer months. Two factors relating to supplement emerged. Mothers' education of a girl/woman, independent of her own education status (OR=2.9), and any attendance at satellite clinic (OR=5.4; SC attendance, was also shown to be associated with needing permission from mother-in-law to attend the health centre).

Conclusion: The majority of primaparous, newly-married women who took iron supplements during pregnancy appeared to be receiving the WHO-recommended daily dosage. However, a small percentage of women was undertaking this regimen; drop-out from supplementation appeared to be high; and supplementation duration appeared to be very low among those who dropped out. Continued progress in universal women's education can improve healthcare behaviours, such as iron supplementation, and further emphasis should be placed on holding satellite clinics according to schedule and motivating pregnant girls and women to attend these.

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Intra-household Food Distribution in a Rural Area of Bangladesh

Rita Das Roy[1], SM Ziauddin Hyder[1], AMR Chowdhury[1], and Alayena Adams[2]

Objective: Explore the sex preference in intra-household food distribution in a rural area of Bangladesh.

Methodology: This in-depth study of the BRAC-ICDDR,B Joint Research Project was conducted in six villages of Matlab thana. School-going brothers and sisters aged 10-14 years from the BRAC and the non-BRAC households were selected for the study. The sample was selected using a quota random-sampling technique. Both quantitative and qualitative research methods were employed for data collection. Three hundred seventy-six brothers and sisters from 188 households were interviewed using a structured questionnaire. Respondents from one village were chosen for direct observation of food distribution behaviour. Six focus group discussions were conducted with the mothers of two villages to understand their views regarding their food distribution behaviour.

Results: The results of the study suggest that there was no major discrimination in the perceived food intake (both regular meal and snacks) between brothers and sisters. The small differences observed in food intake were due to the unavailability of food at home and perhaps due to mothers favouring the sons. During meal observation in one village, it was found that the brothers were given preference over their sisters during food distribution. The mothers' opinion in this subject was that one should treat both daughter and son equally, but it was not always done in practice. Some mothers cited, "sons are the future security for the parents, and they also help their father in work. So, they are offered more food." However, they did not report anything about the contribution of their daughters to household chores. A similar result was observed in the BRAC member and non-member households.

Conclusion: The results of the qualitative and quantitative analysis show that although there was no significant difference in the perceived adequacy of the meals between brothers and sisters, there was a difference in the food distribution pattern by sex.

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Family Planning and Pregnancy-related Nutrition Behaviours and Beliefs among Newly-married Women in Rural Bangladesh

Thomas T Schaetzel and Mohammad Shahjahan

Objective: Determine baseline awareness and knowledge about nutrition in pregnancy among the newly-married women in Bangladesh.

Methodology: A survey of family planning behaviour and knowledge related to nutrition and pregnancy was administered to the 2,259 newly-married women in December 1996 to establish the baseline levels for ongoing monitoring of the pilot intervention among the newly-married couples (NMC) of the Bangladesh Integrated Nutrition Project (BINP). All available newly-married women (married four months or fewer) were interviewed in three rural thanas: one thana implementing the BINP NMC strategy, one thana implementing the standard BINP "total vulnerable group" strategy, and a non-BINP thana.

Results: The mean age of the respondents was 16.1 years, and 50% were 15 years or younger. Of the 2,259 newly-married women, 30.2% were currently using birth control (65.2% of these using oral contraceptives), and family planning was least commonly practised among the youngest age group. Fifty-eight percent of the respondents believed that pregnancy requires an increased food intake, but 25.4% of those who believe food intake should decrease and 57.4% of those who believe food intake should remain as usual stated that a "bigger" or "healthier/stronger baby" would result. Only 30.9% of the respondents felt that pregnancy requires an increased rest, and 41.6% heard of iron/folate supplements, but only 29.9% of these were aware that they were important for pregnancy, and 40% responded that the local doctor/medicine shop was the location to obtain them.

Conclusion: The young age of newly-married women presents an important challenge for targeting family planning and nutrition messages relating to reduction of low birth weight: family planning motivation is particularly essential to delay first pregnancy; adolescents and their families require education on the nutritional needs of adolescent pregnancy and the benefits of increased rest; and information is necessary prior to pregnancy concerning iron supplementation and the free availability of these supplements through the primary healthcare system.

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Prevalence of Vitamin A Deficiency among Adolescent Female Workers

Nabila Hasan[1] and Faruk Ahmed[2]

Objective: Investigate the prevalence of vitamin A deficiency among the adolescent working girls of low-socioeconomic
group.

Methodology: Three hundred eighty-eight girls aged 12-19 years, working at 10 different garment factories of Dhaka city, were selected randomly for this cross-sectional study. Information on socioeconomic conditions, diet, and knowledge and perceptions about vitamin A deficiency and vitamin A-rich foods was obtained through interviews. Anthropometric data and blood samples were also collected following the interview.

Results: By the NCHS standards, 15.5 % of the working girls were thin (Wt/Ht <90%), and nearly 7% were overweight (Wt/Ht >120%). About 44% of the girls were anaemic (Hb <12 g/dl). In about 56%, serum vitamin A was below the adequate level of 30mg/dl, and 14% had frank vitamin A deficiency (<20mg/dl). Food frequency data on vitamin A-rich foods revealed that a large percentage of the girls did not take egg (41%), milk (64%), liver (85%), and sweet pumpkin (85%). However, 40% of the girls took dark-green leafy vegetables, and 17% small fish at least 4 times a week. Only 39% had correct understanding of night blindness, 29% had correct knowledge about the causes of night blindness. The majority of the participants had no idea about the prevention (64%) and treatment (68%) of night blindness. Sixty-eight percent had no knowledge about vitamin A-rich foods. Of those who knew, the majority mentioned dark-green leafy vegetables as a rich source of vitamin A.

Conclusion: The findings suggest that the study population bear a significant public health risk which may be due to their lack of nutritional knowledge and poor dietary habits.

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Intensive Nutrition Education Programme for Adolescent Girls

Sadia A Chowdhury, Zeba Mahmud, and Emily W Counts

Objective: Assess the achievements of the adolescent girls who received intensive nutrition education, including supplementation, aiming at bringing about sound nutritional practices.

Methodology: In one of the thanas, the BRAC's non-formal and primary education (NFPE) schools incorporated health and nutrition education, monthly monitoring of weight, height and mid-upper arm circumference (MUAC), and daily food supplementation (600 calories/day) for the adolescent girls, in addition to the normal curriculum. These activities covered the girls for the total school period of 3 years, and were provided by the female community health workers and the NFPE school teachers.

Results: The mean age of the adolescent girls was 13.4+0.69, and the mean age of menarche was 13.2+0.77. As a group, the mean increase in MUAC was 2.24 cm, and the mean increase in weight and height was 4.52 kg and 5.51 cm respectively. The girls exiting supplementation after two years had better weight gain than the girls of the same age entering supplementation. For example, girls entering at age 10 years exited at age 12 years with a weight of 32.23 kg which was greater than the entry weight of 12-year old girls at 30.5 kg.

Conclusion: Girls receiving two years of intensive nutrition education and supplementation had better weights and were taller, and therefore, an improvement in the growth indicator can be achieved among the adolescent girls with these interventions. Programmes aiming at improving the women's nutritional status, and thereby, their infants should consider focusing on the adolescents as future mothers.

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Effect of Women-Focused Development Programme on Nutritional Status of Rural Women

Sabrina Rasheed[1], Abbas Bhuiya[2], SM Ziauddin Hyder[1], and A.M.R. Chowdhury[1]

Objective: Assess the effect of women-focused development programme on the nutritional status of women measured by the body mass index (BMI).

Methodology: Anthropometric measurements and socioeconomic information of 1,597 women aged 15-55 years were obtained from eight villages of Matlab thana, through the BRAC-ICDDR,B Joint Research Project during April-August 1995. BMI of <16 was used as a cut-off point for severe chronic energy deficiency. The association between the proportion of women with BMI of <16 and the BRAC membership status (categorized as BRAC member poor, BRAC non-member poor and rich) was explored in both bivariate and multivariate analyses. In the multivariate analysis, the effect of age, parity, and education of women was controlled.

Results: According to the multivariate analysis, age, parity, and BRAC membership status of the households showed a significant association with the proportion of severely malnourished women. In the comparative sense, the BRAC-member women had a 35% more chance of being severely malnourished, whereas the BRAC-non-member poor women had a 64% more chance of being severely malnourished than the women from the rich households.

Conclusion: The development programme focused on women has a positive impact on the women's nutritional status.

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Maternal Nutrition and Birth Weight

SM Keramat Ali, AKM Yunus Halim, and Mohammed Yakub Ali

Objective: Determine the weight of rural pregnant women and its effect on birth weight of their babies.

Methodology: One thousand seven hundred and seven pregnant women of Shakipur thana in their 3rd trimester constituted the sample size. Information on height, weight, mid-arm circumference (MAC), and 24-hour dietary intake by these women were collected by interview through house-to-house visit. The birth weight of only 10% babies could be collected.

Results: About 64% of the women were aged 15-25 years. In 81% of the families, the monthly income was Tk 2000. The average birth interval was 24 months. They showed a U-shaped pattern interval of chronic energy deficiency as defined by the body mass index (BMI) of <18.5. The graph showed that with the increase in BMI of the mothers, the birth weight of their babies also increased. About 52% of the mothers had body weight ranging from 41 to 44 kg, and had more low-birth-weight babies. In general, the mothers took low calories contributed by low macro and micronutrients. Mothers having MAC of less than 23 cm had an average body weight of 43.5 kg.

Conclusion: The rural women who ate 1339 food calorie had pregnancy weight (3rd trimester) <43.5 kg, MAC <23m, BMI less than 18.5 gave birth to more low-birth-weight babies.

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Some Socioeconomic Differentials of Weight, Height, and Body Mass Index of Women in Rural Areas of Bangladesh

Yeakub Patwary, Rowshan Jahan, and Shahin Ara Begum

Objective: Examine the levels and regional variations of weight, height, and body mass index (BMI) of approximately 14,000 women from 44 thanas in Bangladesh, and identify their socioeconomic correlates. The correlates are women's age, number of children, family type and size, education, religion, and land owned by the household. Women in rural Bangladesh are of low weight and short stature. Poor maternal health and nutrition status contribute to the high incidence of low birth weight and high infant mortality. Women's poor health and nutrition status may be due to their poor socioeconomic conditions. The strength of association between the household socioeconomic conditions and women's nutrition is little known.

Methodology: Data for the study were collected from 44 thanas in Bangladesh on sampling basis. About 600 households were selected following the multistage cluster sampling methodology to collect information on selected indicators. Weight and height of all available women aged 15-49 years were measured along with some selected socioeconomic, demographic, and health-related information.

Results: The preliminary results of the study showed that 45.5% of the women of childbearing age were malnourished having their BMI <18.5, and 59.6% of the malnourished women had no formal or non-formal education.

Conclusion: There is a strong relationship between women's literacy and maternal nutrition.

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Prevalence of Chronic Energy Deficiency in the Elderly Population of Matlab

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Objective: Study the prevalence of chronic energy deficiency (CED) in the elderly population of Matlab and its association with different socioeconomic status indicators.

Methodology: Socioeconomic and anthropometric measurements of 626 individuals aged 55 years and above, residing in 14 villages of Matlab thana of Chandpur district, were obtained during April-August 1995 using a pre-coded questionnaire. Body mass index was used as an indicator of CED. Bivariate and multivariate analyses were done, and variabls found to be significant in the preliminary bivariate analysis were used as the independent variables in the logistic regression model.

Results: The results of the study showed that about 80% of the elderly people suffered from different degrees of CED, and 35% from severe CED. In both bivariate and multivariate analyses, severe CED was highly prevalent ($p<0.01$) among the elderly people in the BRAC-eligible poor households. According to the logistic regression, severe CED was most prevalent among the unemployed and disabled (56%) and least prevalent among those involved in farming (20%). The elderly people, residing in families with more than five members, seemed to suffer most from severe CED ($p<0.01$).

Conclusion: Severe CED is highly prevalent in the rural elderly population, and was associated with socioeconomic status, employment status, and family size. Therefore, BRAC may be able to provide support services for the elderly people through the existing credit and health programmes.

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The Prevalence of Anaemia among Males and Females in Rural Bangladesh

SM Ziauddin Hyder, Sadia A Chowdhury, and AMR Chowdhury

Objective: Investigate the prevalence of anaemia among males and females in a rural community of Bangladesh.

Methodology: The survey was conducted in a healthy population in March 1996 in 12 villages of Fulbaria thana of Mymensingh district. One hundred six males and 228 non-pregnant females aged 11-48 years were studied. Information on haemoglobin concentration, parasite infestation, and household socioeconomic status was obtained. A HemoCue photometer was used for measuring haemoglobin concentration. Microscopic examination of stool was done in the Microbiology Laboratory of the Mymensingh Medical College.

Results: About 69% of the males and 70% of the females were found to be anaemic according to the WHO criteria. There was no difference in the prevalence of anaemia between males and females. Literacy and economic status were associated with the prevalence of anaemia among the females, but not among the males ($p<0.05$). Anaemia was also more common among those holding little or no land and among those having current Ascaris infestation ($p<0.05$).

Conclusion: Anaemia is highly prevalent in the rural communities of Bangladesh, which affects both males and females equally. Studies should be undertaken to examine the cases of anaemia in different age and sex groups to develop effective preventive and control measures.

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Determinants of Nutritional Status of Pre-school Children in BRAC-eligible Households

Sabah Tarannum and SM Ziauddin Hyder

Objective: Assess the prevalence and determinants of nutritional status of children aged 6-59 months in the BRAC-eligible households.

Methodology: The Nutritional Surveillance Project (NSP) of BRAC and the Helen Keller International collects children's anthropometric and corresponding household's socioeconomic data at an interval of every two months on a cohort of 100 village in 4 rural thanas. During each round, 20 children from each village have been surveyed. The present analysis is based on 11,611 measurements of children aged 6-59 months collected during April 1994-December 1996. Socioeconomic data were collected using a structured questionnaire, and weight and height data were collected using Salter scale and wooden height board. The nutritional status was expressed as stunting which was defined as height-for-age <90% of the NCHS median. BRAC eligibility was defined as the households having land of <50 decimals, and one of the households members sold manual labour at least 100 days in the last one year. Both bivariate and multivariate tools were used for analyzing the data.

Results: The overall prevalence of stunting was 71% which was higher in males (67%) than females (57% ($p<0.01$). The prevalence of stunting was highest (71%) among the 6-24-month age group. The prevalence of stunting was associated with age, point prevalence of acute respiratory infection and diarrhoea, vitamin A capsule coverage, type of latrine, sex of household head, family size, mother's literacy, occupation of main earner, BRAC membership, and per capita monthly food expenditure ($p<0.01$). Multivariate analysis suggests that birth order, point prevalence of diarrhoea, sex of household head, monthly food expenditure, occupation of main earner, and loan received during last month are the significant predictors of stunting of the children among the BRAC-eligible households ($p<0.05$).

Conclusion: Compared to the national average of 46%, a very high proportion of the children in the BRAC eligible households are stunted. Nutritional considerations in monitoring and evaluation of the ongoing poverty Alleviation programmes should be carefully incorporated to incorporated to enhance their impact on nutritional status.

Bangladesh Rural Advancement Committee (BRAC), 75 Mohakhali C/A, Dhaka 1212

Infant Growth Patterns in the Slums of Dhaka in Relation to Birth Weight, Intrauterine Growth Retardation, and Prematurity

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Objective: Describe the patterns of growth in body weight among infants till their first birthday, especially with reference to birth weight, intrauterine growth retardation (IUGR), and prematurity.

Methodology: One thousand six hundred fifty-four infants, born in selected slum areas of Dhaka, were enrolled at birth and followed longitudinally till they reached 12 months of age. Weights, lengths, and baseline information of these infants were measured at enrollment. Anthropometric measurements were taken again at 1, 3, 6, 9 and 12 months of age. Analysis was limited to descriptive statistics based on means and proportions, and comparisons to growth references.

Results: The mean birth weight of the infants was 2,517 g, and 46.6% had low (<2,500 g) birth weight (LBW). Sixty-nine percent were born with IUGR, and 17% were premature. Of the growth-retarded newborns, birth weights of 63% of the infants were proportionate to their lengths according to the ponderal index. This indicates that most IUGR infants were subjected to chronic intrauterine undernourishment. The growth of the infants in the study sample was similar to that of a pooled sample of breastfed infants from affluent countries, in that the study infants closely tracked the -2 SD curve of the "reference" infants. The mean z-scores (based on the breastfed reference) were very similar at birth and 12 months (-2.38 and -2.34) and only showed slight improvements in the first 3 months (-1.72). Differences in weight at birth between infants grouped according to birth weight, IUGR and/or prematurity status were retained throughout infancy.

Conclusion: The infant growth rate in this sample was similar to that observed amongst the breastfed infants in developed countries. Catch-up growth was not seen, and weight at 12 months was largely a function of weight at birth. The data suggest the need to focus on the improvement of birth weight as the principal means for improving nutritional status of infants in this population.

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Knowledge, Attitude and Practice Patterns of Adolescent Girls

Zeba Mahmud and Sadia A Chowdhury

Objective: Understand dietary intake and health beliefs of adolescent girls.

Methodology: The study group comprised 360 randomly selected adolescent girls aged 11-18 years, attending the BRAC's Non-formal Primary Education (NFPE) schools in Muktagacha thana of Mymensingh district. A questionnaire was used for obtaining sociodemographic information and information on knowledge, attitude, and practices regarding their normal diet and diet during illness and menstruation.

Results: The mean age of the adolescent girls was 12±2 years. Ninety-seven percent of the girls felt that they were taken care of and not considered a financial burden. The food intake patterns showed that more than 70% of the girls ate rice 3 times a day. When the girls were asked whether their food intake had increased with age, 78% reported that it increased in terms of quantity, whereas 8% reported that it decreased with age. Data on the nutrition values of food showed that only 59% of the girls could identify energy-containing foods, such as rice, flour, roti, potatoes, and oil. Vegetables were identified as rich source of vitamins by 49%. During menstruation, 32% reported avoiding specific foods, such as milk, sour food, fish, and eggs, and 31% reported decreased food intake during fever and indigestion. Rice was the food most likely to be avoided during fever. Focus group discussions recorded that they learnt food avoidance from the elders in the family.

Conclusion: Although the adolescent girls had a fairly positive perception of their well-being, they were influenced by their families to be self-sacrificing regarding food intake. Therefore, any intervention designed to bring about the behavioural change for better nutrition should include the family members in the target audience.

Bangladesh Rural Advancement Committee (BRAC), 75 Mohakhali C/A, Dhaka 1212, Bangladesh

Intervening Malnutrition in Rural Bangladesh: BRAC Experience

Sadia A. Chowdhury and Zeba Mahmud

Objective: Discuss the experience of BRAC in intervening malnutrition in rural Bangladesh. BRAC operates a community-based, integrated health and nutrition service with the aim of empowering the community with nutritional knowledge.

Methodology: The project in Muktagacha thana of Mymensingh district includes children aged less than two years, adolescent girls, and pregnant/lactating women. One female community health worker in each village (a) visits all households monthly to monitor health and provide education, (b) conducts monthly nutrition and health education meetings, and (c) manages growth-monitoring sessions for children aged less than two years to educate mothers and identifies malnourished/faltering in weight children for the 90-day feeding demonstration which provides 200 kcal/day. Pregnant women receive monthly antenatal care, education, and nutrition assessment. Those with body mass index (BMI) of <18.5 receive a daily food supplement (800 kcal/day) and education at home through 6-month lactation. For adolescent girls of the BRAC schools, the school curriculum added component on health and nutrition education, and monthly monitoring of weight, height and mid-upper arm circumference (MUAC), and daily food supplementation (600 calories/day).

Results: Of the approximately 8,000 children aged less than two years, 85% came to the growth-monitoring sessions. On an average, 2,000 children enter the feeding programme each year. There was no significant difference between the number of female and male children coming into the supplementation. The age of the adolescent girls ranged from 10 to 18 years. The mean weight, length, BMI, and MUAC was far below the 50th NCHS percentile. Even though the weight gain of the pregnant women was slight, their children had a higher birth weight. Moreover, this group had the greatest reduction in low birth weights, i.e. 36% between 1992 and 1994 compared to women from other BRAC areas.

Conclusion: An integrated and intersectoral process with one intersectoral community worker and intensive community participation addresses existing malnutrition, and the community learns to take responsibility for and to correct malnutrition.

Bangladesh Rural Advancement Committee (BRAC), 75 Mohakhali C/A, Dhaka 1212, Bangladesh

Mother's Perception of Colour and Understanding of Growth Curve to Interpret Nutritional Status of Children in Rural and Urban Bangladesh

S Mizan Siddiqi

Objective: Determine the mother's perception of colour and understand the growth curve to interpret the nutritional status of children.

Methodology: The study was conducted in two urban areas of Dhaka city and in one rural area in southern Bangladesh during June-July 1993. In each site, 312 mothers with children aged 6-36 months were interviewed by the trained interviewers. The mothers were asked to indicate colours and curves used in the growth cards to label weight gain, weight loss and static weight, and healthy and malnourished child. Mothers were selected using the EPI cluster-sampling technique.

Results: Red, yellow, and green were the most preferred colours chosen by the mothers to indicate weight gain, static weight, and weight loss respectively. Green was the most favoured colour to label a malnourished child, whereas red was chosen for a healthy child. Most mothers labelled upward curve for weight gain, horizontal curve for static weight, and downward curve for weight loss. Similarly, downward and upward curves were chosen by a majority of the mothers to label a malnourished and a healthy child respectively. A tendency toward bright colours (red and yellow) for labelling a healthy child and darker colours (green and black) for a malnourished child were observed in the study.

Conclusion: The findings of the study suggest that colour selection is culturally sensitive, and should be taken into consideration before introducing any colour as a marker for some meaning.

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Perceptions of the Urban Poor Mothers about Small Babies: A Case Study in Dhaka, Bangladesh

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Objective: Explore perceptions about low-birth-weight babies among the urban poor mothers in Dhaka City, Bangladesh.

Methodology: Focus-group discussions were held with 78 women in the slum and low-income areas. Eight to ten women participated in each session.

Results: Less intake of food, vitamin deficiency, too-much-intake of rice, and evil spirit were recognized as the causes of delivering small babies. Difficult-to-take care and nurse, respiratory and gastrointestinal problems were recognized as the major problems by the respondents. The relationship between malnutrition and low-birth-weight babies were also well recognized. Frequent feeding, less intake of rice, intake of vegetables and fruit, cleanliness, rest, not-to-do hard work during pregnancy and not-to-get cold were the preventive measures suggested by the urban poor mothers.

Conclusion: Findings of the study can be used for designing an intervention on low birth weight.

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Nutritional Status of Children in the BRAC's Urban Primary Schools

S Asiruddin[1], Mamunur Rahman[1], Sadia A Chowdhury[2], and AFM Iqbal Kabir[3]

Objective: Assess the nutritional status of children in the BRAC primary schools in urban areas and its relationship with the surrounding socioeconomic variables.

Methodology: A cross-sectional study on 220 students, selected randomly from 22 BRAC primary schools in Dhaka city, was conducted from October 1996 to February 1997 under a research programme of the JEXCA Community Hospital and was funded by the Essential National Health Research, Bangladesh. Data were collected through a pre-tested partially close-ended questionnaire. Anthropometric measurement was expressed in z-score of the NCHS standard. Biochemical analysis for urinary iodine level was done in the International Centre for Control of Iodine Deficiency Disorders (ICCIDD) Lab, Institute of Nutrition and Food Science, University of Dhaka, and the results were matched with other findings.

Results: Forty-two percent of the male and 58% of the female students were classified in three age groups: 6-8 years (17.7%), 8-10 years (42.3%), and 10-12 years (40%). About thirty-two percent of the children were found stunted and both wasted and stunted, and 8.19% of the children were only wasted. There was a significant relationship of anaemia with angular stomatitis ($p=0.005$), illness ($p=0.005$), weight/age ($p=0.04$) and height/age ($p=0.05$). Considering -2SD as the cut-off point, the incidence of acute malnutrition (Wt./Ht.) was the highest in the 6-8-years age group, and chronic malnutrition (Ht./age) was the highest in the 10-12-year age group. Urine iodine analysis showed only 17.7% children to be suffering from iodine deficiency disorders, while 10.85% of the household salt samples showed no iodine at all. Correlations of vitamin A deficiency with illness and nutritional status were statistically significant. The school attendance of the male students was slightly higher (92.68%) compared to that of the female students (91.48%). Of the 5 city areas, 93.3% of the students in Narayangonj used tubewell water, 90% in Mogbazar used safe latrine, 25% in Jatrabari lived in good houses, and 10% in Moghbazar were engaged in different occupations. This finding will help BRAC's Non-formal Primary Education formulate appropriate socio-health interventions for the students and their families in those areas.

Conclusion: The nutritional status of children in the BRAC primary school in urban Dhaka was found to be lower than the NCHS standard. Vitamin deficiency disorders of the students can be corrected through promoting the school health programme.

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Bangladesh-Australia Child Health Project: A Child-to-Child and Child-to-Parent Approach for Nutrition and Health Education

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Objective: Evaluate the Child-to-Child and Child-to-Parent approach for message communication. This approach was used by the Bangladesh-Australia Child Health (BACH) Project to disseminate health and nutrition education, with special emphasis on growth monitoring, personal hygiene, oral rehydration therapy, and immunization through scouts and guides.

Methodology: The approach was implemented in 25 Bangladeshi villages over a 6-year period (1986-1992). Two hundred and thirty-nine Australian Rovers and Rangers (Scouts and Guides aged 16-26 years) worked in 16 batches with 787 Bangladeshi counterparts. A joint evaluation team, comprising the Bangladeshi and Australian members, visited each BACH village in March-April 1994. The team also included one scientist from ICDDR,B. The team visited every household of the project area to assess the nutrition and health status of children and the impact of the project activities. The parents were interviewed, and each child aged less than five years was measured and weighed.

Results: The growth of children as measured by height and weight showed that the vast majority of the values fell within the 2 SD (standard deviation) of the mean. In respect of measuring the nutritional status by mid-upper arm cicumference (MUAC), 8% of the male children and 13% of the female children were found to be malnourished. The infant mortality rate was found below 30/thousand livebirths. The result also showed a significant coverage in immunization (95%), the use of sanitary latrines, and other aspects of sanitation. Low-cost measures were taken for the implementation of the programme. The Australian rovers and rangers came on their own expenses. On an average, US$ 3,000 were spent for an average of 400 families in each village. The immediate cost appeared one dollar per person.

Conclusion: The Child-to-Child and Child-to-Parent approach for health and nutrition education was found to be attractive and effective. The programme was found to be sustainable, cost-effective, and replicable without using any extra manpower. Behavioural changes in respect of nutrition and health are possible through scouts and guides.

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Home-gardening Approach to Reduce Micronutrient Malnutrition of the Rural Population in Bangladesh

M Eshaque Ali and MA Mannan

Objective: Evaluate the home-gardening approach for better intake of vegetables to reduce current micronutrient malnutrition of the rural population in Bangladesh.

Methodology: Two thousand two hundred five participatory farm families (PFFs) were selected from 35 rural areas of Bangladesh during the three-year project period. Every year, 735 PFF were identified and organized into a group of 21 farmers headed by a Lead Farmer (LF). A benchmark survey was conducted using a structured questionnaire. Six administrative units and six unit officers were responsible for implementation, supervision, and monitoring of the activities of the units. The PFFs were trained on production, processing and preservation of seasonal vegetables, and also on health and other nutritional aspects. The family members were about 6, and the homestead garden size was about 6m/6m. The inputs included seed, seedling, fertilizer, fencing support, spade, and others, particularly educational materials. The yearly progress of production and consumption of vegetables was recorded and analyzed using a computer software.

Results: The monthly PFF's average production and consumption of vegetables in three consecutive years were: 65 kg (SD 40) and 58 kg (SD 18), 73 kg (SD 66) and 62 kg (SD 16), and 63 kg (SD 19) and 61 kg (SD 23) respectively. The average daily vegetable availability at the home level was about 347 g/person (national average is about 125-150 g), and on edible portion, it was about 266 g/person. The PFF's awareness was created through imparting health and nutrition education. The training impact on vegetable processing was found to be 2.1 and 6.6 grade (of 10) respectively during pre- and post-training. The percent impact of seed and seedling production by the PFF at the pre-project ending was 5.5 (SD 2) and 23.4 (SD 11), 4.6 (SD 1.5) and 24.3 (SD 9.8), and 5.7 (SD 1) and 30.8 (SD 9) during 1st year groups, 2nd year groups, and 3rd year groups respectively. Qualitative assessment showed that some behavioural changes were found among the PFFs.

Conclusion: The consumption of vegetables increased among the beneficiaries, even more than that of the national level. This has an effect on deteriorating micronutrient deficiency syndrome and on the reducing micronutrient malnutrition of the rural people. This increased consumption of vegetables was due to awareness of better health and nutrition knowledge among the targeted PFFs. These activities are being sustained in most project areas, and are recommended for replication.

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Changes in Plasma Ceruloplasmin Activity in an Animal Model of Shigellosis

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Objective: Evaluate changes in the ceruloplasmin activity in an animal model of shigellosis.

Methodology: The ceruloplasmin activity was estimated and compared with normal animal in a rabbit model of shigellosis. Colonic infection was induced in adult rabbits by inoculating Shigella flexneri 2a. Blood samples were obtained before and after infection from the 24 to 192-hour experiment period for control and infected animal for measuring the ceruloplasmin activity.

Results: The preliminary results showed that the ceruloplasmin activity (mg/dl, mean±SE) in the plasma was: 17.67±1.02 (control, n=12), 37.08±1.10 (24 hours infected, n=12), 42.04±0.99 (48 hours infected, n=7), 36.80±1.93 (72 hours infected, n=7), 33.11±1.70 (96 hours infected, n=6 hours), 13.88±1.86 (192 hours infected, n=7).

Conclusion: These preliminary data indicated that the serum concentration of the ceruloplasmin activity was significantly elevated during infection of 24 and 48 hours due to shigellosis. However, after 72-192 hours of infection, the ceruloplasmin activity in the plasma was progressively decreased which may be due to the accumulation of copper in the liver and brain and ultimately reached below the baseline values at 192 hours due to copper deficiency.

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Potential Invasive Properties of Vibrio cholerae O139 Bengal in a Rabbit Model: A Preliminary Study

AM Khan[1], MK Bhattacharya[2], GH Rabbani[1], and GJ Fuchs[1]

Objective: Study the invasive properties of Vibrio cholerae O139 Bengal.

Methodology: Adult New Zealand white rabbits were used. Closed intestinal loops of 10-cm length, following appropriate surgical procedures, were constructed. Live cultures of V. cholerae O139 (bacterial count: 109/mL) were inoculated in each loop. Two loops were constructed in the small intestine of an additional rabbit. One loop was challenged with V. cholerae O139, and the other one (control) with bacteria-free culture medium.

Results: After 18 hours, inflammatory changes were noted in the gut wall mucosa of all rabbits. Blood cultures revealed the growth of V. cholerae O139 in one animal, suggesting mucosal invasion followed by bacteraemia. While the gut wall of the control loop showed no signs of inflammation, there were definite signs of inflammation in that of the challenged one.

Conclusion: These preliminary observations indicate that some strains of V. cholerae O139 may have invasive properties in rabbits.

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Prevalence of Intestinal Parasites in the Healthy Adult and Adolescent Population in a Rural Community

SM Ziauddin Hyder[1], M Akram Hossain[2], and Sadia A Chowdhury[1]

Objective: Identify the magnitude of the problem of parasites among healthy adults and adolescents in a rural population of Mymensingh district.

Methodology: Fulbaria thana of Mymensingh district was selected for the study. Eleven villages around the Thana Health Complex (THC) were randomly selected. Thirty samples were collected from each of the 11 villages. Houses were randomly selected. Thus, 330 individuals were interviewed whose stool samples were collected and sent within 6 hours of collection to the Department of Microbiology, Mymensingh Medical College for microscopic examination. Both saline and iodine preparations were done for the samples.

Result: The overall parasitic prevalence was found to be 33.3%, of which 27.9% were among males and 35.8% among females. The adults of the 18-35-years age group had the highest prevalence (40%). Among the infested individuals, Ascaris lumbricoides has got the highest prevalence of 88.3%, followed by Ankylostoma duodenale 4.5%, Strongyloides stercoralis being the lowest 0.9%. Only 30% of the people who washed their hands with soap after defecation suffered from parasitism as against 77% who did not use soap.

Conclusion: The prevalence of intestinal parasites, especially of Ascaris, is very high in the rural communities of Bangladesh. Adequate focus should be given to improve sanitation through information, education and motivation (IEM) to control the spread of intestinal parasites. Importance of using soap to wash hands after defecation should also be popularized.

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Women in Need: Pattern of STD Infection among Street-based Sex Workers of Dhaka City

Swarup Sarkar[1], Ziya Uddin[1], Yasmin Ahmed[2], Nazrul Islam[3], Sushena Reza[1], Fazlul Karim[1], and Maurice Bloem[1]

Objective: Assess the design, development and implementation of an HIV/AIDS and STD intervention targeting the street-based sex workers in Dhaka city.

Methodology: Sex workers were recruited to act as guides during assessment. An extensive ethnographic field observation was used for identifying the locations, and the capture-recapture method was used for estimating the size of street-based female sex workers in the city. Depending on the condom use, the sample size was determined, and 230 sex workers were recruited from the street. The participants in the survey were provided with medical examination by the qualified staff. Of the 230 women, 225 agreed to have clinical examination, as well as endocervical swabs and blood samples taken for laboratory examination. Endocervical swabs for gonorrhoea and blood samples were collected for VDRL tests and confidential and anonymous HIV testing. Samples were simultaneously tested in the Institute of Postgraduate Medicine and Research, Dhaka, and Bolton Sexual Health Center, UK. A cohort of samples was cultured in the International Centre for Diarrhoeal Disease Research, Bangladesh.

Results: Eighty percent reported that they had vaginal discharge, and 40 percent had burning sensation during urination. Thirty-nine percent reported that they had deep dyspareunia. Of the 81% women who had signs of reproductive tract infection, 69% had vaginal discharge, 62% cervical discharge, and 55% both vaginal and cervical discharge. On laboratory examination, TPHA and VDRL were found positive for 52% and 29% respectively. PCR-positive was 53% and 49% for gonorrhoea and chlamydia respectively. There was no positive case for HIV.

Conclusion: Given the prevalence of STD and high-risk behaviours among the street sex workers, a vigorous HIV/AIDS and STD control intervention should be implemented quickly. The nature and size of the target population calls for partnership between different organizations and parties. Satellite and mobile clinics should be established to provide sex workers with outreach services. Sex workers should be recruited and trained as peer educators to disseminate messages and risk reduction materials and work toward creating peer pressure. The sex workers should be provided with appropriate health information, including HIV/AIDS and STD prevention information, education, and condoms.

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Acute Transient Childhood Myositis Resembling Paralytic Illness: A Newly Discovered Entity in Bangladesh

Md. Salim Shakur[1] and Md. Sultan Uddin[2]

Objective: Study the acute transient myositis in children.

Methodology: Ten children (M-6, F-4) aged 3-5 years (mean age 46 months) presented in surgery, during January - July 1996 with sudden onset of significant pain and weakness of lower limbs, with inability to walk (forcing them to become bed ridden), causing great concern to their parents, all of whom were of higher socioeconomic class. All of the patients had muscle tenderness, particularly of lower limb muscles (calves), but no neurological deficit was clinically detected and none showed altered sensorium. Most patients did not walk due to apprehension of enhancement of pain. Most of them had mild constitutional symptoms, including low-grade fever. All patients were investigated for complete blood count, ESR, CRP, creatinine phosphokinase (CPK), and 3 were investigated for blood culture and widal.

Results: All the patients showed sky-high CPK (2297 g/L±194g/L). All except one showed normal ESR; all showed normal white blood cell count. All other investigations were negative. All patients were treated conservatively at home with oral paracetamol, and three received antibiotics. All patients recovered uneventfully within 10 days (mean 7 days) of illness, with complete disappearance of weakness, pain and tenderness of lower limbs, with CPK coming back to normal level within 10 days in parallel with clinical recovery. The conditions showed sudden significant pain and weakness of lower limbs causing complete inability to walk mimicking paralytic illness to be transient reversible phenomena due to acute myositis. The raised serum values were considered to be adequate confirmation of this, and rapid reversion to normal, corresponded to clinical recovery. Although no virology study was done, review of literature suggested viral aetiology, particularly influenza B virus, as the most likely aetiology of this condition.

Conclusion: Sudden onset of severe pain and tenderness in lower limb muscles in children, causing inability to walk may be due to acute myosities which is a transient reversible phenomena and not a panicky condition like paralytic illness. Revised serum CPK should not be misinterpritated as collagen disease or onset of heriditary myopathic illness.

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Acute Viral Hepatitis in Pregnancy

Rokeya Begum and Syeda Nurjahan Bhuiyan

Objective: Analyze the factors relating to the maternal and perinatal outcome of pregnancy with viral hepatitis.

Methodology: A prospective study was carried out during January-November 1997 in the Department of Obstetrics and Gynaecology, Chittagong Medical College Hospital. This study includes all pregnant women in any gestational age with the presence of a clinical syndrome of acute viral hepatitis with or without complications. The cases suggestive of chronic liver disease were excluded. Data were recorded on predesigned protocol, including the well- known prognostic variables. All investigations available in the setup were done. Daily clinical follow-up consisted of recording of maternal and foetal well-being as well as looking for new complications. Patients were followed till discharge/death. Survivors were tested by USG for foetal well-being.

Results: The majority (70.5%) of women were aged ranging from 25 to 35 years. Fourteen (82.3%) of them were multiparous. Forty percent of the pregnant women had only one time antenatal visit and rest had no antenatal check-up. Ten (58.8%) cases came from urban areas. Jaundice was detected in 15 women (88.2%) in 3rd trimester of pregnancy. Eighty-eight percent of the women were admitted to hospital within two weeks of detection of jaundice. Five (29.4%) cases had hepatic failure at the time of admission. It has been seen that prothrombin time over 20 seconds above control, bilirubin level more than 20 mg/dl, and serum transaminase level above 1000 IU/L were associated with high mortality. As no specific treatment exists for viral hepatitis, patients were managed by supportive treatment only. Of the 17 caes in in this series, 13 (76.4%) were died. Eleven cases (84.6%) died due to hepatic failure and 2 cases died due to PPH. The majority of foetuses (76.4%) were stillborn and rest had normal babies. It has been observed that, during the rainy season, 76% of the cases were admitted and had high mortality. The important risk factors associated with poor outcome were third timester of pregnancy, fulminating hepatic failure, deep jaundice, prolong thrombin time, on set of labour and any obstetrical complication.

Conclusion: Viral hepatitis in pregnancy is devastating specially in last trimester and is an important cause of maternal death in Bangladesh. Presence of complications and associated risk factors carried almost 100% mortality. Prevention of enteral modes of transmission of viral hepatitis is essential for pregnant women in Bangladesh.

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Parasites in Healthy City Dwellers

MA Samad Talukder

Objective: Investigate faecal parasites in healthy Bangladeshi people who normally reside in Dhaka city.

Methodology: Direct microscopic examination of wet film of faeces in normal saline and Lugol's iodine on microscopic slide with cover slip was done without floatation or concentration method as the subjects were healthy adult population for routine medical check-up.

Results: Of the two hundred seventy-eight patients, including four women, submitted their stool specimens for examination, 71 (25.54 %) had 80 parasites. None of the subjects had any complaints. A single infection was present in 63 subjects (88.73%), double infection in 7 (9.89%), and treble infection in 1 (1.41%). The parasites isolated were Ascaris lumbricoides (including one larva, 51.25%), Trichuris trichiura 14 (17.5%), cysts of Giardia lamblia 12 (15%), cysts of Entamoeba histolytica 7 (9.89%), Ancylostoma duodenale, 3 (3.75%), Trichomonas hominis 2 (2.5%), and Fasciolopsis buski 1 (1.25%).

Conclusion: The prevalence of faecal parasites in healthy city dwellers was 25.54%, and just over 50% were A. lumbricoides. It was found that even healthy asymptomatic people (having no complaints) harbour parasites in endemic areas where warm climate may help the development of ova to infective form and infect even the healthy population.

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Seroprevalence of Syphilis, Hepatitis B, and HIV Infections

AKM Shariful Islam[1], Osul Ahmed Chowdhury[1], M Shibbir Ahmed[1], and Ahmed Kabir Chowdhury[2]

Objective: Determine seroprevalence of syphilis, hepatitis B, and HIV infections among patients with sexually transmitted disease (STD)/sexual health problems (SHP).

Methodology: A sequential cross-sectional study was done at the Skin and VD outdoor of the Sylhet M.A.G. Osmani Medical College Hospital during September 1996-August 1997. Sociodemographic information and data on clinical diagnoses, age of first sexual experience, number of sexual partners, casual relations, and sexual practices were collected confidentially by one of the researchers in a purposively designed pre-tested questionnaire after personal interview and clinical examination of the randomly selected patients (n=468) who gave verbal consent to participate in the study. Blood samples of these patients were collected and tested at the Microbiology Department of the College by the commercially available kits.

Results: Of the 468 patients tested, 460 were male and 8 were female. The mean age of the patients was 24.2 years with a range 9-46 years. Of these, 358 (76.5%) were unmarried, 109 (23.3%) married, and 1 (0.2%) widowed. In total, 44 (9.4%), 34 (7.3%) and 79 (16.9%) were reactive/positive in VDRL, TPHA and HBsAg tests respectively. Of the 44 VDRL-reactive cases, 34 (77.3%) were confirmed to be syphilis by the TPHA test. The overall seroprevalence for syphilis and hepatitis B was 7.3% and 16.9% respectively. No HIV antibody was detected in any patient. The major diagnoses were 163 (34.8%) genital ulcerative diseases (GUD), 245 (52.4%) genital discharge diseases (GDD), and 60 (12.8%) sexual health problems (SHP). VDRL, TPHA, and HBsAg tests were reactive/positive in 22.7%, 19.1%, and 14.7% cases in GUD compared to 2.5%, 1.2%, and 18.4% cases in GDD respectively. Only 1.7% VDRL and 16.7% HBsAg tests were reactive/positive in the SHP patients. These differences were statistically significant.

Conclusion: Seroprevalence of syphilis was more confirmed in GUDs than in GDDs, whereas the seroprevalence of hepatitis B was more confirmed in GDDs than in GUDs. Besides, reactive/positive results of these tests were significantly higher in the STD patients than in the SHP patients. Because of these high rates of STDs and great potential for the spread of HIV infection, surveillance of STD patients should receive priority for the control and management of STDs in Bangladesh.

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Plasmid Fingerprinting for the Investigation of Inter-household Spread of Multiresistant Faecal Bacteria in Rural Bangladesh

Kazi Selim Anwar[1] and Paul Shears[2]

Objective: Determine the household spread and epidemiology of multiple drug-resistant (MDR) faecal coliform bacteria from human and environmental sources employing plasmid fingerprinting.

Methodology: Thirty-three water specimens of kolshi (storage pot), 13 tubewell and 71 faecal samples were collected from 17 randomly selected households of two villages of Rajbari. Lactose-fermenting coliforms (LFC) were cultured, and MDR strains, after antibiogram (Stokes, 1972), were subjected for in vitro conjugation. Plasmid from donor LFCs and their transconjugants were extracted following the method of Boirnboim and Doly. Restriction endonuclease (REA) fragments were generated by digesting the large 98 Mda transconjugant plasmid using HindIII. Dendrogram was also employed to compare its findings with the conventional (manual) plotting method.

Results: Ninety-four percent of the faecal samples, 21 of the 33 kolshi, and only two of the tubewell samples were contaminated with LFC. Overall, 78% of the isolates were resistant to more than 3 antibacterials; R-pattern 'RRRSS' genes [(resistant to tetracycline (Tc), ampicillin (Am), and trimethoprim (Tm), and sensitive to chloramphenicol and nalidixic acid (Na)] were the commonest and prevalent in all types of samples and from almost every household. Conjugation experiment showed a complete transfer of 'RRRSS' genes which were carried most commonly by a large 98-Mda plasmid, although not all of these were genetically identical as revealed by REA. However, similar or different plasmid/transconjugant/REA profiles were observed in the same or different households or baris (cluster of households) of both the study areas.

Conclusion: It was evident that a wide range of MDR LFCs (as a marker of Enterobacteriaceae) was circulating in both the villages, within the households and among different baris. Water from kolshi, but not from tubewell, played a vital role in the household spread of these bacteria.

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Efficacy of Erythromycin, Ampicillin, and Tetracycline in the Treatment of Cholera in Children

SK Roy, A Islam, R Ali, E Islam, RA Khan, SH Ara, NM Saifuddin, and GJ Fuchs

Objectives: Compare the clinical outcome(s) of treatment of cholera in children with ampicillin, erythromycin, and tetracycline.

Methodology: In a double-blind randomized 4-cell trial, 184 children aged 1-5 years whose weight-for-age was more than 80% of the NCHS standard were given either tetracycline, erythromycin, ampicillin, or placebo for 3 days (the dosage suggested by the World Health Organization) in a diarrhoeal disease hospital in Dhaka. Selection criteria included diarrhoea of less than 48 hours duration, signs of some or severe dehydration, a dark-field stool microscopy demonstrating the presence of Vibrio cholerae and baseline purging rate more than 4 mL.kg/h (over 6 hours).

Results: After three days of antibiotic treatment, the mean±SEM stool output was significantly reduced in each of the three groups who received antibiotics compared to the placebo group. The mean duration of recovery was 66% longer in the placebo group (p=0.000), 25% in the ampicillin group (p=0.017), and 9% in the erythromycin group (p=0.37) compared to the tetracycline group. The clinical recovery rate by 96 hours was 75% (p=0.001) in the placebo group, 91.3% in the ampicillin group (p=0.16), and 95.7% in the erythromycin group (p=0.04) compared to the tetracycline group. The stool output in mL.kg.body weight was: 318±50, 335±30, 323±25, and 498±37 respectively in tetracycline, ampicillin, erythromycin, and placebo groups.

Conclusion: The results of the study indicate that the clinical efficacy of tetracycline, ampicillin, and erythromycin in the treatment of cholera in children was comparable. It is recommended that, where test for V. cholerae is positive to ampicillin, it can be used as an effective alternative antibiotic for the treatment of cholera and acute respiratory tract infections.

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Desire for Children and Subsequent Abortion in Matlab, Bangladesh

Abdur Razzaque, Kapil Ahmed, Nurul Alam, and Jeroen van Ginneken

Objective: Investigate the desire for children and subsequent abortion in the MCH-FP and comparison areas of Matlab, Bangladesh.

Methodology: Data of the in-depth survey 1984, KAP survey 1990, and the Demographic Surveillance System (1984-1994) were used.

Results: During 1984-1994, the incidence of abortion increased substantially in both comparison and intervention areas, and such increase was due to those who wanted no more children. After controlling for all the variables in the logistic regression, the probability of subsequent abortion was higher among those who wanted no more children than those who wanted more in both MCH-FP (5.2 times) and comparison (8.9 times) areas. The incidence of abortion was lower in the MCH-FP area than that in the comparison area and was lower among the illiterates, users of contraception, and the Muslims in both the areas compared to the educated, non-users of contraception, and the Hindus.

Conclusion: The findings of the study suggest that there is a need to improve the quality of family planning services, particularly for those who want no more children to reduce abortion and abortion-related deaths.

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Implementation of the Essential Services Package through Standardized Service Delivery Protocols

Selina Amin[1], Cristobal Tuñón[1], SE Arifeen[1], AH Baqui[1], Rasheda Khanam[2], and Samina Manaf[1]

Objective: Evaluate the range and quality of services delivered from the urban primary-care clinics through the adaptation and implementation of appropriate and practical service delivery protocols.

Methodology: The study was on a quasi-experimental design. Based on the national priorities, epidemiological data, implementation feasibility, and client preferences, eight components of essential services were identified. The existing national and international guidelines and protocols were reviewed and adapted. Providers from three clinics of an NGO and three government dispensaries (GOD) were trained on the newly-adapted protocols. These clinics were monitored by a physician regularly. For comparison, the activities of the clinic staff at the two non-intervention NGO sites and two non-intervention government sites were also monitored. A mid-term evaluation, conducted after a year of implementation, was based on the data from the pre- and post-training knowledge tests, structured observations of provider-client interactions, analysis of the clinic records, and interviews with providers and with clients.

Results: The results of the evaluation indicated that the intervention markedly improved the diagnostic and treatment practices of the service providers. There were marked improvements in the prescription patterns, with a reduced misuse of antibiotics for the management of diarrhoea, acute respiratory infection (ARI), and reproductive tract infection along the lines suggested by the protocols. After the introduction of the protocols, inappropriate use of metronidazole was reduced from 86% to 31% in diarrhoea cases, and inappropriate use of antihistamine was reduced from 77% to 18% in ARI cases. These changes were not observed or were less pronounced in the comparison clinics. However, the providers stated that the protocols were easy to follow, but had increased the waiting time at the clinics.

Conclusion: The practice of following standard protocols improves the quality of services. However, the comments of the providers need to be analyzed further. Complementary subsystem interventions (quality of the physical facility, logistics, information and management support system) are needed to implement the protocols fully.

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Use of Antenatal Care in an Urban Area of Dhaka City

Quamrun Nahar

Objective: Describe the types, patterns and use of antenatal care (ANC) in an urban area of Dhaka city.

Methodology: A community-based study of antenatal care-seeking behaviour was conducted during February-June 1996. A sample of 200 women who were pregnant for at least six months was identified from an ongoing health and demographic surveillance system set up in Zone 3 of the Dhaka city. A pre-tested structured questionnaire was used for collecting information on the sociodemographic characteristics, reproductive history, and the ANC use patterns. In-depth interviews were also conducted among a subsample (n=16) of these women to understand the process of seeking ANC.

Results: Most study women (88%) received some form of ANC. However, about a quarter received only tetanus immunization (TT), and less than 10% received all the necessary elements of ANC as recommended by the Government of Bangladesh. In addition, half of the women made only one or two visits, and only one-third made the first visit during their first trimester. A diverse variety of health-care providers was used by the study women. While most women obtained ANC from modern providers, about a quarter used traditional providers, either alone or as an adjunct to the care given by modern providers. Factors affecting the use of ANC suggest that the women who were more educated had fewer children, and whose husbands had more schooling and who had higher monthly income were more likely to use ANC ($p<0.05$). Women's ANC-seeking behaviour seems to follow a four-stage process: recognition of the importance of ANC, stance to seek ANC, selection of a provider, and finally, seeking ANC.

Conclusion: Although the findings of the study reveal that the TT coverage among the pregnant women was high in urban Dhaka, other ANC services were very weak. The results of the study also suggest that there is still ample room for improvement in the delivery and organization of antenatal care, particularly in the process of client-provider interaction.

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Incorporation of Checklists in Clinic Information System Supports the Delivery of Quality Essential Health Services

SM Tariq Azim[1], Sangeeta Mookherji[2], and AH Baqui[1]

Objective: Assess the impact of introducing a revised clinic information system on the quality of essential health services provided at the urban NGO clinics.

Methodology: A card-based client-oriented information system for the urban primary-level NGO clinics was developed that incorporated screening checklists on key elements of a number of essential services. Thus, there were checklists on screening for family planning methods, antenatal and postnatal check-up, assessment of reproductive tract infection cases, and assessment of children with diarrhoeal disease and acute respiratory tract infections. The system was tested in 1996 at the two primary-level clinics of a non-governmental organization (NGO) in Dhaka city. The study was quasi-experimental with another two urban primary-level clinics where service records were kept in registers, serving as comparison. Data for evaluation were collected through independent observations and review of the clinic cards.

Results: In the intervention clinics, in nearly 85% of the new clients seeking injectable contraceptives, the paramedics carried out the minimum required screening. Almost all (98%) of the pregnant women were screened as per the organization's guidelines, and important physical examinations were done in about 69% of the cases. In the comparison clinics, none of the clients who came either for injectable contraceptives or for antenatal check-up received the full range of screening procedures. Before the introduction of the protocol for syndromic diagnosis of RTI cases in the intervention clinics and after the training on the protocol, the diagnosis made by the paramedics was based mostly on the amount of vaginal discharge and the condition of cervix. With the introduction of checklist on RTI, in about 92% of the cases with vaginal discharge, syndromic diagnosis was made according to the protocol, based primarily on the characteristics of vaginal discharge and the partner's symptoms.

Conclusion: Incorporating checklists in a client-oriented routine record-keeping system assisted service providers to follow assessment protocols according to the organizational standards.

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Reducing Drug Costs through Rationalization of Diarrhoea and ARI Case Management in Urban Areas

Zahidul Quayyum, Selina Amin, AH Baqui, and Samina Manaf

Objective: Examine the possibility of reducing the drug costs through rationalization of diarrhoea and acute respiratory infection (ARI) case management in urban areas.

Methodology: Based on the WHO guidelines, protocols were adapted for the management of diarrhoeal diseases and ARI cases at the primary healthcare (PHC) level. Service providers at three selected clinics of the Concerned Women for Family Planning in urban Dhaka were trained on the protocols. The diagnosis and treatment patterns for diarrhoeal diseases and ARI cases before and after the introduction of standardized procedures were examined. Such information was collected from the clinic registers for six months before and six months after the introduction of the protocols. The drugs that were dispensed by the providers for the treatment were costed, and the drug cost per client for the treatment was estimated in two different situations, i.e. treatment with and without the use of standard protocols. These were then compared to determine whether the cost per client decreased after the protocols were introduced.

Results: The diagnosis pattern changed both in the case of diarrhoeal diseases and ARI cases. Before the introduction of the protocols, 50% of the patients with diarrhoeal diseases were inappropriately diagnosed. This had implication on the treatment procedures. Following introduction of the protocols, the use of oral rehydration solutions (ORS) increased, and metronidazole was not used for treating the diarrhoea cases. Similarly, cotrimaxazole was appropriately used for treating dysentery. The drug cost of treating diarrhoeal diseases declined by Tk 1.96 (12%) per client after introduction of the protocol. For the ARI cases, the drug cost was reduced by Tk 11.9 (53%) per client. This has helped reduce the total drug cost for diarrhoea and ARI cases by about 32%, saving Tk 1,688 for the clinics in the six-month period.

Conclusion: Inappropriate diagnosis and treatment pattern increase the cost of services for the providers. The use of standard protocols may help reduce the drug cost for treating cases of ARI and diarrhoeal diseases.

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Factors Contributing to Low Immunization Coverage among Urban Slum Children in Bangladesh

Jahanara Khatun

Objective: Describe the extent of immunization coverage in the urban slum of Zone 3 of the Dhaka City Corporation, and identify the factors contributing to the low immunization coverage.

Methodology: Childhood immunization coverage and socioeconomic data were collected from 651 women who had a child aged 12-23 months from the Urban Panel Survey (UPS) of the Urban MCH-FP Extension Project of ICDDR,B. Thirteen immunization service providers were interviewed, and 33 children were observed when obtaining their vaccination. Bivariate analysis was done to identify the association between the low immunization coverage and the sociodemographic characteristics.

Results: The results of the study showed that the complete immunization coverage of the children aged 12-23 months in the study area was 60.2%. However, in the slum area, the immunization coverage was 48%, whereas in the non-slum cluster, it was 67%. The drop-out rate in the slum cluster from DPT1 to DPT3 was also higher than that in the non-slum cluster (21.7% vs. 9.3%). The characteristics of the urban slum children who completed their immunization series were strongly associated with the following variables: maternal education, maternal employment, family income, father's occupation, household possessions, and the number of living children. Provision-related factors that influence the low immunization coverage in urban slums were: inadequate location and timing of clinics, improper supervision, lack of referral, missed opportunities for vaccination, and lack of coordination among different service provider organizations.

Conclusion: The immunization coverage should be improved through strengthening routine immunization services and increasing the integration and coordination among the service providers. The Expanded Programme on Immunization (EPI) should develop a "Slum Strategy" to ensure that high-risk slum children are vaccinated properly. The EPI services should also be linked with development organizations to improve the overall health status of the slum dwellers. Further, health system research is needed to identify operational problems of EPI.

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Maternal Morbidity in Rural Bangladesh: Where Do Women Go for Care?

Parveen A Khanum, Shameem Ahmed, Ariful Islam, and Sadia D Parveen

Objective: Assess the complications experienced and subsequent care-seeking behaviour of rural women and their knowledge about the complications of pregnancy and childbirth.

Methodology: A structured questionnaire was used for interviewing 2,105 rural Bangladeshi women who delivered within one year of the survey. They were interviewed in their homes between May and August 1996. In this study, maternal morbidity refers to any compilations reported by women during their last pregnancy, delivery and/or within 42 days after delivery.

Results: Obstetric complications were experienced by 66% of the women, and commonest among these were prolonged labour, fever, bleeding, and oedema. The older and higher parity women, and those with less education, were more likely to develop complications, and stillbirths were four times higher among those with complications. Among all the women who had complications, 41% consulted village practitioners, 18% went to homeopaths, and 6% went to traditional healers. Thirty-seven percent of the women went to nobody. Husbands were the principal decision-makers for consultation with service providers. Use of institutional facilities and/or trained providers was positively associated with women's education, parity, their knowledge of obstetric complications and average monthly family expenditure. Women's knowledge about complications of pregnancy and childbirth was limited. Most women knew about prolonged labour and malpresentation, but very few knew about bleeding, retained placenta, and convulsion. A majority knew nothing about postpartum complications. Thirty-seven percent of the women received antenatal care from medically trained personnel, like paramedic, and doctors. Ninety-two percent of all the deliveries took place at home, and only 7% of the complicated cases delivered at the health facility. Eighty-nine percent of the women had a livebirths, nearly 3% had stillbirths and rest had either induced or spontaneous abortion as the outcome of their last pregnancy.

Conclusion: Very few women in rural Bangladesh know about the common complications of pregnancy and childbirth, and most do not seek medical help for these. Also, use of government health facilities for the management of obstetric complications is poor. Therefore, efforts need to be strengthened for raising community awareness emphasizing on the importance of seeking medical help for obstetric emergencies. As an effort toward this the Operations Research Project of ICDDR,B has designed an intervention on emergency obstetric care at the thana level.

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Neonatal Morbidity and Care-seeking Behaviour in Two Rural Areas of Bangladesh

Shameem Ahmed, Farzana Sobhan, and Ariful Islam

Objective: Assess the pattern of neonatal morbidity and subsequent care-seeking behaviour in rural Bangladesh.

Methodology: Data were collected from 3,030 women who had livebirths between May 1995 and February 1997 in two rural subdistricts—Abhoynagar and Mirsarai—the field sites of the Operations Research Project of ICDDR,B. The women were interviewed in their homes using a semi-structured questionnaire. Bivariate analysis was done to assess the relationship between the different variables.

Results: More than two-thirds of the neonates were reported to have problems. The most common complaint was fever (40%), followed by respiratory distress (25%). Complications during pregnancy were found to be associated with increased neonatal morbidity ($p<0.001$). About 42% of the women did not seek help from any health service providers when their newborns had problems. Significant sex differential was observed among the male and female neonates for whom services were sought ($p<0.001$). In majority of the cases (48%), village doctors were consulted, followed by homeopaths in Mirsarai, whereas in Abhoynagar, the opposite trend was seen. Only a negligible percentage attended the government facilities, like Satellite Clinic, Health and Family Welfare Centre, and Thana Health Complex. However, 30% of the mothers consulted private practitioners. It was found that health care-seeking behaviour was associated with mothers' education ($p<0.01$).

Conclusion: The government facilities for neonatal care are under-used, and efforts should be made to raise awareness among mothers regarding this. Steps to reduce maternal morbidity by raising awareness of complications during pregnancy may result in decreased neonatal morbidity.

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Perceptions and Involvement of Members of Zonal Health and Family Planning Coordination Committees of Dhaka City Corporation

J Uddin, MA Bhuiyan, SU Alamgir, and Cristobal Tuñón

Objective: Assess the perception and involvement of members of the zonal health and family planning coordination committees formed by the Dhaka City Corporation.

Methodology: The Dhaka City Corporation formed zonal health and family planning coordination committees in all 10 zones of the city to link all the local service providers and to establish a mechanism for local-level planning to ensure the effective use of the available local resources through minimizing gaps and overlaps in the health and family planning service delivery system. Of the 181 registered members of the zonal committees, 126 were selected for interview using quota sampling methodology. Data were collected through individual interviews using a structured questionnaire with both open- and close-ended questions. Data were also collected by field observations on the activities of the committees. Secondary data from the minutes of meetings, work plans, and registers were also analyzed. Data were processed and analyzed using the EPI Info statistical software package.

Results: Over 70% of the respondents could mention the most important purpose of formation of the zonal committees, i.e. the zonal committee is intended to strengthen promotional activities, establish coordination among the government and non-governmental organizations, and establish a regular exchange of service statistics and information on health services at the zone level. The main health problems perceived by the respondents were lack of safe drinking water and sanitation facilities, unplanned growth of slums, improper garbage cleaning and drainage system, mosquitoes, and environmental pollution. About 67% of the scheduled zonal committee meetings were held, and on an average, 62% of the members attended the meetings. It revealed from the study that the committees contributed in coordinating implementation of national measles and neonatal tetanus campaign and National Immunization Day at the local level. Important activities were initiated and were partially implemented. These include reorganization of service-delivery points to bring services close to the slum dwellers and minimizing gaps and overlaps, installation of incinerators for clinical waste disposal, and formation of the ward committees.

Conclusion: The zonal committee was found to be an effective forum for mobilizing support from service providers and community leaders in planning and coordinating delivery of urban health services.

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Improving Aailability of and Access to an Essential Health Services Package in Urban Dhaka, Bangladesh

SU Alamgir, Cristobal Tuñón, SE Arifeen, AH Baqui, MA Bhuiyan, and J Uddin

Objective: Improve access to and the availability of essential health services, and also improve the use of individual clinics and overall use at the zone level by reorganizing the government and non-governmental organization (NGO) facilities. The urban primary healthcare (PHC) facilities are managed by multiple organizations, i.e. two directorates of the Government; NGOs; Dhaka City Corporation; Ministry of Local Government, Rural Development and Cooperatives; and the for-profit commercial sector. In the government and NGO facilities, clients rarely obtain a combination of essential services. The distribution of these facilities is not optimal and results in some areas with "excess" of facilities often providing similar services while other areas are underserved, resulting in less access to service, creation of missed opportunities, and increase in cost of service provision and use.

Methodology: The Urban Extension Project (UEP) of ICDDR,B implemented an intervention in two zones of the Dhaka City Corporation (DCC). The UEP developed a methodology as a part of this intervention for reorganizing the government and NGO facilities in the two zones. The methodology was based on the locally available data (inventory, mapping, service use) and participatory workshops involving managers and decision-makers of the government and NGO facilities. The workshop for Zone 3 was held in August 1996 and that of Zone 8 in November 1996. The workshops resulted in specific ward-wise redistribution plans which had four elements: (1) relocation of certain facilities, (2) bringing facilities and/or services preferably under one roof, (3) expansion of the range of services, and (4) improving referral among the neighbouring facilities. A mid-term evaluation was recently conducted.

Results: The findings of the evaluation indicate that reorganization is possible using a participatory planning methodology. Six of the 14 specific recommendations for changes have already been implemented in Zone 3, whereas 7 of the 14 specific recommendations have so far been implemented in Zone 8. Improvement in service use was also observed, which indicates the possibility of improving the use of facilities at the clinic and the zone level after the reorganization of facilities.

Conclusion: Redistribution plans of the PHC facilities based on the intervention methodology can significantly reduce inadequacies, gaps and overlaps of essential services, and improve access to and the availability of essential services in the urban areas.

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Health Promotion Campaigns and Urban Women in Bangladesh

Cristobal Tuñón, Md. Abdul Quaiyum, Nazma Begum, and Selina Amin

Objective: Assess differences in awareness about ongoing health promotion campaigns among urban women.

Methodology: Sociodemographic information and data on awareness of recent health promotion campaigns were collected from respondents in the stratified multistage cluster sample of households from the Panel Survey based on the population in the Bakshi Bazar, Lalbagh and Rayer Bazar areas of Dhaka city. Samples of 525 and 2,636 women with children aged less than five years were interviewed in slum and non-slum areas after the National Immunisation Days (NIDs) in June 1995 and in June 1996 respectively. Subsequently in March 1997, a random sample of 601 women from the same panel was interviewed after the launching of a national campaign to promote the use of MCH-FP clinic.

Results: Over 60% knowledge of the slum women, in contrast to only 23% of the non-slum women, said that they had never seen or heard about the logo of the clinic promotion campaign at that stage. There were also significant differences among the slum and non-slum women in terms of their awareness of the national immunization day campaign. While 44% of the women in the non-slum areas linked the NID campaigns with polio prevention and erradication, only 25% made this link in the slum areas. Nearly 90% of those who were aware of the clinic promotion campaign had acquired this information from TV. In contrast, less than a third of the slum dwellers had heard about NIDs from TV messages. The main source of information about NIDs among slum dwellers are clinic outreach staff delivering domiciliary services, friends, relatives, and neighbours.

Conclusion: The majority of the women interviewed were aware of both campaigns. The most established immunization campaigns were better known by both slum and non-slum dwellers. As expected, the proportion of women who knew about the clinic promotion campaigns was greater in the non-slum areas. Nevertheless, most slum and non-slum dwellers mentioned TV as their source of information about the clinic promotion campaign. The findings suggest links between awareness and access to TV. The more mature immunisation campaigns revealed contrasting findings about the sources of information of the slum and non-slum women. Although television was the most frequent source of information mentioned by all respondents, there were significant differences among the slum and non-slum dwellers. The findings suggest the use of combined channels of communication in health promotion campaigns to reach slum women.

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Accessible STD Care for Street-based Sex Workers of Dhaka City: Potentials and Experience of Partnership

Swarup Sarkar[1], Yasmin Ahmed[2], Ziya Uddin[1], Sushena Reza[1], Enamul Haque[1], Fazlul Karim[1], and Maurice Bloem[1]

Objective: Examine the effectiveness of the partnership between the SHAKTI-Project, CARE-Bangladesh, and the Marie Stopes Clinic Society (MSCS) in providing reproductive healthcare, including care for sexually transmitted diseases (STD) to street-based sex workers of Dhaka city through satellite clinics.

Methodology: The MSCS and the SHAKTI-Project agreed that the SHAKTI would undertake the outreach activity for the street-based sex workers, while the MSCS would provide necessary clinical services. Four satellite clinics were planned jointly. SHAKTI will provide space in their storefront, furniture, and fixtures. The sex workers' peer educator of SHAKTI will refer the suspected cases to the satellite clinic. The MSCS will provide staff and drugs at a subsidized cost. Preliminary data collected from three satellite clinics have been examined. The mandate of the two projects was examined. In-depth interviews and focus-group discussions were held with beneficiaries as well as the members of the staff of both the projects. Analysis of data is in progress.

Results: The preliminary information and observations showed that a transparent partnership worked toward the avoidance of duplicate use of resources, and facilitated the use of one's area of expertise and a faster implementation of activities. In addition, it helped create a congenial environment between the two projects, and stimulated the formation of broader teams in the field of STD/AIDS prevention.

Conclusion: For faster and economic implementation of activities, different organizations can work together to reduce duplication of work, avoid the wastage of resources, and strengthen the ultimate coordination between different organizations working in the same field.

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An Assessment of Risk Perceptions of STD/HIV/AIDS and Presence of Risk Behaviours among Street-based Sex Workers in Dhaka City

Swarup Sarkar[1], Ziya Uddin1, Nazrul Islam[2], Sushena Reza[1], Enamul Haque[1], Fazlul Karim[1], and Maurice Bloem[1]

Objective: Assess the risk of behaviour and perception of risk regarding STD/HIV/AIDS by the street-based sex workers in the Dhaka city.

Methodology: Sex workers were recruited to assist in accessing to other street-based sex workers, identifying their locations, estimating their size and to act as guides. A capture-recapture technique was used for estimating the size of street-based sex workers. Of the 15 thanas of Dhaka city, 13 were selected. Both qualitative and quantitative methods were used as data collection techniques. Extensive ethnographic observations were made, and in-depth interview of the key informants and focus-group discussions were held. A quantitative survey was conducted with 230 sex workers recruited from the street. A strict quality control procedure was adopted to ensure consistency and minimize errors.

Results: Thirty-four percent of the women reported having more than one sex partner in the last 24 hours. More than half (56%) had sex during menstruation. One-sixth reported ever practising anal sex. Sixty-eight percent had sex more than 3 times per day. Only 50% reported that they occasionally used condoms. An insignificant proportion (2%) reported consistent condom use, and 34% used less than 50% of the time. Forty-three percent and 57% had knowledge about sexual transmission of STDs and AIDS respectively, while 76% and 44% were sure that had condoms been used they would not have got or transmitted STDs and AIDS respectively.

Discussion: Risk of behaviour exists among the street sex workers, and risk perceptions are not adequate. HIV/AIDS and STD prevention programme targeting high-risk behaviour groups should include strategies to raise awareness through regular contact using peers and should be supplemented by provision of means of behaviour change, endorsement by the target group and the society. To promote safer behaviour, the intervention should work to create an environment which will facilitate the behavioural change among the street-based sex workers.

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Impact of Community-based Intervention on Diarrhoea through Oral Rehydration Therapy on Hospitalization of Children Aged Less Than Five Years in Rural Bangladesh

DS Alam, M Yunus, A Rahman, HR Chowdhury, and J Patrick Vaughan

Objective: Examine the impact of community-based intervention on diarrhoea through oral rehydration therapy (ORT) on hospitalization of children aged less than five years in rural Bangladesh.

Methodology: The Maternal and Child Health and Family Planning (MCH-FP) programme has been involved in community-based intervention on diarrhoea through active promotion of ORT, covering nearly half of the ICDDR,B study area in Matlab. In the other half (the comparison area), ORS is available with the community health workers on demand. Children were considered genuinely eligible for hospitalization if they met any of the following criteria: (1) severe dehydration, (2) moderate dehydration with vomiting, (3) bloody stool, or (4) diarrhoea for more than three days. Data from the Matlab Diarrhoea Treatment Centre (DTC) were analyzed in relation to the area (MCH-FP/comparison) and eligibility criteria for hospitalization.

Results: Data on 1,760 (95% of the total admissions) children (61% male) with no (8%), mild (75%), moderate (14%), and severe (3%) dehydration at admission were included in the analysis. Age, sex, nutritional status, and the type of diarrhoea of the admitted children were comparable between the study areas. About 12% of the children presented with moderate dehydration with vomiting, 18% with bloody diarrhoea, 24% with diarrhoea for more than three days, and 10% of the patients required intravenous fluid within 24 hours of admission. Forty-seven percent of the children did not meet any of the criteria for hospitalization, and that proportion was similar in both the areas. Overall, 66% of the children took ORS at home, with a significantly higher proportion of the children from the MCH-FP area compared to the comparison area (72% vs. 64%, $c2 = 25$, $p<0.01$). The volume of ORS intake was significantly higher in the MCH-FP area compared to that of the comparison area (mean ±SD 2.23±2.3) vs. 1.72±2.1, $p<0.01$).

Conclusion: The findings of the study indicate that the home use of ORS is greater and significantly influenced in the areas served by MCH-FP. Nearly a half of the children had no valid criterion for admission. This suggests that mothers in the study areas are yet to be convinced that uncomplicated diarrhoea can be effectively managed at home through ORT. Further intensification and modification of the educational programmes are required.

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), GPO Box 128, Dhaka 1000, Bangladesh

Molecular Analysis of Shigella dysenteriae Type 1 Strains by Using Pulsed-Field Gel Electrophoresis

KA Talukder and MJ Albert

Objective: Determine the use of pulsed-field gel electrophoresis (PFGE) in molecular typing of Shigella dysenteriae type 1 strains from sporadic outbreaks and epidemic periods from different geographical location of the world.

Methodology: Genomic DNA of S. dysenteriae type 1 strains was analyzed using PFGE. The use of PFGE in a Not-I-digested DNA fragments clearly distinguished isolates involved in the epidemic from the non-epidemic strains. Genomic DNA was digested by Not-I restriction enzyme and the fragments separated using the contour-clamped homogenous electric field method on a CHEF-DRII system on 1% agarose.

Results: One hundred thirteen isolates of S. dysenteriae type 1 (18 from epidemic and 95 from sporadic outbreaks) were typed by the PFGE method. These isolates were classified into 8 PFGE type A to H, comprising 1 to 22 patterns, and 25 patterns were identified in total. The major groups consisted of A and B. Type A was predominantly detected (in 51 of 56 strains) among the strains isolated in Bangladesh, while type B was rare (in 2 of 56 strains) and isolated only from Rangpur during the epidemic period in 1984. Among the very recent isolates (1995-1997) in Bangladesh, there were no type A pattern 2 isolates. This pattern was present among the epidemic strains isolated in Rangpur and in the Hooghly district of West Bengal, in 1984.

Conclusion: The results of the analysis suggest that a clonal relationship existed between these strains during the epidemic period in Bangladesh and West Bengal in 1984. Thus, the PFGE technique could be used as an epidemiologic tool for identifying epidemic-associated strains as well as for molecular subtyping of epidemiologically unrelated strains.

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Clonal Groups of Enteropathogenic Escherichia coli Isolated in Case-Control Studies on Diarrhoea in Bangladesh

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Objective: Investigate the clonal status of enteropathogenic Escherichia coli (EPEC) strains isolated from case-control studies in Bangladesh.

Methodology: Eighty EPEC isolates from children with diarrhoea and 14 isolates from matched healthy controls from two case-control studies were analyzed. The first study, conducted during 1991-1992, comprised 451 children aged up to five years with diarrhoea and 602 matched control children without diarrhoea. The second study, conducted during 1993-1994, comprised 546 children with diarrhoea and 215 matched healthy children recruited from the same neighbourhood. The EPEC isolates were characterized by serogrouping, enterobacterial repetitive intergenic consensus (ERIC) sequence PCR, and biochemical fingerprinting method (the automated phene plate or PhP system).

Results: Twelve EPEC serogroups were found with O114 (n=19) and O127 (n=23) being the dominant serogroups. Most strains of O114 serogroup belonged to the same PhP and PCR types. Strains of O127 serogroup contained those producing cytolethal distending toxin CDT (n=16) and those which did not (n= 7). Both were found among the patients and the controls. The results of PCR and PhP typing showed that the CDT-positive strains belonged to the same clonal group and were related to one of the two PhP/PCR types of CDT-negative O127 strains. Thirty-one O-non-typable EPEC strains and 21 strains of other less prevalent serogroups belonged to diverse Ph/PCR types. Furthermore, they did not show any similarity to the strains of the two major serogroups: O114 and O127.

Conclusion: Recent case-control studies in Bangladesh showed a high prevalence of EPEC strains associated with childhood diarrhoea. The results of the present study suggest that single clonal groups of EPEC strains belonging to serogroup O114 and O127 are predominantly associated with childhood diarrhoea in Bangladesh.

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Economic Benefits of Diabetes Control in Bangladesh

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Objective: Estimate the direct medical-care costs associated with diabetes, and indicate how the medical-care costs increased with the progression of the disease from the initial stage.

Methodology: This study was based on the data derived from the "Guidebook" of diabetic patients registered at the BIRDEM hospital. The sample consists of the registered patients in the hospital since December 1985. The surveillance collected two types of information: baseline information on patient's demographic characteristics and follow-up information of revisits later on. The baseline data also report health-related information at the first visit. The follow-up data include height, weight, blood pressure, blood sugar level, treatment recommended, and complications observed for patients. Using the blood sugar level cut-off points, the severity of diabetes was defined for the patients, and all patients were grouped into three severity categories. The use of medical services by different categories of diabetic patients was compared to estimate the effect of increased blood sugar levels on the medical-care costs. An attempt was also made to identify the costs not related to diabetes to estimate the economic benefits of preventing the disease.

Results: Although the prevalence of diabetes is low in Bangladesh, it is already the 10th most expensive disease in terms of total healthcare cost allocated for an illness. Diabetes imposes significant additional costs on individuals, and the cost of diabetes tends to increase with time since onset. The severity of diabetes is also associated with increased direct medical-care cost implying that even a modest control of diabetes reduces the direct medical cost for patients.

Conclusion: The cost of diabetes is likely to increase at a rapid rate in the future due to its expected higher prevalence and the increased cost of medical interventions. An early diagnosis and control should significantly lower the future costs of medical care associated with diabetes. The Bangladesh health sector should consider the incorporation of diabetes screening as an important public health intervention. However, the degree of financial subsidy needed for this purpose should be determined by carefully examining its prevalence rate among the different socioeconomic groups.

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Evaluating the Alternative Strategies for Hepatitis A and B Vaccination in Bangladesh: An Economic Analysis

Disha Ali and M Mahmud Khan

Objective: Compare the costs and benefits of adopting alternative strategies for hepatitis A and B vaccination in Bangladesh to determine the most cost-effective approach of preventing the infection.

Methodology: Three alternative strategies that have been evaluated are: (a) vaccinate all individuals in the society without testing, (b) vaccinate only those who are not immune to hepatitis A and B, and (c) vaccinate the non-immune cases among the high-risk population. A simple economic model, explaining the costs and benefits associated with screening and vaccination, was developed to examine the alternatives. The model predicts the costs and benefits at different levels of prevalence, cost of identification of the high-risk groups, sensitivity and specificity of the tests, the direct and indirect economic cost of infections, and effectiveness of the vaccinations in Bangladesh context. Costs of tests and vaccinations were obtained through the private provider survey in Dhaka, and the direct medical benefits of preventing the infections were derived from the expert opinion surveys. The indirect economic costs were estimated by considering the degree and duration of morbidity, and case-specific fatality information was obtained either from literature or through expert opinion surveys. The costing implicitly assumed that successive three doses of immunization would provide life-long immunity to an individual.

Results: Hepatitis A vaccination, due to its low direct and indirect cost, turned out to be a low-priority intervention for Bangladesh. Hepatitis B-screening tests and vaccinations are relatively costly. However, the benefit of preventing hepatitis B is also high. Despite the high cost of screening and vaccination, the prevention of hepatitis B remains a highly desirable intervention for Bangladesh.

Conclusion: Immunization against hepatitis B appears beneficial to the society at a reasonable range of prevalence. Economic benefits of preventing hepatitis B are high, but the intervention appears too costly to be funded through the public sector alone. Alternative methods of funding hepatitis B vaccination (HBV) should be considered. Therefore, in the short run, a mass campaign on HBV will not be feasible due to the financial resource constraints.

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), GPO Box 128, Dhaka 1000, Bangladesh

Evaluation of Rice-based Reduced Osmolarity Oral Rehydration Solution in Children with Severe Persistent Diarrhoea

SA Sarkar[1], NH Alam[1], D Mahalanabis[2], and GJ Fuchs[1]

Objective: Evaluate and compare the efficacy of a standard (WHO) oral rehydration solution (ORS) and a rice-based reduced osmolarity ORS in children with severe persistent diarrhoea. Persistent diarrhoea accounts for 7-21% of all childhood diarrhoeal episodes and 32-62% of all diarrhoea-related deaths in developing countries. Fluid and electrolyte balance is an important part in the management of persistent diarrhoea.

Methodology: This randomized controlled clinical trial was conducted on 64 children with severe persistent diarrhoea (duration >14 days, stool output >80 mL.kg.d). After a one-day observation period to confirm the diagnosis and severity, they were assigned to either standard WHO-ORS (sodium 90, potassium 20, chloride 80, citrate 10, glucose 111, osmolarity 311; all in mmo1/L or to a rice-based reduced osmolarity ORS (rice powder 33 g; sodium 60, potassium 14, chloride 57, citrate 6, osmolarity 137; all in mmol/L) for replacement of ongoing stool loss for seven days. Stool output and frequency, ORS, and food intakes were monitored daily. Serum electrolytes were also determined on study day 3 and 7. Daily and total (day 1-7) food intakes were comparable among the study groups. The stool volume (mL.kg.d) mean±SEM) was significantly less in infants receiving rice-based reduced osmolality ORS than the WHO-ORS on day 4 (86±11 vs. 44±28, p=0.05), 5 (73±10 vs. 144± 31, p=0.04), 6 (60±9 vs. 139±31 p=0.02), 7(59±11 vs. 120±59, p=0.04), as well as for the entire (1-7 d) study period (523±54 vs. 932±177, p=0.04). The median stool frequency (number/d) during the entire study period in children receiving reduced osmolality ORS was also significantly less than those in the WHO-ORS group (70±5 vs. 92±10, p=0.05). Furthermore, the children belonging to reduced osmolarity ORS required less amount of ORS in total compared to the WHO-ORS groups. Children in both the groups maintained normal serum electrolytes as determined on day 3 and 7.

Conclusion: It is concluded that rice-based reduced osmolarity ORS is more effective than WHO-ORS for replacement and reducing ongoing stool loss, and therefore, may be useful in the management of children with persistent diarrhoea.

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Intestinal Transport of Different Electrolyte Solutions Across Small Intestine of Rabbit in vivo

S Islam, A Rahman, GJ Fuchs, AK Chowdhury, MA Wahed, and GH Rabbani

Objective: Determine the relative effects of different electrolyte solutions in optimizing intestinal absorption of water and electrolytes.

Methodology: The rice electrolyte solutions, called CeraLyte 90 contained 40 g rice and 90 mM sodium, and CeraLyte 70 contained 40 g rice and 70 mM sodium per litre of solution. Different concentrations of carboxymethyl cellulose CMC (10.0, 5.0, and 2.5 g/L) were added to standard oral rehydration solutions (Std ORS) to increase its viscosity. Fifty-centimetre small intestinal segments were perfused with different electrolyte solutions, containing 6 g/L polyethylene glycol (MW 4000) as a non-absorbable marker.

Results: Mean±SE of water and sodium ion absorption with standard ORS vs. CeraLyte 90 was 1.53±0.11 vs. 1.59±0.09 mL/min/g of dry intestine (NS) and 0.24±0.21 vs. 0.58±0.09 mM/min/g of dry intestine respectively. The absorption of potassium and chloride ions was not different when compared among Std ORS, CeraLyte 90, and CeraLyte 70. Secretion of sodium ion was found when the rabbit was perfused with the electrolyte solutions, containing different concentrations of CMC. Water absorption and sodium ion secretion from electrolyte solution with 5 g/L CMC was significantly different when compared with the solution with 10 g/L CMC (p=0.01 and 0.0004).

Conclusion: It is concluded that CeraLyte 90 and CeraLyte 70 have no additional absorption-promoting effect on water and electrolytes transport. Increasing viscosity of electrolyte solution may cause stimulation of secretion across the small intestine of rabbit.

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), GPO Box 128, Dhaka 1000, Bangladesh

Evaluation of the Plant Extract (Hirtacin) in a Rabbit Model of Shigellosis

Qazi Khaleda Rahman[1], Chowdhury Rafiqul Ahsan[1], Kamaluddin Ahmed[1], and GH Rabbani[2]

Objective: Evaluate the efficacy of the plant extract hirtacin in a rabbit model of shigellosis.

Methodology: Cecal-ligated rabbits were inoculated with Shigella flexneri 2a, and after 24 hours, three groups of rabbits were treated with hirtacin, ciprofloxacin, and placebo respectively for five days.

Results: The plant extract hirtacin and ciprofloxacin significantly reduced myeloperoxidase (MPO) level in plasma which serves as a marker of inflammation, while the MPO level was still high in the placebo group after five days of treatment. Also the clinical features of both the drug-treated groups showed complete remission from dysentery, while dysenteric symptoms still persisted in the placebo group after five days.

Conclusion: It is concluded that the plant extract hirtacin has a potent therapeutic activity in shigellosis.

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"To evaluate the impact of *Cryptosporidium* infection on diarrheal disease burden and nutrition status, a nested case-control study was done among children who were followed from birth in Fortaleza, Brazil. The diarrhea history and growth records of 43 children with a symptomatic diarrhea episode of cryptosporidiosis (case-children) were compared with those of 43 age-matched controls with no history of cryptosporidiosis. After *Cryptosporidium* infection, case children =1 year old experienced an excessive and protracted (nearly 2 years) diarrheal disease burden. Case-children =1 year old with no history of diarrhea prior to their *Cryptosporidium* infection also experienced a subsequent increased diarrheal disease burden with an associated decline in growth. Control subjects experienced no change in their diarrhea burden over time. This study suggests that an episode of symptomatic *Cryptosporidium* infection in children =1 year of age is a marker for increased diarrhea morbidity."

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"Gastrointestinal food allergy still poses a challenge to the clinician because of its variable symptomatology and lack of reliable diagnostic tests. Its prevalence is estimated at 2~5%, higher in children than in older age-groups. Allergy to food usually diminishes with advancing age. Although a wide variety of foods can cause allergic reactions, cow's milk is the most common cause of food allergy in infants and young children. Depending upon the speed of onset of symptoms, immediate and delayed types of food allergy have been described. Gastrointestinal symptoms in food allergy have been explained by alterations in transport across the intestinal wall (increased secretory and/or decreased absorptive functions), increased permeability, and motility of the intestine. The exact pathogenesis of food allergy is still not clear. However, immediate type of food allergy is believed to be mediated by type I hypersensitivity reaction, involving mast cells and food-specific IgE antibodies. The diagnosis of food allergy is based upon a favorable response to an elimination diet and a response to a challenge with the suspected food. The condition is treated by eliminating the allergenic food from diet for as long as 9-12 months in case of cow's milk allergy. While exclusive breast-feeding for the initial four months or more reduces the chances of development of food allergy, the role of diet restrictions in the mother in reducing the incidence of food allergy in the infant is controversial. Data on food allergy from developing countries are limited. This may be due to lack of diagnosis or less attention given to the condition relative to other diseases including infectious diarrheas and acute respiratory infections. The role of cow's milk allergy in the pathogenesis of persistent diarrhoea, a major problem in the developing world, remains speculative. Frequent intestinal infections and reduced secretory IgA, which are associated with malnutrition, alter intestinal permeability and result in an increased uptake of food antigens. The increased antigenic load combined with factors such as an atopic predisposition may initiate an abnormal mucosal immune response resulting in chronic enteropathy."

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"Awareness and knowledge of oral rehydration therapy (ORT) and preparation abilities of salt-sugar solution (SSS) were investigated by means of focus group discussions and complemented by a structured questionnaire survey of mothers in rural and urban areas of north-eastern Nigeria. ORT awareness was high with some intra-regional variations. Perception of ORT function was, however, grossly unrealistic, with a third

to four-fifth of mothers expecting ORT to stop diarrhoea. At least one quarter of mothers lacked adequate SSS preparation abilities and the materials and ingredients required for its preparation. Re-evaluation of the content and method of imparting health education messages in ORT promotion is recommended. Such messages should emphasise the function of ORT. It is also recommended that standardised cups for water, salt and sugar measurements be provided to households as a ready means of ensuring the correct preparation of SSS in the home-based management of diarrhoea."

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"The study was aimed at learning about home management of infant and young child diarrhoea in Lesotho. Focus groups and individual interviews were conducted with mothers, grandmothers and nurses during two phases of field work in three geographically different locations. It was found that home management of diarrhoea traditionally had emphasised feeding. While medical advice in the past recommended that, except for breast feeding, food should be withheld during diarrhoea, mothers, grandmothers and even nurses had been reluctant to follow this advice. Mothers and grandmothers saw feeding during diarrhoea as so essential that they would make special efforts to feed a child with diarrhoea. Since most foods contain protein and carbohydrates which stimulate intestinal fluid absorption, feeding during diarrhoea, besides maintaining nutrition, will help maintain hydration. When such beneficial feeding practices are protected and supported there is less need to promote new practices and less risk of producing harmful effects."

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"**Background:** The etiology of persistent diarrhea in children is multifactorial. The objective of the current study was to ascertain the role of microorganisms in the etilogy and pathogenesis of persistent diarrhea in a group of children in Bangladesh. **Methods:** Enteric pathogens and total aerobic microflora were studied in the duodenal aspirates of 100 children with persistent diarrhea and compared with those in aspirates of 30 children with acute diarrhea, and those in aspirates of 15 healthy control children. The enteric pathogens in the stools of these children and in stools of an additional 38 patients with persistent diarrhea and 12 with acute diarrhea were also studied. **Results:** Approximately two thirds of the patients with acute diarrhea and

persistent diarrhea, and half of the control subjects had more than 10^5 organisms per milliliter of duodenal fluid. Significantly, more patients with persistent diarrhea had a greater variety of flora than did patients with acute diarrhea and control subjects. The predominant organisms in patients with acute diarrhea and in those with persistent diarrhea were gram-negative rods, whereas those in control subjects were gram-positive cocci. Significantly more acute diarrhea patients and persistent diarrhea patients had enteric pathogens isolated from stool than did control subjects. Diarrheagenic *Escherichia coli*, as a whole, were present in significantly more persistent diarrhea patients than in acute diarrhea patients and control subjects. Among diarrheagenic *E. coli*, enteroaggregative *E. coli* were significantly associated only with persistent diarrhea. Other organisms significantly associated with persistent diarrhea were *Aeromonas* spp. and *Klebsiella* spp. Some patients in the acute diarrhea and the persistent diarrhea groups had the same pathogens isolated from both the duodenal fluid and stool. **Conclusions:** In accordance with results of other studies, an association between enteroaggregative *E. coli* and persistent diarrhea was found in the present study. This suggests that therapy directed against enteroaggregative *E. coli* can be evaluated for management of some cases of persistent diarrhea."

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"We have investigated the capacity of live attenuated *Shigella flexneri* strains to act as vectors for the induction of local and systemic antibody responses against heterologous epitopes. The *S. flexneri* IpaC antigen was selected as a carrier protein into which the C3 neutralizing epitope of the poliovirus VPI protein was inserted in eight sites distributed along IpaC. The resulting IpaC-C3 hybrid proteins were expressed from recombinant plasmids in the *S. flexneri* 2a vaccine candidate, SC602. Their production was similar to that of wild-type IpaC. All of the hybrid proteins but one were secreted as efficiently as wild-type IpaC. Immunization of mice with each of the recombinant SC602 derivatives reveals that one construct is able to induce serum and local anti-C3 antibodies, showing that at least one permissive site of insertion within IpaC can be defined. Furthermore, mouse-to-mouse variability in the anti-C3 response indicates that the amount of hybrid proteins produced in the host by SC602 should be improved for optimal use of *S. flexneri* live attenuated strains as mucosal vectors for foreign epitopes."

012 Basu A, Mukhopadhyay AK, Chakrabarti AK, Niyogi SK, Saha MR, Yamasaki S, Takeda Y, Nair GB. Comparison between the multiplex PCR, sensitivity to biotype specific phages & polymyxin B for biotyping of *Vibrio cholerae* O1. Indian J Med Res 1997 Dec;106:491-3. 10 ref, Eng. Department of Microbiology, National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

013 Bhandari N, Bahl R, Sazawal S, Bhan MK. Breast-feeding status alters the effect of vitamin A treatment during acute diarrhea in children. J Nutr 1997 Jan;127(1):59-63. 29 ref, Eng. Division of Gastroenterology and Nutrition, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110029, India

"Vitamin A administration in children reduces the incidence of severe diarrhea during the subsequent few months. We therefore examined the effect of treatment with vitamin A during acute diarrhea on the episode duration and severity. In a double-blind controlled field trial, 900 children 1 to 5 y of age with acute diarrhea of = 7 d duration were randomly assigned to receive vitamin A (60 mg) or a placebo. Children followed up at home every alternate day until they recovered from the diarrheal episode. In all study children, those treated with vitamin A had a significantly lower risk of persistent diarrhea [odds ratio (OR) 0.30, 95% confidence interval (CI) 0.07-0.97], but there was no effect on the mean diarrheal duration or the mean stool frequency. In the subgroup of children who were not breast-fed, the mean diarrheal duration [ratio of geometric means (GM) 0.84, 95% CI 0.72-0.97], mean number of stools passed after the intervention (ratio of GM 0.73, 95% Cl 0.56-0.95), the proportion of episodes lasting =14 d (P=0.002) and the percentage of children who passed watery stools on any study day (OR 0.40, 95% Cl 0.21-0.77) were significantly lower in those treated with vitamin A. We conclude that administration of vitamin A during acute diarrhea may reduce the severity of the episode and the risk of persistent diarrhea in non-breast-fed children. Similar benefit was not seen in breast-fed children."

014 Bhattacharya MK, Bhattacharya SK, Dutta D, Deb AK, Deb M, Dutta A, Choudhury AS, Nair GB, Mahalanabis D. Efficacy of oral hyposmolar glucose-based and rice-based oral rehydration salt solutions in the treatment of cholera in adults. Scand J Gastroenterol 1998 Feb;33(2):159-63. 17 ref, Eng. Clinical Division, National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

"*Background:* Recent animal experiments and clinical trials have shown that both osmolarity and rice as the organic components are important factors for net intestinal absorption of an oral rehydration salt solution. *Methods:* In a controlled clinical trial 123 male adult patients with severe cholera, after initial rehydration with intravenous Ringer's lactate solution, were randomly assigned to receive one of the four oral rehydration salt solutions: WHO ORS, ORS containing 70 mmol/l Na^+ and 16.2 g/l glucose, rice ORS containing 50 g/l rice and 90 mmol/l Na^+, and rice ORS containing 50 g/l rice and 70 mmol/l Na^+. All patients received 300 mg of doxycycline as a single dose. *Results:* Patients who received rice-low-sodium ORS subsequently had lower ($P < 0.05$) stool output, ORS consumption, and diarrhoea duration than the other three ORS groups. *Conclusions:* We conclude that rice-based low-sodium ORS is superior for treating adult cholera."

015 Bhutta ZA, Nizami SQ, Thobani S, Issani Z. Risk factors for mortality among hospitalized children with persistent diarrhoea in Pakistan. J Trop Pediatr 1997 Dec;43(6):330-6. 35 ref, Eng. Department of Paediatrics, The Aga Khan University Medical Center, Stadium Road, PO Box 3500, Pakistan

"We evaluated factors associated with mortality among a cohort of malnourished children with persistent diarrhoea (PD) admitted for nutritional rehabilitation with a defined rice-lentil (Khitchri) and yoghurt diet. Of 302 children consecutively admitted with PD, 13 (4 per cent) died, mostly (62 per cent) within 72 h of admission. Univariate analysis of risk factors at admission associated with mortality indicated significantly increased risk of death with severe stunting [relative risk (RR) 3.1, 95 per cent confidence interval (Cl) 1.1-9.0], hypo-albuminaemia (RR 4.3, 95 per cent CI 1.5-12.3), stool frequency >12/day (RR 6.0, 96 per cent CI 2.0-17.6), stool volume >100 g/kg/day (RR 10.7, 95 per cent CI 3.0-37.6) and severe dehydration (RR 7.5, 95 per cent CI 2.6-21.8). Children who died also had comparatively shorter duration of diarrhoea at admission, and were also associated with higher rate of bacteremia at admission (Fisher's exact test $P < 0.01$). The logistic regression model evaluating multivariate risk of mortality identified weight-for-age z-score and sepsis as significant risk factors. Our data suggest that severe malnutrition and sepsis are associated with significantly increased risk of mortality in children with PD. Stringent screening for infections and recognition of subgroups with severe malnutrition and severe diarrhoea may improve screening and case management strategies for this disorder."

016 Bini EJ, Weinshel EH. Endoscopic evaluation of chronic human immunodeficiency virus-related diarrhea: is colonoscopy superior to flexible sigmoidoscopy? Am J Gastroenterol 1998 Jan;93(1):56-60. 24 ref, Eng. Division of Gastroenterology, Department of Veterans Affairs Medical Center, 423 East 23rd Street, New York, NY 10010, USA

"*Objective:* In patients with chronic human immunodeficiency virus (HIV)-related diarrhea undergoing lower endoscopy, the decision to perform flexible sigmoidoscopy or colonoscopy is controversial. The purpose of this study is twofold: 1) to evaluate the diagnostic yield of colonoscopy in a large group of patients with chronic HIV-related diarrhea and negative stool studies, and 2) to determine whether colonoscopy is superior to flexible sigmoidoscopy in this setting. *Methods:* All HIV-infected patients with chronic diarrhea who were referred for diagnostic colonoscopy at Bellevue Hospital Center between January 1992 and December 1996 were identified. Patient charts, pathology reports, and endoscopy records were reviewed. *Results:* During the 5-yr study period, 317 consecutive patients with chronic unexplained diarrhea undergoing colonoscopy were identified. A potential cause of diarrhea was found in 116 patients (36.6%). Cytomegalovirus was the most common pathogen detected (24%). The yield of colonoscopy was significantly higher in patients with a CD4 count of <100 cells/mm^3 than in those with higher CD4 counts (44.8% vs 6.4%, $P<0.0001$). Thirty percent of pathogens and 75% lymphomas were identified only on biopsies taken from the proximal colon, well beyond the reach of the flexible sigmoidoscopy. Importantly, 94% of the pathogens that were found only in the proximal colon were organisms for which effective therapy is currently available. *Conclusions:* Colonoscopy is superior to

flexible sigmoidoscopy in HIV-infected patients with chronic unexplained diarrhea. If flexible sigmoidoscopy had been performed instead of colonoscopy, 30% of pathogens would have been missed and 75% of lymphomas would have escaped detection."

017 Boehm P, Nassimbeni G, Ventura A. Chronic non-specific diarrhoea in childhood: how often is it iatrogenic? Acta Paediatr 1998 Mar;87(3):268-71. 20 ref, Eng. Istituto di Clinica Pediatrica, Ospedale Infantile Burlo Garofolo, Via dell'Istria, 65, 34100 Trieste, Italy

"Epidemiological data, the appropriateness of the medical approach, the effectiveness of unrestricted diet and the subsequent course of chronic non-specific diarrhoea (CNSD) were evaluated in 20 children, mean age at diagnosis 4.7 y, and mean duration of diarrhoea 24 months. A mean of 2.4 previous hospital admissions and a mean of 2.5 diagnoses other than CNSD were recorded per child before admission. On admission, 14/20 were following an elimination diet and 8/20 had an inadequate caloric intake; 16/20 had a weight/height ratio below the 50th percentile. In all cases a normal diet prescribed during hospitalization improved the diarrhoea and increased weight. A telephone interview performed 5.6 y after discharge revealed that in 10/20 of the cases, the parents were disappointed with the unrestricted diet prescribed during hospitalization. Nevertheless they reported that CNSD stopped spontaneously in a mean time of 1.7 y."

018 Bonapace ES, Jr., Fisher RS. Constipation and diarrhea in pregnancy. Gastroenterol Clin North Am 1998 Mar;27(1):197-211. 77 ref, Eng. Gastroenterology Section, Department of Medicine, Temple University Hospital, 3401 North Broad Street, Philadelphia, PA 19140, USA

019 Buch NA, Bashir SA. Medical practitioners and their practices in acute diarrhea (brief report). Indian Pediatr 1997 Jun;34(6):530-4. 12 ref, Eng. Pediatric Specialist, King Fahd Hospital, Gizan, PO Box 204, Kingdom of Saudi Arabia

020 Buysse JM, Dunyak DS, Hartman AB, Venkatesan MM*. Identification and molecular characterization of a 27 kDa *Shigella flexneri* invasion plasmid antigen, IpaJ. Microbial Pathogen 1997 Dec;23(6):357-69. 52 ref, Eng. *Department of Enteric Infections, Bldg. 40, Room B020, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC 20307-5100, USA

021 Carlson SJ, Webster C, Craig RM*. Urinary recovery of lactulose compared to D-xylose absorption kinetics in HIV patients with diarrhea and weight loss. Dig Dis Sci 1997 Dec;42(12):2599-2602. 15 ref, Eng. *Gastroenterology Division, Searle 10541, Northwestern University Medical School, 303 E. Chicago, Chicago, Illinois 60611, USA

"Using a kinetic model of D-xylose absorption, we have previously shown that there is severely impaired absorption of D-xylose in HIV patients with diarrhea and weight loss. The absorptive defect is characterized by an increased rate constant for nonabsorptive loss of D-xylose, K_o, and a decreased absorptive rate constant, K_a, and is unrelated to histology or the presence of pathogens. It is not known if there is also abnormal paracellular transport in these patients. We have extended our observations in these patients by including a measurement of paracellular transport, lactulose absorption. Nine HIV patients with chronic diarrhea, weight loss, and no detectable intestinal pathogens, two healthy volunteers, and three non-HIV patients with chronic diarrhea (two functional and one with scleroderma) were enrolled. Of the nine HIV patients, six had diminished bioavailability of D-xylose, F (range: 19-52%, normal >70%), and elevated rate constant for nonabsorptive loss, K_o (range: 0.54-1.35/hr, normal <0.353/min). Four of the six also had decreased K_a (range: 0.09-0.36/hr, normal >0.634/min). Only one of these six had increased lactulose recovery (3.51%, normal <0.5%). Two of three patients with normal kinetic parameters of D-xylose absorption had increased lactulose urinary recovery (1.92%, 2.61%). In conclusion, lactulose absorption is increased in some patients with HIV-related diarrhea who have normal D-xylose absorption, suggesting a paracellular mechanism for diarrhea in some patients with AIDS enteropathy."

022 Casey TA, Herring CJ, Schneider RA, Bosworth BT, Whipp SC. Expression of heat-stable enterotoxin STb by adherent *Escherichia coli* is not sufficient to cause severe diarrhea in neonatal pigs (note). Infect Immun 1998 Mar;66(3):1270-2. 21 ref, Eng. USDA, Agricultural Research Service, National Animal Disease Center, PO Box 70, Ames, IA 50010, USA

023 Chatterjee S, Mondal AK, Begum NA, Roychoudhury S*, Das J. Ordered cloned DNA map of the genome of *Vibrio cholerae* 569B and localization of genetic markers. J Bacteriol 1998 Feb;180(4):901-8. 45 ref, Eng. *Biophysics Division, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Calcutta 700032, India

024 Cheasty T, Skinner JA, Rowe B, Threlfall EJ. Increasing incidence of antibiotic resistance in shigellas from humans in England and Wales: recommendations for therapy. Microbial Drug Resist 1998;4(1):57-60. 12 ref, Eng. Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

"Since 1983 the incidence of resistance to ampicillin in *Shigella dysenteriae, Sh. flexneri*, and *Sh. boydii* infections in England and Wales has increased from 42% to 65% and the incidence of resistance to trimethoprim, from 6% to 64%. Furthermore, of 1524 strains received in 1995-1996, 46% were resistant to both of these antimicrobials. For *Sh. sonnei* almost 50% of isolates were resistant to ampicillin or trimethoprim and 15% were resistant to both of these antimicrobials. These results demonstrate that if antibiotic therapy had been indicated for infections with *Sh. dysenteriae*, *Sh. flexneri*, and *Sh. boydii*, then treatment with either ampicillin or trimethoprim may have been ineffective in almost 50% of cases and for *Sh. sonnei*, in 15% of cases. It is concluded that if it is necessary to commence treatment before the results of laboratory-based sensitivity tests are available, the best options would be to use nalidixic acid for children and a fluoroquinolone antibiotic such as ciprofloxacin or ofloxacin, for adults."

025 Chen I, Finn TM, Yanqing L, Guoming Q, Rappuoli R, Pizza M. A recombinant live attenuated strain of *Vibrio cholerae* induces immunity against tetanus toxin and *Bordetella pertussis* tracheal colonization factor. Infect Immun 1998 Apr;66(4):1648-53. 37 ref, Eng. IRIS, Chiron Vaccines Immunobiological Research Institute in Siena, Via Fiorentina, 1, 53100 Siena, Italy

026 Chu Y-W, Houang ETS, Lyon DJ*, Ling JM, NG T-k, Cheng AFB. Antimicrobial resistance in *Shigella flexneri* and *Shigella sonnei* in Hong Kong, 1986 to 1995 (note). Antimicrob Agents Chermother 1998 Feb;42(2):440-3. 19 ref, Eng. *Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Ngan Shing St., Shatin, New Territories, Hong Kong

027 Chunder N, Bhattacharya SK, Biswas D, Niyogi SK, Kumar R. Isolation of a fluoroquinolone resistant *Shigella dysenteriae* 1 strain from Calcutta. Indian J Med Res 1997 Dec;106:494-6. 11 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

028 Cohen SH, Tang YJ, Hansen B, Silva J, Jr. Isolation of a toxin B-deficient mutant strain of *Clostridium difficile* in a case of recurrent *C. difficile*-associated diarrhea. Clin Infect Dis 1998 Feb;26(2):410-2. 10 ref, Eng. Division of Infectious Diseases, University of California-Davis Medical Center, 4301 X Street, Suite 2410, Sacramento, California 95817, USA

029 Collington GK, Booth IW, Knutton S. Rapid modulation of electrolyte transport in Caco-2 cell monolayers by enteropathogenic *Escherichia coli* (EPEC) infection. Gut 1998 Feb;42(2):200-7. 39 ref, Eng. Institute of Child Health, University of Birmingham, Francis Road, Birmingham B16 8ET, UK

"*Background and aims* - The pathophysiology of enteropathogenic *Escherichia coli* (EPEC) diarrhoea remains uncertain. EPEC adhere to enterocytes and transduce signals which produce a characteristic "attaching and effacing" (A/E) lesion in the brush border membrane. The present in vitro study was designed to determine whether signal transduction by EPEC also influences electrolyte transport. *Methods* - Caco-2 cell monolayers were rapidly infected with wild type EPEC strain E2348/69, or the signal transduction-defective mutant 14.2.1(1), and mounted in Ussing chambers. *Results* - Strain E2348/69 stimulated a rapid but transient increase in short circuit current (*Isc*) which coincided with A/E lesion formation; this *Isc* response was absent on infection with strain 14.2.1(1). While the initial rise in *Isc* induced by E2348/69 was partially (~35%) dependent on chloride, the remainder possibly represents an influx of sodium and amino acid(s) across the apical membrane. *Conclusions* - The study directly shows that, after initial adhesion, EPEC induce major alterations in host cell electrolyte transport. The observed *Isc* responses indicate a rapid modulation of electrolyte transport in Caco-2 cells by EPEC, including stimulation of chloride secretion, for which signal transduction to host cells is a prerequisite."

030 Covone MG, Brocchi M, Palla E, da Silveira WD, Rappuoli R, Galeotti CL*. Levels of expression and immunogenicity of attenuated *Salmonella enterica* serovar typhimurium strains expressing *Escherichia coli* mutant heat-labile enterotoxin. Infect Immun 1998 Jan;66(1):224-31. 40 ref, Eng. *Immunobiology Research Institute Siena, Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy

031 Dalla-costa LM, Irino K*, Rodrigues J, Rivera ING, Trabulsi LR. Characterisation of diarrhoeagenic *Escherichia coli* clones by ribotyping and ERIC-PCR. J Med Microbiol 1998 Mar;47(3):227-34. 26 ref, Eng. *Instituto Adolfo Lutz, Sao Paulo SP, Brazil

032 Davies-Adetugbo AA, Adetugbo K, Orewole Y, Fabiyi AK. Breast-feeding promotion in a diarrhoea programme in rural communities. J Diarrhoeal Dis Res 1997 Sep;15(3):161-6. 24 ref, Eng. College of Health Sciences, Obafemi Awolowo University, Ile Ife, Nigeria

"Breast-feeding promotion is an important intervention for the control of infant diarrhoea. This study assesses the impact of a breast-feeding counselling programme on the prevalence of exclusive breast feeding in rural communities in Nigeria. Mothers attending primary care facilities because their infants had acute diarrhoea were randomised into two groups. The study group (n=82) received individual, focused breast-feeding counselling, while the controls (n=79) had routine advice for diarrhoea. Both groups were monitored and followed with home visits to determine infant-feeding practices. The results showed marked increases in exclusive breast-feeding prevalence for the intervention group at day 7 (49% vs. 6% control; $p<0.0001$) and day 21 (46% vs. 8%; $p<0.0001$). Moreover, diarrhoea re-occurrence at day 21 was less in the intervention group (12%) than controls (18%). It is concluded that focused breast-feeding counselling can increase exclusive breast feeding and reduce the prevalence of diarrhoea in rural communities."

033 Desjeux J-F, Heyman M. The acute infectious diarrhoeas as diseases of the intestinal mucosa. J Diarrhoeal Dis Res 1997 Dec;15(4):224-31. 57 ref, Eng. Institut Nationale Sante & Recherche Medicale, Unit 290 75003 Paris, France

"One of the intriguing aspects of the acute infectious diarrhoeas is that, while resulting from an infection of the intestinal tract, they only last from a few hours to a few days. The study of the interactions between infectious agents and intestinal epithelium has allowed a better understanding of the cellular and molecular mechanisms that cause the sudden loss of water and electrolytes, the hallmark of acute diarrhoea. These interactions have also led to formulating oral rehydration solutions used worldwide now. They do not, however, allow understanding the short course of most intestinal infections. For that, one has to consider the intestinal epithelium as part of an anatomical and functional system that, including the many types of cells present in the lamina propria, constitutes the intestinal mucosa. Infectious agents interact with the whole of the mucosa, including the cells of the *lamina propria*. This leads, among other things, to a change in the functions of the epithelial cells and accelerates their turnover. The pathophysiology of the intestinal mucosa leads to better understand the short and benign course of most intestinal infections. It also leads to better understand the physiology of the intestinal mucosa, and the interactions between the body and its nutritional environment."

034 Desjeux J-F, Briend A, Butzner JD. Oral rehydration solution in the year 2000: pathophysiology, efficacy and effectiveness. Bailliere Clin Gastroenterol 1997 Sep;11(3):509-27. 88 ref, Eng. Institut Nationale Sante & Recherche Medicale U290, Conservatoire National des Arts et Metiers, 2 rue Conte, 75003 Paris, France

035 Devereaux CE. Human monocytic ehrlichiosis presenting as febrile diarrhea. J Clin Gastroenterol 1997 Oct;25(3):544-5. 5 ref, Eng. MC, USN, c/o Clinical Investigation Department, Naval Medical Center, 34800 Bob Wilson Drive, San Diego, CA 92134-5000, USA

036 Do AN, Fridkin SK, Yechouron A, Banerjee SN, Killgore GE, Bourgault AM, Jolivet M, Jarvis WR*. Risk factors for early recurrent *Clostridium difficile*-associated diarrhea. Clin Infect Dis 1998 Apr;26(4):954-9. 38 ref, Eng. *Hospital Infections Program, Centers for Disease Control and Prevention, Mail Stop E-69, Atlanta, Georgia 30333, USA

037 Dutta P, Mitra U, Rasaily R, Saha MR, Manna B, Chatterjee MK, Garai T, Sengupta M, Bhattacharya SK. Multi-drug resistant typhoid fever with diarrhea. Indian Pediatr 1997 Oct;34(10):891-9. 43 ref, Eng. Division of Clinical

Medicine, National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

"***Objective:*** To provide information about the characteristics of diarrheal stool in multi-drug resistant typhoid fever and observe the clinical course after treatment with furazolidone or ciprofloxacin. ***Setting:*** Hospital based. ***Subjects and Methods:*** Twenty one male children who were positive for multi-drug resistant *S. typhi* by blood and stool cultures, having diarrhea at the time of hospitalization comprised the subjects. Serum and stool electrolytes were estimated. Stool samples were also processed to detect established enteropathogens, leukocytes and red blood cells. Children were treated either with furazolidone or ciprofloxacin and evaluated till recovery. ***Results:*** Mean (±SD) pre-admission duration of fever and diarrhea of these cases were 19.1 (±5.6) and 15.8 (±4.6) days, respectively. Stool character in 81% of the patients was watery with mean (±SD) volume of stool 51.4 (±25.1) ml per kg body weight in the first 24 hours of observation. Leukocyte count varied between 20-49 per high power field in 66.7% stool samples. Occult blood was present in only 19% cases. Fecal red blood cells in high power field were detected in 52.4% cases. Mean fecal electrolytes (mmol/liter) were as follows: sodium-53.8, potassium-51.4, chloride-41.6 and total Co_2-24.3. Most of the children (71.4%) had no dehydration and had normal serum electrolytes. The isolated strains of *S. typhi* were multi-drug resistant. These children were treated successfully either with furazolidone or ciprofloxacin. ***Conclusion***: The stools of multi-drug resistant typhoid fever patients were watery with little blood. Their electrolyte contents were more similar to the diarrheal stool seen in shigellosis rather than cholera. Uncontrolled observations revealed that children recovered with furazolidone or ciprofloxacin therapy."

038 Dutta S, Mondal SK, Saha MR, Sengupta PG, Gupta DN, Ghosh S, Sircar BK. Isolation of *Escherichia coli* to detect faecal contamination of infants and their mothers in West Bengal. J Diarrhoeal Dis Res 1997 Sep;15(3):173-6. 16 ref, Eng. Department of Microbiology, National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, PO Box 177, Beliaghata, Calcutta 700010, India

"The present study was undertaken to gain insight into the sources of faecal contamination of infants in rural Bengal. It was carried out in three villages near Calcutta, India, from June 1993 to August 1995 among 148 infants and their mothers. *Escherichia coli* was used as an indicator of faecal pollution. A total of 725 samples, including hand rinsings of children and mothers, feeding utensils and leftover food were examined. The total isolation rate of faecal *E. coli* was 30%. The isolation rates from hands of children and mothers were 17% and 40% respectively. The germs from 30% of utensils and 59% of leftover food and drinks were recovered further. The study highlights the precarious hygiene in rural Bengal."

039 Ehara M, Iwami M, Ichinose Y, Hirayama T, Albert MJ, Sack RB, Shimodori S. Induction of fimbriated *Vibrio cholerae* O139. Clin Diagnostic Lab Immunol 1998 Jan;5(1):65-9. 14 ref, Eng. Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University, 1-chome 12-4, Sakamoto, Nagasaki 852, Japan

"Several fimbriated phases of *Vibrio cholerae* 0139 strains were selectively induced and compared immunologically and biochemically with those of *V. cholerae* 01. Fimbrial antigens were detected on the surfaces of vibrio cells colonizing the epithelial cells of a rabbit small intestine. Convalescent-phase sera from six individuals infected with *V. cholerae* 0139 revealed the development of antibody against the fimbrillin. These findings suggest that the fimbriae of *V. cholerae* 01 and 0139 are expressed in vivo during infection and that consideration must be given to the use of fimbrial antigens as components of vaccines against cholera."

040 Elliott SJ, Srinivas S, Albert MJ, Alam K, Robins-Browne RM, Gunzburg ST, Mee BJ, Chang BJ. Characterization of the roles of hemolysin and other toxins in enteropathy caused by alpha-hemolytic *Escherichia coli* linked to human diarrhea. Infect Immun 1998 May;66(5):2040-51. 45 ref, Eng. Center for Vaccine Development, University of Maryland School of Medicine, 685 W Baltimore St., Baltimore, MD 21201, USA

"*Escherichia coli* strains producing alpha-hemolysin have been associated with diarrhea in several studies, but it has not been clearly demonstrated that these strains are enteropathogens or that alpha-hemolysin is an enteric virulence factor. Such strains are generally regarded as avirulent commensals. We examined a collection of diarrhea-associated hemolytic *E. coli* (DHEC) strains for virulence factors. No strain

produced classic enterotoxins, but they all produced an alpha-hemolysin that was indistinguishable from that of uropathogenic *E. coli* strains. DHEC strains also produced other toxins including cytotoxic necrotizing factor 1 (CNF1) and novel toxins, including a cell-detaching cytotoxin and a toxin that causes HeLa cell elongation. DHEC strains were enteropathogenic in the RITARD (reversible intestinal tie adult rabbit diarrhea) model of diarrhea, causing characteristic enteropathies, including inflammation, necrosis, and colonic cell hyperplasia in both small and large intestine. Alpha-hemolysin appeared to be a major virulence factor in this model since it conferred virulence to nonpathogenic *E. coli* strains. Other virulence factors also appear to be contributing to virulence. These findings support the epidemiologic link to diarrhea and suggest that further research into the role of DHEC and alpha-hemolysin in enteric disease is warranted."

041 Evans CAW, Gilman RH, Rabbani GH, Salazar G, Ali A. Gastric acid secretion and enteric infection in Bangladesh. Trans R Soc Trop Med Hyg 1997 Nov-Dec;91(6):681-5. 34 ref, Eng. Room 5521, Department of International Health, The Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA

"In developing countries many enteric infections are caused by acid-sensitive pathogens. Failure of the gastric acid barrier to infection has been reported in cholera but gastric acid secretion has been little studied in other enteric infections. We therefore studied basal and stimulated gastric acid in 185 Bangladeshi men admitted to hospital for the treatment of enteric infection. Patients with dysentery (amoebiasis, n=24 and shigellosis, n=19) and culture-negative diarrhoea (n=69) had similar mean gastric acid levels (basal, 3-5 mmol/h; stimulated, 11-17 mmol/h), which remained stable in those patients studied throughout 12 weeks of convalescence. In contrast, patients with secretory diarrhoea caused by cholera or enterotoxigenic *Escherichia coli* (ETEC) had low gastric acid levels ($P<0.05$ compared with other groups) (cholera, n=34: basal mean 1.8 mmol/h [SD=2.2], stimulated mean 7.9 mmol/h [SD=6.4]; ETEC, n=39; basal mean 2.7 mmol/h [SD=2.8], stimulated mean 9.4 mmol/h [SD=7.5]). Cholera patients' gastric acid level rose during convalescence to similar levels to the dysentery patients'. Low gastric acid level was associated with severe disease in patients with cholera ($P<0.02$) or ETEC ($P<0.05$). Gastric acid level fell with increasing age ($P<0.007$) but this did not account for the differences between groups. Gastric acid levels were not associated with *Giardia duodenalis* or *Strongyloides stercoralis* co-infection, fever, use of tobacco, or chewing betel nut. Cholera and secretory diarrhoea caused by ETEC may, therefore, partly result from a reduction in gastric acid level which does not occur during dysentery. Factors which impair gastric acid secretion may predispose to diarrhoeal disease in developing countries."

042 Faruque ASG, Hoque SS, Fuchs GJ, Mahalanabis D. Randomized, controlled, clinical trial of rice versus glucose oral rehydration solutions in infants and young children with acute watery diarrhoea. Acta Paediatr 1997 Dec;86(12):1308-11. 12 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"A randomized clinical trial was carried out to compare a packaged ready-to-mix rice oral rehydration solution (ORS) to the standard glucose ORS for the treatment of childhood diarrhoea. Children were of either gender, aged 3-35 months, presenting with a history of watery diarrhoea for 72 h or less. The main outcomes examined were stool output, ORS intake, duration of diarrhoea and nutritional recovery during follow-up at 16 d of illness. Stool output in the first 24 h (106 vs 107 g kg^{-1}), ORS intake in clinic (93 vs 102 ml per motion) and duration of diarrhoea (88 h vs 81 h) were similar in the two treatment groups. The few episodes that became persistent were similar (2%) in the two groups. The weight gain during follow-up was similar in the two ORS groups."

043 Foley JE, Solnick JV, Lapointe J-M, Jang S, Pedersen NC. Identification of a novel enteric *Helicobacter* species in a kitten with severe diarrhea. J Clin Microbiol 1998 Apr;36(4):908-12. 25 ref, Eng. Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

044 Foudraine NA, Weverling GJ, van Gool T, Roos MTL, de Wolf F, Koopmans PP, van den Broek PJ, Meenhorst PL, van Leeuwen R, Lange JMA, Reiss P. Improvement of chronic diarrhoea in patients with advanced HIV-1 infection during potent antiretroviral therapy. AIDS 1998 Jan 1;12(1):35-41. 19 ref, Eng. National AIDS Therapy Evaluation Center, Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine, and AIDS,

Academic Medical Center, University of Amsterdam, Room F5-108, Box 22700, 1100 DE Amsterdam, The Netherlands

045 Frost JA, Oza AN, Thwaites RT, Rowe B. Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat-stable antigens. J Clin Microbiol 1998 Feb; 36(2):335-9. 27 ref, Eng. Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Ave., London NW9 5HT, UK

046 Goulet OJ, Brousse N, Canioni D, Walker-Smith JA, Schmitz J, Phillips AD*. Syndrome of intractable diarrhoea with persistent villous atrophy in early childhood: a clinicopathological survey of 47 cases. J Pediatr Gastroenterol Nutr 1998 Feb;26(2):151-61. 47 ref, Eng. *University Department of Paediatric Gastroenterology, Third Floor, Royal Free Hospital, Pond Street, London NW3 2QG, UK

"**Background:** The syndrome of intractable diarrhoea of infancy is heterogeneous and includes several diseases with diverse aetiologies. This study determines whether diagnostic categories can be defined on the basis of clinicopathological analysis. **Methods:** European Society of Paediatric Gastroenterology, Hepatology and Nutrition members were surveyed to identify cases of intractable diarrhoea with persisting small intestinal enteropathy. A retrospective clinicopathological analysis was performed on cases showing life-threatening diarrhoea within the first 24 mo of life and requiring total parenteral nutrition, which were characterized by persistent villous atrophy, and resistance to therapy. **Results:** Forty-seven infants were identified with intractable diarrhoea. Villous atrophy was of varying degrees with (group I, n=24) or without (group II, n=18) lamina propria mononuclear cell infiltration. Group I presented later, had gut autoantibodies, and a higher prevalence of protein-losing enteropathy; a subset (group Ia, n=12) also had extraintestinal symptoms of autoimmunity associated with a later onset of larger volume diarrhoea. Group II presented early; 8 cases (group IIa) had phenotypic abnormalities and a low birth weight; the remaining 10 (group IIb) showed mild-to-moderate villous atrophy, epithelial tufting, and abnormal crypts. Group III included five patients in whom no specific features were recognised. Twenty-one (45%) died at a median age of 24 months, 20 (43%) remained dependent on parenteral (n=16) or enteral tube (n=4) feeding, 4(9%) received elimination diets plus other therapies, and 2 (4%) were lost to follow-up. **Conclusions:** Clinicopathological analysis allowed distinct disease groups to be identified, allowing a provisional classification to be made. This straightforward approach forms a basis for future research in this exceptionally difficult paediatric condition."

047 Helander A, Hansson GC, Svennerholm A-M*. Binding of enterotoxigenic *Escherichia coli* to isolated enterocytes and intestinal mucus. Microbial Pathogen 1997 Dec;23(6):335-46. 45 ref, Eng. *Department of Medical Microbiology and Immunology, Göteborg University, Guldhedsgatan 10A, Göteborg 413 46, Sweden

"Binding of human enterotoxigenic *Escherichia coli* (ETEC) to the small intestine is a prerequisite for colonization and is mediated by colonization factor (CF) antigens. Coli surface antigen 6 (CS6) is considered a CF but binding to isolated enterocytes has not been established. In this study bacteria expressing CS6 were analysed for binding to enterocytes from human and rabbit small intestine, isolated using either an EDTA-containing buffer or a buffer devoid of EDTA. We found that the bacteria bound to enterocytes from rabbit ileum and human duodenum, but only when the cells had been isolated in the absence of EDTA. Pretreatment of rabbit enterocytes with *meta*-periodate resulted in a decreased proportion of cells with bound bacteria. Purified CS6, and for comparison other ETEC CFs, were also tested for binding to different human and rabbit mucus fractions. These analyses showed that purified CS6 bound to mucus from rabbit duodenum and ileum as well as from human duodenum, jejunum and ileum and that this binding was abolished by pretreatment of the mucus material with *meta*-periodate or proteinase K. CFA/1 CS1 to CS5, CS7, CS17, putative CF(PCF) 0159 (CS12), PCF0166 (CS14), and CFA/111 (CS8) also bound to the rabbit mucus material although with different patterns; the binding of CS2 and CS5 was abolished by *meta*-periodate treatment. Thus, ETEC bacteria expressing CS6 might bind to carbohydrate-containing structure(s) in the apical membrane of isolated rabbit ileal and human duodenal enterocytes that could probably be released by EDTA treatment. In addition, CS6 and other ETEC CFs bind to component(s), in some instances protein-associated carbohydrate structures, in mucus fractions from small intestine."

048 Heyman SN, Ginosar Y, Shapiro M, Kluger Y, Marx N, Maayan S. Diarrheal epidemics among

Rwandan refugees in 1994: management and outcome in a field hospital. J Clin Gastroenterol 1997 Dec;25(4):595-601. 34 ref, Eng. Department of Medicine, Hadassah University Hospital, Mount Scopus, PO Box 24035, Jerusalem 91240, Israel

049 Hofinger C, Karch H, Schmidt H*. Structure and function of plasmid pColD157 of enterohemorrhagic *Escherichia coli* O157 and its distribution among strains from patients with diarrhea and hemolytic-uremic syndrome. J Clin Microbiol 1998 Jan;36(1):24-9. 46 ref, Eng. *Institut fur Hygiene und Mikrobiologie der Universitat Wurzburg, Josef-Schneider-Str. 2, D-97080 Wurzburg, Germany

"In this study, pColD157, a 6.7-kb colicinogenic plasmid of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strain CL40 cu, was characterized by restriction mapping and determination of its complete nucleotide sequence. The sequence consists of 6,675 bp and shows a high degree of similarity to the nucleotide sequence of colicinogenic plasmids pColD-CA23 and pColK. Seven potential genes were located on pColD157, three of which were closely related (>97.9%) to the colicin D structural gene and the corresponding immunity and lysis genes of plasmid pColD-CA23, and these were therefore designated *cda*, *cdi*, and *cdl*, respectively, using the reference extension -CL40 for differentiation. The adjacent 3' region is related to the origin of replication of pColD-CA23. In contrast, the remaining part of the plasmid harbors a cluster of genes, closely related to the mobilization genes of pColK, which is followed by a 0.3-kb stretch homologous to the pColK resolution function. These determinants were designated *mbdA*, *mbdB*, *mbdC*, and *mbdD* and *cdr*, respectively. Southern blot anlaysis was performed with a probe specific for the *cda* gene of pColD157 and two groups of EHEC 0157:H7 isolates from patients with diarrhea or hemolytic-uremic syndrome resident in Germany. Whereas 16 of 46 *E. coli* O157 strains isolated between 1987 and 1991 harbored plasmid pColD157, only 1 of 50 strains isolated during 1996 carried this plasmid. In addition, all strains harboring plasmid pColD157 were shown to have colicinogenic activity."

050 Hoge CW, Gambel JM*, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clin Infect Dis* **1998 Feb;26(2):341-5. 22 ref, Eng.** *Division of Preventive Medicine, Walter Reed Army Institute of Research, Building 40, Washington, DC 20307-5100, USA

"Antibiotic resistance trends were examined for *Shigella* species, nontyphoidal *Salmonella* species, enterotoxigenic *Escherichia coli* (ETEC), and *Campylobacter* species isolates from indigenous persons and travelers in Thailand for up to 15 years. Resistance to trimethoprim-sulfamethoxazole was found in >90% of *Shigella* and 40% of ETEC and nontyphoidal *Salmonella* isolates. Resistance to nalidixic acid was found in 97%-100% of *Shigella dysenteriae* 1 strains isolated between 1992 and 1995. Ciprofloxacin resistance was detected in 1% of ETEC isolates in 1994 and 1995 and in one of 349 nontyphoidal *Salmonella* isolates in 1995. Ciprofloxacin resistance among *Campylobacter* species increased from zero before 1991 to 84% in 1995 (P<.0001). Azithromycin resistance was found in 7%-15% of *Campylobacter* isolates in 1994 and 1995, as well as 15% of ETEC and 3% of *Salmonella* isolates in 1995. Enteric pathogens in Thailand have developed resistance to virtually all antibiotics routinely used in the treatment of diarrhea, as well as the newer fluoroquinolone and macrolide classes of drugs."

051 Hossain MI, Kabir I, Fuchs GJ, McCutcheon MJ, Alvarez JO, Khaled MA. Intra- and extracellular water dynamics on rehydration in cholera and noncholera patients. Dig Dis Sci 1998 Mar;43(3):663-7. 15 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"To estimate the intra- and extracellular body water compartments during rehydration of patients with cholera and noncholera diarrhea by bioimpedance analyzer, we studied 30 patients with acute watery diarrhea. Total body water (TBW), intracellular water (ICW), and extracellular water (ECW) of severely dehydrated adult patients were measured with a dual frequency bioimpedance analyzer at different phases of rehydration. Fluid compartments between cholera and noncholera patients were compared. Cholera patients gained more TBW than noncholera patients during recovery. Unlike patients with noncholera diarrhea, the gain in cholera patients was mainly contributed by the ICW (1.5 $\pm$ 1.6 vs 3.0 $\pm$ 1.2 liters, respectively, P<0.01). It was also observed that the recovery of the ICW compartment in cholera patients occurred rapidly within the first 2 hr after infusion. Differential dynamics of body water compartments in cholera compared to

noncholera patients as observed in this study may contribute further to understanding the mechanism of dehydration in diarrheal disease, which might help in improving case management."

052 Isogai E, Isogai H, Kimura K, Hayashi S, Kubota T, Fujii N, Takeshi K. Role of tumor necrosis factor alpha in gnotobiotic mice infected with an *Escherichia coli* O157:H7 strain. Infect Immun 1998 Jan;66(1):197-202. 27 ref, Eng. Department of Preventive Dentistry, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu 1757, Hokkaido 061-02, Japan

053 Iwanaga M, Honma Y, Enami M. Molecular epidemiology of *Vibrio cholerae* O1 isolated from sporadic cholera cases in Okinawa, Japan. *Microbiol Immunol* **1997;41(11):861-4. 6 ref, Eng.** Department of Bacteriology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan

054 Jertborn M, Ahren C, Holmgren J, Svennerholm A-M. Safety and immunogenicity of an oral inactivated enterotoxigenic *Escherichia coli* vaccine. Vaccine 1998 Jan/Feb;16(2/3):255-60. 31 ref, Eng. Department of Medical Microbiology and Immunology, Göteborg University, Guldhedsgatan 10, S-413 46 Göteborg, Sweden

"The safety and immunogenicity of two different lots 001 and 003, of an oral inactivated enterotoxigenic *Escherichia coli* (ETEC) vaccine consisting of a mixture of formalin-killed whole bacteria expressing the most prevalent colonisation factor antigens, i.e. CFA/I, CFA/II and CFA/IV and recombinantly produced cholera B subunit (rCTB) have been evaluated in Swedish volunteers. Neither of the two vaccine preparations, containing different CFA/II-expressing strains but otherwise identical, gave rise to any significant side-effects. Musocal immune responses, as reflected in antibody-secreting cell (ASC) responses in peripheral blood, were studied after two doses of vaccine and did not differ significantly for the two vaccine lots. Vaccination induced high levels of CTB-specific IgA ASCs in 100% of the volunteers, and significant IgA ASC responses (9- to 36-fold) were noted in 84% of them against CFA/I, in 87% against CFA/II subcomponents CS1-CS3 and in 91% against CFA/IV subfactors CS4 and/or CS5. The frequencies and magnitudes of CFA IgA ASC responses were similar when giving the vaccine with a 1 or 2 week interval. Results from serological analyses showed that the local IgA responses against CFAs are only infrequently associated with serum antibody titre rises."

055 Johansson E-L, Rask C, Fredriksson M, Eriksson K, Czerkinsky C, Holmgren J*. Antibodies and antibody-secreting cells in the female genital tract after vaginal or intranasal immunization with cholera toxin B subunit or conjugates. Infect Immun 1998 Feb;66(2):514-20. 39 ref, Eng. *Department of Medical Microbiology and Immunology, Göteborg University, Guldhedsgatan 10A, S-413 46 Göteborg, Sweden

056 Kalambaheti T, Chaisri U, Srimanote P, Pongponratn E, Chaicumpa W. Immunogenicity and protective role of three formulations of oral cholera vaccine. Vaccine 1998 Jan/Feb;16(2/3):201-7. 25 ref, Eng. Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand

"Three formulations of oral cholera vaccine were compared with respect to their immunogenicity and protective ability in a rat ileal loop model. Eight-week-old Wistar rats were divided into five groups. The first group received orally vaccine A consisting of liposome-associated *V. cholerae* lipopolysaccharide, fimbriae and procholeragenoid, whereas the rats of groups 2 and 3 received orally vaccines B and C consisting of heat-killed fimbriated and non-fimbriated whole cell *V. cholerae*, respectively. Rats of groups 4 and 5 were controls that received orally liposomes alone and normal saline solution, respectively. It was found that vaccine A elicited stronger immune responses to all three *V. cholerae* antigens. The antibody responses were detected in both serum and intestinal lavage samples. Vaccine B elicited only modest serum and intestinal responses to *V. cholerae* fimbriae (anti-F). No detectable immune response was found in rats of group 3 immunized with vaccine C. Rats immunized with vaccines A and B had a similar order of magnitude of numbers of vibrios adhered to their intestinal mucosa. These numbers were less than those associated with the intestinal tissues of control rats of groups 4 and 5 by about two orders of magnitude. Although without any detectable immune response, rats of group 3 that were immunized with vaccine C showed some reduction in numbers of vibrios associated with their intestinal mucosa. The numbers of vibrios recovered from the intestinal segments of rats

of all treatment groups were in the order group 1 = 2<4 = 5. Electron micrography also revealed patches of vibrio colonization on the mucosa of rats of groups 3, 4 and 5. These features were not found in the groups vaccinated with vaccines A and B. The inhibition of vibrio colonization afforded by the vaccines was biotype- and serotype-non-specific. The results suggest that the heat-killed whole cell fimbriated *V. cholerae* may be an alternative vaccine preparation to the liposome-associated refined antigen vaccine at a lower cost."

057 Kilhamn J, Jertborn M*, Svennerholm A-M. Kinetics of local and systemic immune responses to an oral cholera vaccine given alone or together with acetylcysteine. Clin Diagn Lab Immunol 1998 Mar;5(2):247-50. 33 ref, Eng. *Department of Medical Microbiology and Immunology, Göteborg University, Guldhedsgatan 10, S-413 46 Göteborg, Sweden

"The possibility that a mucolytic drug, i.e., acctylcysteine, given orally may enhance the gut mucosal or systemic immune response to an oral B-subunit-whole-cell (B-WC) cholera vaccine was evaluated for 40 adult Swedish volunteers, and the kinetics of the immune responses were monitored for responding volunteers. Two doses of vaccine induced similar frequencies of immunoglobulin A (IgA) and IgG antitoxin responses (80 to 90%) and vibriocidal titer increases (60 to 65%) in serum irrespective of whether the vaccine was given alone or together with 2 g of acetylcysteine. In feces the frequencies of IgA antitoxin (67%) and antibacterial (33 to 40%) antibody responses were also comparable in the two immunization groups. Six months after vaccination, IgA and IgG antitoxin as well as vibriocidal antibody titer increases in serum could still be detected in approximately 80% of initially responding vaccinees. Significantly elevated fecal antitoxin and antibacterial IgA antibody levels were found in, respectively, 50 and 43% of those volunteers who initially had responded to the vaccine. Determination of IgA antibodies in feces does not seem to offer any advantages compared to determination in serum for assessment of immune responses after immunization with inactivated cholera vaccine."

058 Klose KE, Mekalanos JJ*. Differential regulation of multiple flagellins in *Vibrio cholerae*. J Bacteriol 1998 Jan;180(2):303-16. 56 ref, Eng. *Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115, USA

"*Vibrio cholerae*, the causative agent of the human diarrheal disease cholera, is a motile bacterium with a single polar flagellum. Motility has been implicated as a virulence determinent in some animal models of cholera, but the relationship between motility and virulence has not yet been clearly defined. We have begun to define the regulatory circuitry controlling motility. We have identified five *V. cholerae* flagellin genes, arranged in two chromosomal loci, *flaAC* and *flaEDB*; all five genes have their own promoters. The predicted gene products have a high degree of homology of each other. A strain containing a single mutation in *flaA* is nonmotile and lacks a flagellum, while strains containing multiple mutations in the other four flagellin genes, including a *flaCEDB* strain, remain motile. Measurement of *fla* promoter-*lacZ* fusions reveals that all five flagellin promoters are transcribed at high levels in both wild-type and *flaA* strains. Measurement of the flagellin promoter-*lacZ* fusions in *Salmonella typhimurium* indicates that the promoter for *flaA* is transcribed by the σ^{-54} holoenzyme form of RNA polymerase while the *flaE, flaD*, and *flaB* promoters are transcribed by the σ^{-28} holoenzyme. These results reveal that the *V. cholerae* flagellum is a complex structure with multiple flagellin subunits including FlaA, which is essential for flagellar synthesis and is differentially regulated from the other flagellins."

059 Kücükbay H, Yakinci C, Kücükbay FZ, Turgut M. Serum vitamin A and beta-carotene levels in children with recurrent acute respiratory infections and diarrhoea in Malatya. J Trop Pediatr 1997 Dec;43(6):337-40. 17 ref, Eng. Faculty of Science and Arts, Inönü University, 44069, Malatya, Turkey

"Deficiency of serum vitamin A is one of the widespread public health problems among pre-school children in developing countries. A limited number of studies have been done about this problem in Turkey and there is no similar work done in Malatya. Serum vitamin A and beta-carotene levels in 56 pre-school age children who had recurrent acute respiratory infections (ARI) or recurrent diarrhoea were determined by a UV/VIS spectrometer. The results obtained were compared with 35 healthy pre-school age children. Serum vitamin A (51.66 ± 8.10 µg/dL) and beta-carotene (82.88 ± 18.5 µg/dL) levels in children with ARI's were found

significantly lower than the control group (58.14 ± 9.07 µg/dL and 131.43 ± 22.38 µg/dL, respectively) (P<0.001). Serum vitamin A (47.21 ± 8.27 µg/dL) and beta-carotene (81.63 ± 15.41 µg/dL) levels in children with recurrent diarrhoea were also found significantly lower than the control group (58.14 ± 9.07 µg/dL and 131.43 ± 22.38 µg/dL, respectively) (P<0.001)."

060 Lacaille F, Emile J-F, Canioni D, Pierre P, Vaerman J-P, Brousse N, Schmitz J*. Chronic diarrhea with massive intestinal plasma cell infiltration and high polyclonal immunoglobulin A serum level. J Pediatr Gastroenterol Nutr 1998 Mar;26(3):345-50. 22 ref, Eng. *Department of Paediatrics, Necker-Enfants Malades Hospital, 149 rue de Sevres, 75743 Paris Cedex 15, France

061 Lazar S, Waldor MK*. ToxR-independent expression of cholera toxin from the replicative form of CTXf (note). Infect Immun 1998 Jan;66(1):394-7. 22 ref, Eng. *New England Medical Center, No. 041,750 Washington St., Boston, MA 02111, USA

062 Mahfouz AAR, El-Morshedy H. Ecological determinants of intestinal parasitic infections among pre-school children in an urban squatter settlement of Egypt. J Trop Pediatr 1997 Dec;43(6):341-4. 18 ref, Eng. Department of Epidemiology, High Institute of Public Health, Alexandria University, 165 El-Horreya Avenue, Alexandria, Egypt

063 Mathewson JJ, Salameh BM, DuPont HL, Jiang ZD, Nelson AC, Arduino R, Smith MA, Masozera N. HEp-2 cell-adherent *Escherichia coli* and intestinal secretory immune response to human immunodeficiency virus (HIV) in outpatients with HIV-associated diarrhea. Clin Diagnostic Lab Immunol 1998 Jan;5(1):87-90. 16 ref, Eng. Center for Infectious Diseases, The University of Texas Medical School and School of Public Health, 1200 Herman Pressler, Houston, TX 77030, USA

"HEp-2 cell-adherent *Escherichia coli* and the human immunodeficiency virus (HIV) itself have recently been incriminated as causes of chronic HIV-associated diarrhea. This study sought to determine the prevalence of these two agents among HIV-infected patients with diarrhea in an outpatient setting in the United States and to compare their prevalence to that of other commonly recognized enteropathogens known to be present in this population. HEp-2 cell-adherent *E. coli* was found in 20 of 83 (24.1%) patients with diarrhea. A diffuse pattern of adherence was the most common, found in 14 of 20 (70%) patients, followed by a localized adherence pattern (6 of 20; 30%). An intestinal secretory immune response against the p24 antigen of HIV was found in 9 of 34 (27.5%) patients with HIV-associated diarrhea. The following pathogens or products were also detected in lower frequencies: *Cryptosporidium* spp. (10.8%), *Clostridium difficile* toxin (8.8%), microsporidia (6%), *Isospora belli* (3.6%), *Blastocystis hominis* (2.4%), *Giardia* spp. (1.2%), *Salmonella* spp. (1.2%), and *Mycobacterium* spp. (1.2%). The role of HEp-2 cell-adherent *E. coli* and HIV enteric infections in patients with HIV-associated diarrhea deserves further study."

064 Mazumder RN, Hoque SS, Ashraf H, Kabir I, Wahed MA. Early feeding of an energy dense diet during acute shigellosis enhances growth in malnourished children. J Nutr 1997 Jan;127(1):51-4. 33 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"In a controlled clinical trial, we examined the effect of the short-term feeding of an energy-dense milk cereal formula in malnourished children with clinically severe dysentery due to acute shigellosis. Seventy-five malnourished children, aged 12-48 mo, passing blood or blood with mucous in the stool for =96 h, were offered a hospital diet. In addition, study children (n=36) were offered a milk-cereal formula with an energy of 5 kj/g (an 11% protein diet); similarly control children (n=39) were offered a milk-cereal formula with an energy content of 2.5 kj/g (an 11% protein diet). Patients were admitted to the metabolic ward of the Clinical Research and Service Centre, Dhaka, at the International Centre for Diarrhoeal Disease Research, Bangladesh. Patients were studied for 10 hospital days and were then followed up at home after 30 d. After 10 d of dietary intervention, children in the study group had a significantly greater increase vs. controls in weight-for-age (6 vs. 3%, P<0.001) and in weight-for-height (7 vs. 3%, P<0.001). Serum prealbumin concentrations were significantly higher (study vs. control) after 5 d (0.214 vs. 0.170 g/L, P=0.01) and after 10 d (0.244 vs. 0.193 g/L, P=0.006) of the study. Greater weight-for-age was sustained at home 1 mo after discharge (8 vs. 5%, P=0.005) from the hospital. Similarly, higher weight-for-height was sustained 1 mo after discharge (8 vs. 5%, P=0.01). During their stay

at home, there was no dietary intervention. The results of this study suggest that short-term feeding of an energy-dense diet enhances growth in malnourished children with acute dysentery due to shigellosis."

065 Meqdam MMM, Youssef MT, Nimri LF, Shurman AA, Rawashdeh MO, Al-Khdour MS. Viral gastroenteritis among young children in northern Jordan. J Trop Pediatr 1997 Dec;43(6):349-52. 29 ref, Eng. Department of Applied Biology, Jordan University of Science and Technology, PO Box 3030, Irbid, Jordan

066 Mintz ED, Weber JT, Guris D, Puhr N, Wells JG, Yashuk JC, Curtis M, Tauxe RV. An outbreak of brainerd diarrhea among travelers to the Galapagos Islands. J Infect Dis 1998 Apr;177(4):1041-5. 14 ref, Eng. Foodborne and Diarrheal Diseases Branch, M/S A-38, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

067 Moolasart P, Sangsujja J, Eampokalap B, Ratanasrithong M, Likanonsakul S. Nontyphoidal *Salmonella* diarrhea in Thai children: a study at Bamrasnaradura Hospital, Nonthaburi, Thailand. *J Med Assoc Thai* **1997 Oct;80(10):613-7. 29 ref, Eng.** Department of Immunology, Bamrasnaradura Infectious Disease Hospital, Nonthaburi 11000, Thailand

068 Moser CA, Speaker TJ, Offit PA. Effect of water-based microencapsulation on protection against EDIM rotavirus challenge in mice. *J Virol* **1998 May;72(5):3859-62. 21 ref, Eng.** Section of Infectious Diseases, The Children's Hospital of Philadelphia, Abramson Research Center, Rm. 1205A, 34th St. and Civic Center Blvd., Philadelphia, PA 19104, USA

69 Mukhopadhyay AK, Basu I, Bhattacharya SK, Bhattacharya MK, Nair GB. Emergence of fluoroquinolone resistance in strains of *Vibrio cholerae* isolated from hospitalized patients with acute diarrhea in Calcutta, India (letter). Antimicrob Agents Chemother 1998 Jan;42(1):206-7. 9 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

070 Murch SH. The molecular basis of intractable diarrhoea of infancy. Bailliere Clin Gastroenterol 1997 Sep;11(3):413-40. 122 ref, Eng. University Department of Paediatric Gastroenterology, Royal Free Hospital, Pond Street, Hampstead, London NW3 2QG, UK

071 Musafija A, Barzilal A, Ramon J, Rubinstein E*. Effect of cholera Toxin on intestinal elimination of ciprofloxacin in rabbits. Antimicrob Agents Chemother 1998 Feb;42(2):473-4. 8 ref, Eng. *Infectious Diseases Unit, Chaim Sheba Medical Center, Tel Hashomer 52621, Israel

072 Mwenye S, Gumbo N, Mavela M, Peterson DE, Siziya S, Woelk G. Risk factors associated with contracting dysentery during *Shigella dysenteriae* type 1 outbreak in Harare, 1993. Cent Afr J Med 1997 Apr;43(4):111-2. 8 ref, Eng. Harare City Health Department, Ministry of Health and Child Welfare, PO Box CY1122, Causeway, Harare, Zimbabwe

073 Myaux JA, Ali M, Chakraborty J, de Francisco A. Flood control embankments contribute to the improvement of the health status of children in rural Bangladesh. Bull WHO 1997;75(6):533-9. 21 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"Every year, Bangladesh experiences major floods that inundate about one-third of the country. Therefore, flood control projects that comprise earthen dikes and irrigation/drainage systems are built along the major rivers to protect the people living in low-lying areas, stabilize the river banks and improve agricultural productivity. However, the adverse effects of these projects are regularly emphasized, such as environmental degradation and reduction of fishing supplies. The Demographic Surveillance System of the International Centre for Diarrhoeal Disease Research, (ICDDR,B) was used to assess the effect of a flood control programme on the mortality of 0-4-year-old children residing in the Matlab study area. Adjusted mortality rates were used in comparing four adjacent child populations residing either inside or outside a flood-control embankment and according to the type of health services provided in this area. Between the periods 1983-86 and 1989-92, the crude child mortality in the total study area decreased by 37%, from 185.9 per 100 live births to 117.9 per 1000 live births. Following the construction of the embankment, death rates outside were up to 29% higher in 1-4-year-old children and 9% higher for 0-4-year age group compared to the flood-protected area ($P < 0.001$). Simultaneously, in the same study area, health interventions contributed to a 40%

reduction in mortality among children less than 5 years of age in all causes of deaths (P <0.001). Migration patterns and the effect of distances to the hospital are discussed."

074 Nash SV, Bourgeault R, Sands M. Colonic disease associated with a positive assay for *Clostridium difficile* toxin: a retrospective study. J Clin Gastroenterol 1997 Sep;25(2):476-9. 24 ref, Eng. Department of Pathology, Baystate Medical Center, Springfield, MA 01199, USA

075 Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998 Jan;11(1):142-201. 719 ref, Eng. Center for Vaccine Development, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

076 Obi CL, Coker AO, Epoke J, Ndip RN. Enteric bacterial pathogens in stools of residents of urban and rural regions in Nigeria: a comparison of patients with and without diarrhoea and controls without diarrhoea. J Diarrhoeal Dis Res 1997 Dec;15(4):241-7. 29 ref, Eng. Department of Medical Microbiology, Medical School, University of Zimbabwe, PO Box A178, Avondale, Harare, Zimbabwe

"A total of 2,400 stool samples comprising 1,200 from patients with diarrhoea (600 each from urban and rural area) and 1,200 similarly divided controls were obtained from school children and clinic attendants of government and private clinics around three designated study centres of Edo, Lagos and Cross River states, Nigeria. These were screened for the prevalence of bacteria that could cause diarrhoea. Diarrhoea cases in urban areas had a high prevalence rate for *Campylobacter* spp. (28%), followed by enteropathogenic *Escherichia coli* (22%), *Salmonella* spp. (17%), *Shigella* spp. (14%), *Aeromonas* spp. (5%), and *Yersinia enterocolitica* (4%), whereas in rural areas *E. coli* was the most frequently encountered pathogen (18%), followed by *Salmonella* spp. (16%), *Aeromonas* spp. (15%), *Shigella* spp. (9%), *Campylobacter* spp. (8%), and *Plesiomonas shigelloides* (8%). A similar distribution but with lower rates was noted for controls in both urban and rural areas, however, no *P. shigelloides* was isolated. Results highlight a possible difference between the prevalence of enteric bacteria in rural and urban areas and reveals the strong association of *Aeromonas* and *Plesiomonas* species with cases of diarrhoea in Nigeria."

077 Okhuysen PC, Chappell CL, Sterling CR, Jakubowski W, DuPont HL. Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. Infect Immun 1998 Feb;66(2):441-3. 21 ref, Eng. Division of Infectious Diseases, University of Texas Medical School and School of Public Health, 6431 Fannin, 1.728 JFB, Houston, TX 77030, USA

078 Oli MW, Petschow BW, Buddington RK*. Evaluation of fructooligosaccharide supplementation of oral electrolyte solutions for treatment of diarrhea. Dig Dis Sci 1998 Jan;43(1):138-47. 40 ref, Eng. *Department of Biological Sciences, Mississippi State University, Mississippi State, Mississippi 39762, USA

"Although oral electrolyte solutions (OES) replenish salts and water lost during diarrhea, present formulations do not address disturbances of the normal intestinal microbiota. Therefore, we evaluated the efficacy of an OES with and without fructooligosaccharide (FOS) for treatment of pigs with acute secretory diarrhea induced by cholera toxin. Before, during, and after diarrhea, bacteriologic evaluation was made of contents collected from the mid small intestine, cecum, and distal colon and mucosa scraped from the mid small intestine. Diarrhea caused significant declines in total bacterial counts of contents from all three regions, with less of an impact on bacteria associated with the mucosa. Although total bacterial counts recovered within 24 hr, regardless of treatment, densities of enterobacteriaceae were higher in pigs treated with OES whereas those receiving FOS had more lactobacilli. Our results show that secretory diarrhea disturbs the normal densities and relative species abundance of the microbiota, with the influences more pronounced for contents relative to the mucosa, and that adding FOS to OES accelerates the recovery of bacteria perceived as beneficial while potentially slowing the recovery of pathogenic forms."

079 Pantosti A, Malpeli M, Wilks M, Menozzi MG, D'Ambrosio F. Detection of enterotoxigenic *Bacteroides fragilis* by PCR. J Clin Microbiol 1997 Oct;35(10):2482-6. 31 ref, Eng. Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanita, Viale Regina Elena 299,00161 Rome, Italy

"Strains of enterotoxigenic *Bacteroides fragilis* (ETBF) are associated with diarrhea in young farm animals and, at least in particular settings, in children. Enterotoxin production by ETBF is currently detected by a tissue culture assay with HT-29 cells. We have developed a

PCR assay based on the detection of the enterotoxin gene to identify ETBF in culture and in stool samples. Overall, 113 bacterial strains were examined, including 3 *B. fragilis*, and 15 strains belonging to other genera. Complete agreement was found between the results of the tissue culture assay and those of the PCR for our strains. PCR was also used to detect ETBF directly in fecal samples. Stools from two healthy volunteers were spiked with known numbers of ETBF and were processed by three different methods. A culture method, which required inoculation of the stools on selective plates and the collection of the whole bacterial growth ("sweeps"), was found to be the most sensitive. PCR performed with the plate sweeps yielded amplification products with a detection limit of 10^5 to 10^4 CFU/g of feces. By this method 18 samples of diarrheic stools (10 positive and 8 negative for ETBF) were examined. The results of the PCR were in accordance with the culture results in all cases. The proposed PCR assay represents a diagnostic tool for the rapid identification of ETBF in culture as well as in fecal samples."

080 Parashar UD, Kilgore PE, Holman RC, Clarke MJ, Bresee JS, Glass RI. Diarrheal mortality in US infants: influence of birth weight on risk factors for death. Arch Pediatr Adolesc Med 1998 Jan;152(1):47-51. 16 ref, Eng. Viral Gastroenteritis Section, Mailstop-G04, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

081 Parashar UD, Holman RC, Clarke MJ, Bresee JS, Glass RI. Hospitalizations associated with rotavirus diarrhea in the United States, 1993 through 1995: surveillance based on the new ICD-9-CM rotavirus-specific diagnostic code. *J Infect Dis* **1998 Jan;177(1):13-7. 14 ref, Eng.** Viral Gastroenteritis Unit, Mailstop G-04, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, GA 30333, USA

"The introduction of a specific international classification of diseases code for rotavirus diarrhea in 1992 prompted examination of the National Hospital Discharge Survey (NHDS) for trends in rotavirus-associated hospitalizations among US children aged 1 month through 4 years. During 1993-1995, 13.5% of hospitalizations were associated with diarrhea (n=162,478/year). Rotavirus was the most common pathogen identified, coded in 16.5% of diarrhea cases (n=26.798/year), and increased from 13.3% in 1993 to 18.9% in 1995. The age distribution and seasonality of hospitalizations of presumed noninfectious and viral etiology resembled those associated with rotavirus. Rotavirus was reported as a cause of diarrhea more frequently by hospitals that were large (=100 beds), proprietary-owned, or in the West/Midwest. Although these findings suggest incomplete detection of rotavirus diarrhea cases, the large number of rotavirus-associated hospitalizations underscores the need for vaccines and indicates that NHDS data could be used to monitor the impact of a US rotavirus immunization program."

082 Paton AW, Paton JC*. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{0111}, and rfb_{0157} (note). J Clin Microbiol 1998 Feb;36(2):598-602. 35 ref, Eng. *Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, S.A. 5006, Australia

083 Peracchi M, Bardella MT, Conte D. Late-onset idiopathic hypoparathyroidism as a cause of diarrhoea (case report). Eur J Gastroenterol Hepatol 1998 Feb;10(2):163-5. 14 ref, Eng. Istituto di Scienze Mediche, Padiglione Granelli, Via F, Sforza 35, 20122 Milan, Italy

084 Phillips AD, Frankel G. Mechanisms of gut damage by *Escherichia coli*. Bailliere Clin Gastroenterol 1997 Sep;11(3):465-83. 112 ref, Eng. University Department of Paediatric Gastroenterology, Third Floor, Royal Free Hospital, Pond Street, London NW3 2QG, UK

085 Radhakutty G, Sircar BK, Mondal SK, Mukhopadhyay AK, Mitra RK, Basu A, Ichhpujani RL, Nair GB, Bhattacharya SK. Investigation of the outbreak of cholera in Alleppey & Palghat districts, South India. Indian J Med Res 1997 Nov;106:455-7. 6 ref, Eng. Department of Microbiology, National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

"In May 1996, a massive outbreak of cholera occurred in Alleppey district of Kerala which spread to Palghat district by July 1996. Of the 575 patients hospitalized at the Alleppey Medical College hospital between May 1 and August 2, 1996, 30 deaths occurred with a case fatality rate of 5.2 per cent while of the 638 diarrhoea patients admitted at Agali PHC of Attapadi area in Palghat district, 30 (4.7%) deaths were recorded. Clinically, the patients had profuse watery diarrhoea

with vomiting. The epidemic of cholera in Alleppey and Palghat districts was caused by *V. cholerae* 01 of the El Tor biotype, Ogawa serotype which possessed both the *ctxA* and *tcpA* genes when examined by multiplex PCR. Gross contamination of water sources was incriminated as the cause of the epidemic."

086 Rahman MM, Mahalanabis D, Ali M, Mazumder RN, Wahed MA, Fuchs GJ. Absorption of macronutrients and nitrogen balance in children with dysentery fed an amylase-treated energy-dense porridge. *Acta Paediatr* **1997 Dec;86(12):1312-6. 26 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"The aim of this study was to determine the absorption of macronutrients and energy from an energy-dense diet liquefied with amylase from germinated wheat (ARF) in children suffering from acute dysentery. Thirty male children aged 6-35 months presenting with acute dysentery were randomly assigned to receive either an ARF-treated porridge or a standard porridge liquefied with water to make its consistency similar to the ARF porridge. After 24-h stablization a 72-h metabolic balance was performed. Sixteen children received an ARF-treated porridge and 14 received a standard porridge liquefied with water. The mean ± SD coefficients of absorption (%) of carbohydrate, fat, protein and energy (ARF porridge vs regular porridge) were 81.4 ± 11 vs 86.9 ± 7,86.1 ± 10 vs 82.8 ± 15,57.3 ± 12 vs 48.4 ± 24 and 81.4 ± 9 vs 83.1 ± 8 respectively. The stool loss of carbohydrate, protein, fat and energy was similar in the two groups. The net absorption of energy was substantially greater in the ARF-fed than regular porridge-fed children (by 28%, p=0.01). The nitrogen balance was 6.9 ± 3.4 mg $kg^{-1}d^{-1}$ in the ARF porridge group and 1.1 ± 6.7 mg $kg^{-1}d^{-1}$ in the regular porridge group (p=0.01). These results show that, despite being hyperosmolar, an amylase-treated liquefied energy-dense porridge is absorbed as well as a regular porridge by malnourished children with severe dysentery. Consequently, its use substantially increased the absorption of a net amount of macronutrients and resulted in a better nitrogen balance. These results further support this innovative approach of feeding sick children in developing countries."

087 Roels TH, Proctor ME*, Robinson LC, Hulbert K, Bopp CA, Davis JP. Clinical features of infections due to *Escherichia coli* producing heat-stable toxin during an outbreak in Wisconsin: a rarely suspected cause of diarrhea in the United States. Clin Infect Dis 1998 Apr;26(4):898-902. 15 ref, Eng. *Communicable Diseases Section, Bureau of Public Health, Wisconsin Division of Health, 1414 East Washington Avenue, Room 167, Madison, Wisconsin 53703-3044, USA

"In September 1994, a foodborne outbreak of enterotoxigenic *Escherichia coli* (ETEC) infection occurred in attendees of a banquet in Milwaukee. *E. coli* was isolates from stool specimens from 13 patients that were comprehensively tested; isolated from five patients were positive for *E. coli* producing heat-stable toxin, were biochemically identified and serotyped as *E. coli* 0153:H45, and were all resistant to tetracycline, ampicillin, sulfisoxazole, and streptomycin. Diarrhea (100%) and abdominal cramps (83%) were the most prevalent symptoms in 205 cases; vomiting (13%) and fever (19%) were less common. The median duration of diarrhea and abdominal cramps was 6 days and 5 days, respectively. In the United States, health care providers rarely consider ETEC as a possible cause of diarrhea in their patients, and few laboratories offer testing to identify ETEC. Hence, outbreaks of ETEC infection may be underdiagnosed and underreported. As in this outbreak, the relatively high prevalence of diarrhea and cramps lasting =4 days and the low prevalence of vomiting and fever can help distinguish ETEC infection from Norwalk-like virus infection and gastroenteritis due to other causes with incubation times of ³15 hours and can provide direction for confirmatory laboratory testing."

088 Ruggeri FM, Johansen K, Basile G, Kraehenbuhl J-P, Svensson L*. Antirotavirus immunoglobulin A neutralizes virus in vitro after transcytosis through epithelial cells and protects infant mice from diarrhea. J Virol 1998 Apr;72(4):2708-14. 46 ref, Eng. *Department of Virology, Smittskyddsinstitutet, 105 21 Stockholm, Sweden

"Rotaviruses are the major cause of severe diarrhea in infants and young children worldwide. Due to their restricted site of replication, i.e., mature enterocytes, local intestinal antibodies have been proposed to play a major role in protective immunity. Whether secretory immunoglobulin A (IgA) antibodies alone can provide protection against rotavirus diarrhea has not been fully established. To address this question, a library of IgA monoclonal antibodies (MAbs) previously developed

against different proteins of rhesus rotavirus was used. A murine hybridoma "backpack tumor" model was established to examine if a single MAb secreted onto mucosal surfaces via the normal epithelial transport pathway was capable of protecting mice against diarrhea upon oral challenge with rotavirus. Of several IgA and IgG MAbs directed against VP8 and VP6 of rotavirus, only IgA VP8 MAbs (four of four) were found to protect newborn mice from diarrhea. An IgG MAb recognizing the same epitope as one of the IgA MAbs tested failed to protect mice from diarrhea. We also investigated of antibodies could be transcytosed in a biologically active form from the basolateral domain to the apical domain through filter-grown Madin-Darby canine kidney (MDCK) cells expressing the polymeric immunoglobulin receptor. Only IgA antibodies with VP8 specificity (four of four) neutralized apically administered virus. The results support the hypothesis that secretory IgA antibodies play a major role in preventing rotavirus diarrhea. Furthermore, the results show that the in vivo and in vitro methods described are useful tools for exploring the mechanisms of viral mucosal immunity."

089 Sack DA, Tacket CO, Cohen MB, Sack RB, Losonsky GA, Shimko J, Nataro JP, Edelman R, Levine MM, Giannella RA, Schiff G, Lang D. Validation of a volunteer model of cholera with frozen bacteria as the challenge. Infect Immun 1998 May;66(5):1968-72. 17 ref, Eng. Johns Hopkins University Vaccine Testing Unit, 550 N. Broadway, Suite 1001, Baltimore, MD 21205, USA

"To evaluate a standardized inoculum of *Vibrio cholerae* for volunteer challenge studies, 40 healthy adult volunteers were challenged at three different institutions with a standard inoculum prepared directly from vials of frozen, virulent, El Tor Inaba *V. cholerae* N16961, with no further incubation. Groups of 5 volunteers, with each group including 2 volunteers with blood group O, were given a dose of 10^5 CFU, and 34 of the 40 volunteers developed diarrhea (mean incubation time, 28 h). Transient fevers occurred in 15 (37.5%) of the volunteers. *V. cholerae* was excreted by 36 of 40 volunteers. Five additional volunteers received 10^4 CFU, and four developed diarrhea but with a lower average purging rate than required for the model. Of the 40 volunteers, 37 developed rises in their vibriocidal and antitoxin titers similar to those in previous groups challenged with freshly harvested bacteria. We conclude that challenge with frozen bacteria results in a reproducible illness similar to that induced by freshly harvested bacteria. Use of this model should minimize differences in attack rates or severity when groups are challenged at different times and in different institutions."

090 Savarino SJ, Brown FM, Hall E, Bassily S, Youssef F, Wierzba T, Peruski L, El-Masry NA, Safwat M, Rao M, Jertborn M, Svennerholm A-M, Lee YJ, Clemens JD. Safety and immunogenicity of an oral, killed enterotoxigenic *Escherichia coli*-cholera toxin B subunit vaccine in Egyptian adults. J Infect Dis 1998 Mar;177(3):796-9. 13 ref, Eng. US Naval Medical Research Unit No. 3, PSC 452, Box 127, FPO AE, 09835-0007, USA

"Enterotoxigenic *Escherichia coli* (ETEC) is the leading cause of bacterial diarrhea in young children in developing countries. The safety and immunogenicity of a killed, oral ETEC vaccine consisting of whole cells plus recombinantly produced cholera toxin B subunit (rCTB) was evaluated in Egypt, which is endemic for ETEC diarrhea. Seventy-four healthy Egyptian adults (21-45 years old) were randomized and received two doses of the ETEC/rCTB vaccine (E003) or placebo 2 weeks apart. The frequency of adverse events after either dose did not differ by treatment group, and no severe adverse events were reported. After vaccination, peripheral blood IgA B cell responses to CTB (100%) and to vaccine colonization factor antigens CFA/I (94%), CS4 (100%), CS2 (81%), and CS1 (69%) were significantly higher than response rates for the placebo group. These favorable results in Egyptian adults indicate that the ETEC/rCTB vaccine is a promising candidate for evaluation in younger age groups in this setting."

091 Sengupta PG, Sircar BK, Mondal S, Gupta DN, Ghosh S, De SP. Epidemiologic profile of actue diarrhoeal diseases of a cohort of rural underfive children : a three years longitudinal observation. Indian J Public Health 1997 Jul-Sep;41(3):79-81. 6 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

092 Shamir R, Zahavi I, Abramowich T, Poraz I, Tal D, Pollak S, Dinari G. Management of acute gastroenteritis in children in Israel. *Pediatrics* **1998 May;101(5):892-4. 15 ref, Eng.** Division of Gastroenterology and Nutrition, Schneider Children's Medical Center, 14 Kaplan St., Petah Tikva, 49202 Israel

"***Objective.*** Diarrheal diseases are a major cause of morbidity and mortality worldwide. Guidelines about the use of oral rehydration solutions (ORS) and dietary management of acute gastroenteritis (GE) were recently revised and published by the American Academy of Pediatrics (AAP). Study aims were to examine Israeli pediatricians' knowledge and implementation of the revised AAP guidelines, the effect of medical school (Israeli versus foreign medical graduates) on the physicians' practice, and the effect of the type of practice (community vs hospital-based) on the management of gastroenteritis. ***Methods.*** A multiple-choice, written questionnaire was distributed at two pediatrics annual meetings. ***Results.*** A total of 87 pediatricians completed the questionnaire. They were aware of 73% of the current AAP guidelines and followed 60% of the guidelines. Most pediatricians (83%) use ORS for treatment of dehydration in GE, but 60% of pediatricians believe that full-strength feeds are inappropriate in the presence of GE, and 67% of them do not recommend full-strength formulas. In addition, 37% of the pediatrians stop feeding temporarily in the presence of GE, in contrast to the 1996 AAP guidelines. There were no differences in knowledge and management practices among pediatricians graduating in Israel, Europe, or the United States, and no differences between pediatricians working in an ambulatory setting or in a hospital.

Conclusions. Pediatricians in Israel, regardless of country of origin, medical school, or place of practice, are aware of the correct use of ORS but do not follow nutritional practices recommended recently by the AAP. These findings suggest that steps for implementing the guidelines are needed in Israel and most probably world-wide."

093 Sharma C, Thungapathra M, Ghosh A, Mukhopadhyay AK, Basu A, Mitra R, Basu I, Bhattacharya SK, Shimada T, Ramamurthy T, Takeda T, Yamasaki S, Takeda Y, Nair GB*. Molecular analysis of non-01, non-O139 *Vibrio cholerae* associated with an unusual upsurge in the incidence of cholera-like disease in Calcutta, India. J Clin Microbiol 1998 Mar;36(3):756-63. 36 ref, Eng. *National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

"There was an inexplicable upsurge in the incidence of non-01, non-0139 *Vibrio cholerae* among hospitalized patients admitted to the Infectious Diseases Hospital, Calcutta, India, between February and March 1996. Of the 18 strains of *V. cholerae* isolated during this period, 15 belonged to the non-01, non-0139 serogroups (4 belonged to 0144, 3 belonged to 011, 1 each belonged to 06, 08, 012, 019, 039, and 058, and 2 strains could not be typed), 2 belonged to the 0139 serogroup, and 1 belonged to the 01 serogroup. Cell-free culture supernatants of 13 representative non-01, non-0139 *V. cholerae* strains evoked a distinct cytotoxic effect on CHO and HeLa cells, and the strains examined produced the nonmembrane-damaging cytotoxin. By several PCR assays, it was determined that none of the non-01, non-0139 strains were positive for the *ctxA*, *zot*, *ace*, and *tepA* genes and for the genes representing the heat-labile toxin, heat-stable toxin, and verotoxin of *Escherichia coli* and the various variants of these genes. Studies on the colonality of non-01, non-0139 *V. cholerae* strains by restriction fragment length polymorphism (RFLP) analysis of rRNA genes and of other genes (*hlyA*, *hlyU*, *hlx*, *toxR*, and *attRSI*) and by pulsed-field gel electrophoresis (PFGE) collectively indicate that the upsurge which occurred in February and March 1996 was caused by strains belonging to different clones. Overall, there was an excellent correlation between the results of ribotyping, RFLP analysis of various genes, and PFGE, with strains belonging to a particular serogroup showing nearly identical restriction patterns and PFGE profiles. It is clear from this study that some serogroups of *V. cholerae* can cause diarrhea by a mechanism quite different from that of toxigenic *V. cholerae* 01 and 0139, and we have proposed the nomenclature of enteropathogenic *V. cholerae* to include these serogroups."

094 Sharma C, Ghosh A, Dalsgaard A, Forslund A, Ghosh RK, Bhattacharya SK, Nair GB. Molecular evidence that a distinct *Vibrio cholerae* O1 biotype El Tor strain in Calcutta may have spread to the African continent (note). J Clin Microbiol 1998 Mar;36(3):843-4. 9 ref, Eng. *National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

095 Sobottka I, Schwartz DA*, Schottelius J, Visvesvara GS, Pieniazek NJ, Schmetz C, Kock NP, Laufs R, Albrecht H. Prevalence and clinical significance of intestinal microsporidiosis in human immunodeficiency virus-infected patients with and without diarrhea in Germany: a prospective coprodiagnostic study. Clin Infect Dis 1998 Feb;26(2):475-80. 39 ref, Eng. *Department of Pathology, Grady Memorial Hospital, 80 Butler Street SE, Atlanta, Georgia 30335, USA

"The prevalence of intestinal microsporidiosis among human immunodeficiency virus (HIV)-infected persons with chronic diarrhea varies from 7% to 50%; thus, microsporidia are a significant source of morbidity and, occasionally, mortality among these patients. Anecdotal reports suggest that intestinal microsporidiosis is also an important infection in patients with AIDS in Germany. To determine the prevalence of microsporidiosis among HIV-infected patients in Germany, we performed a prospective coprodiagnostic study of 97 consecutive HIV-infected patients. Microsporidia were the most common enteropathogen identified in 18 (36.0%) of 50 patients with diarrhea and 2 (4.3%) of 47 patients without diarrhea ($P<.001$; X^2 test). Microsporidia were present in 60% of patients with chronic diarrhea and 5.9% of patients with acute diarrhea. The etiologic agent was *Enterocytozoon bieneusi* in 18 patients and *Encephalitozoon intestinalis* in two patients. The prevalence of intestinal microsporidiosis in this cohort of German patients with AIDS and diarrhea is one of the highest to be reported anywhere in the world. Microisporidiosis seems to represent one of the most important causes of diarrhea in HIV-infected patients in Germany and thus must be considered in the differential diagnosis for all AIDS patients presenting with diarrhea."

096 Sodeinde O, Adeyemo AA, Gbadegesin RA, Ademowo OG. Persistent diarrhoea in Nigerian children aged less than five years: a hospital-based study. J Diarrhoeal Dis Res 1997 Sep;15(3):155-60. 15 ref, Eng. Department of Paediatrics, University College Hospital, Ibadan, Nigeria

"To identify possible risk factors for persistent diarrhoea, 307 children with acute diarrhoea presenting at the University College Hospital, Ibadan, Nigeria over a 10-month period from July 1993 to April 1994 were followed up prospectively until the resolution of the illness. The children were aged 6-60 months. In 36 (11.7%) of them, diarrhoea became persistent (i.e. lasted more than 14 days). This hospital frequency of 11.7% of persistent diarrhoea is, as expected, higher than the figures from previous community-based studies of diarrhoea from Nigeria. The major factor associated with persistent diarrhoea was poor nutritional status. Mean *z* scores of weight-for-height and weight-for-age were significantly lower in the persistent diarrhoea group, while mean *z* scores of height-for-age were similar in the two groups. The frequencies of occurrence of undernutrition, marasmus and kwashiorkor were also higher in the persistent diarrhoea group. Therefore, in common with studies from other regions of the world, malnutrition is an important risk factor for persistent diarrhoea in this group of Nigerian children. The implication of these findings is that reduction in the prevalence of malnutrition may be associated with reduction in the proportion of acute diarrhoeal episodes that eventually progress to persistent diarrhoea."

097 Steele AD, Phillips J, Smit TK, Peenze I, Jiang X. Snow mountain-like virus identified in young children with winter vomiting disease in South Africa. J Diarrhoeal Dis Res 1997 Sep;15(3):177-82. 24 ref, Eng. MRC/MEDUNSA Diarrhoeal Pathogens Research Unit, Department of Virology, Medical University of Southern Africa, PO Box 173, MEDUNSA 0204, South Africa

"Human caliciviruses have been reported to be associated with both epidemics of acute diarrhoeal illness and with sporadic cases of gastroenteritis in children. In this study, we report the identification of genogroup II small round-structured viruses or human caliciviruses associated with an outbreak of winter vomiting disease in South Africa. The virus was initially identified by electron microscopic examination of the stools and then further characterised by recombinant immunoassay with expressed capsid proteins to human caliciviruses from genogroups I and II. Both antigenically by the EIA and by sequence analysis of a region of the RNA-dependent RNA polymerase gene, the virus was shown to belong to genogroup II of the human *Caliciviridae*."

098 Stepan VM, Hammer HF, Krejs GJ. Hyperkalaemia and diarrhoea in a patient with surreptitious ingestion of potassium sparing diuretics. Eur J Gastroenterol Hepatol 1997 Oct;(10):1001-4. 35 ref, Eng. Department of Internal Medicine, Karl Franzens University, Auenbruggerplatz 15, A-8036 Graz, Austria

099 Stockmann M, Fromm M, Schmitz H, Schmidt W, Riecken E-O, Schulzke J-D. Duodenal biopsies of HIV-infected patients with diarrhoea exhibit epithelial barrier defects but no active secretion. AIDS 1998 Jan 1;12(1):43-51. 45 ref, Eng. Medizinische Klinik 1, Abt. für Gastroenterologie und Infektiologie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, D-12200 Berlin, Germany

100 Sultan F, Jin-L-L, Jobling MG, Holmes RK, Stanley SL, Jr*. Mucosal immunogenicity of a holotoxin-like molecule containing the serine-rich *Entamoeba histolytica* protein (SREHP) fused to the A_2 domain of cholera toxin. Infect Immun 1998 Feb;66(2):462-8. 31 ref, Eng. *Washington University School of Medicine, Campus Box 8051, 660 S. Euclid Ave., St. Louis, MO 63110, USA

101 Tacket CO, Taylor RK, Losonsky G, Lim Y, Nataro JP, Kaper JB, Levine MM. Investigation of the roles of toxin-coregulated pili and mannose-sensitive hemagglutinin pili in the pathogenesis of *Vibrio cholerae* O139 infection. Infect Immun 1998 Feb;66(2):692-5. 24 ref, Eng. Center for Vaccine Development, 685 West Baltimore St., Room 480, Baltimore, MD 21201, USA

"In this study, adult volunteers were fed *tcpA* and *mshA* deletion mutants of *V. cholerae* 0139 strain CVD 112 to determine the role of toxin-coregulated pili (TCP) and mannose-sensitive hemagglutinin (MSHA) in intestinal colonization. Eight of 10 volunteers who received CVD 112 or CVD 112 D*mshA* shed the vaccine strains in their stools; the geometric mean peak excretion for both groups was 1.4×10^5 CFU/g of stool. In contrast, only one of nine recipients of CVD 112 D*tcpA* shed vibrios in his stool ($P < 0.01$); during the first 24 h after inoculation, 3×10^2 CFU/g was recovered from this volunteer. All recipients of CVD 112 and 8 (80%) of the recipients of CVD 112 D*mshA* developed at least a fourfold rise in vibriocidal titer after immunization. In contrast, only one (11%) of the nine recipients of CVD 112 D*tcpA* developed a fourfold rise in vibriocidal titer ($P < 0.01$). We conclude that TCP are an important colonization factor of *V. cholerae* 0139 and probably of El Tor *V. cholerae* 01. In contrast, MSHA does not appear to promote intestinal colonization in humans."

102 Tucker AW, Haddix AC, Bresee JS, Holman RC, Parashar UD, Glass RI. Cost-effectiveness analysis of a rotavirus immunization program for the United States. JAMA 1998 May 6;279(17):1371-6. 37 ref, Eng. Viral Gastroenteritis Section, Centers for Disease Control and Prevention, US Department of Health and Human Services, 1600 Clifton Rd NE, Mailstop GO4, Atlanta, GA 30333, USA

"**Context.** - Rotavirus is the most common cause of severe diarrhea in children, and a live, oral vaccine may soon be licensed for prevention. **Objective.** - To estimate the economic impact of a national rotavirus immunization program in the United States. **Design.** - Cost-effectiveness was analyzed from the perspectives of the health care system and society. A decision tree used estimates of disease burden, costs, vaccine coverage, efficacy, and price obtained from published and unpublished sources. **Intervention.** - The proposed vaccine would be administered to infants at ages 2, 4, and 6 months as part of the routine schedule of childhood immunizations. **Main Outcome Measures.** - Total costs, outcomes prevented, and incremental cost-effectiveness. **Results.** - A routine, universal rotavirus immunization program would prevent 1.08 million cases of diarrhea, avoiding 34000 hospitalizations, 95000 emergency department visits, and 227000 physician visits in the first 5 years of life. AT $20 per dose, the program would cost $289 million and realize a net loss of $107 million to the health care system - $103 per case prevented. The program would provide a net savings of $296 million to society. Threshold analysis identified a break-even price per dose of $9 for the health care system and $51 for the societal perspective. Greater disease burden and greater vaccine efficacy and lower vaccine price increased cost-effectiveness.

Conclusions. - A US rotavirus immunization program would be cost-effective from the perspectives of society and the health care system, although the cost of the immunization program would not be fully offset by the reduction in health care cost of rotavirus diarrhea unless the price fell to $9 per dose."

103 Turbyfill KR, Mertz JA, Mallett CP, Oaks EV. Identification of epitope and surface-exposed domains of *Shigella flexneri* invasion plasmid antigen D(IpaD). Infect Immun 1998 May;66(5)1999-2006. 33 ref, Eng. Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC 20307, USA

104 Unicomb LE, Kilgore PE, Faruque ASG, Hamadani JD, Fuchs GJ, Albert MJ, Glass RI. Anticipating rotavirus vaccines: hospital-based surveillance for rotavirus diarrhea and estimates of disease burden in Bangladesh. Pediatr Infect Dis J 1997 Oct;16(10):947-51. 27 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

Objectives. Rotavirus is the most common cause of severe diarrhea in children worldwide, and a vaccine

may soon be licensed and available for use in immunization programms. To assess the need for a rotavirus vaccine in Bangladesh, we estimated the disease burden of rotavirus diarrhea from national vital statistics for births and diarrheal deaths, together with hospital surveillance data on the proportion of severe childhood diarrhea attributed to rotavirus. ***Methods***. From 1990 through 1993, hospital surveillance was conducted of a systematic, random 4% sample of >80000 patients with diarrhea who sought care each year at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). ***Results***. Rotavirus was detected in 20% (1561 of 7709) of fecal specimens from children with diarrhea <5 years of age; 92% of all cases (1436) occurred in children <2 years of age, but only 3% (50) of cases occurred in infants <3 months of age. Children infected with rotavirus were more likely to have watery stools (P<0.001), severe vomiting (P<0.001) but less severe dehydration (P=0.007) than children infected with other enteropathogens. ***Conclusions***. We estimate that in this setting, where 18% of children die by age 5 and about 25% of these succumb to diarrhea, between 14850 and 27000 of the 3 million Bangladeshi children born in 1994 will die of rotavirus by the age of 5 years, equivalent to 1 rotavirus death per 111 to 203 children. The estimated burden of rotavirus diarrhea in Bangladesh is sufficiently great to warrant field testing of rotavirus vaccines for possible inclusion in the current immunization program."

105 Washington N, Harris M, Mussellwhite A, Spiller RC. Moderation of lactulose-induced diarrhea by psyllium: effects on motility and fermentation. Am J Clin Nutr 1998 Feb;67(2):317-21. 35 ref, Eng. Department of Surgery, E. Floor, West Block, Queen's Medical Centre, Nottingham, NG7 2UH, UK

"Psyllium has been reported to inhibit lactulose-induced colonic mass movements and to benefit patients with irritable bowel syndrome, improving both constipation and diarrhea. Our aim was to define how psyllium modified the whole-gut transit of a radiolabeled lactulose-containing test meal by using gamma scintigraphy. Eight subjects participated in a randomized crossover study comparing gastric emptying and small bowel and colonic transit after consumption of 20 mL lactulose three times daily with or without 3.5 g psyllium three times daily. Psyllium significantly delayed gastric emptying: the time to 50% emptying increased from a control value of 69 ± 9 to 87 ± 11 min (` C ± SEM; P <0.05, n=8). Small bowel transit was unaltered. However, progression through the colon was delayed with an increase in the percentage of the dose at 24 h in the ascending (control group: 2 ± 3%, psyllium group: 11 ± 8%; P <0.02) and transverse colon (control group: 5 ± 12%, psyllium group: 21 ± 14%) with correspondingly less in the descending colon. Although the time for 50% of the isotope to reach the colon was not significantly different with psyllium, psyllium significantly delayed the rise in breath-hydrogen concentrations, which reached 50% of their peak at 217 ± 34 min compared with control values of 155 ± 27 min (P <0.05). Psyllium delays gastric emptying, probably by increasing meal viscosity, and reduces the acceleration of colon transit, possibly by delaying the production of gaseous fermentation products."

106 Way SS, Borczuk AC, Dominitz R, Goldberg MB*. An essential role for gamma interferon in innate resistance to *Shigella flexneri* infection. Infect Immun 1998 Apr;66(4):1342-8. 31 ref, Eng. *Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461-1602, USA

"*Shigella* spp. are the major cause of bacillary dysentery worldwide. To identify immune effectors associated with protection of the naive host during infection, the susceptibility to pulmonary *Shigella* infection of each of various mouse strains that have a targeted deletion in a specific aspect of the immune system was evaluated. Our results demonstrate that mice deficient in gamma interferon are 5 orders of magnitude more susceptible to *Shigella* than are wild-type mice, whereas mice deficient in B and T lymphocytes or in T lymphocytes alone exhibit no difference in susceptibility. Significantly lower numbers of shigellae were recovered from immuno-competent compared with gamma-interferon-deficient mice after infection. While immunocompetent mice were able to clear a sublethal *Shigella* inoculum by day 5 postinfection, progressively increasing numbers of shigellae were cultured from the lungs of gamma interferon-deficient mice over the same period. Histopathology of the lungs from immunocompetent mice infected with a sublethal *Shigella* inoculum showed mild inflammatory changes, whereas the lungs from gamma interferon-deficient mice demonstrated progressively worsening acute bronchiolitis with ulceration. Further, the time to death in gamma interferon-deficient mice correlates inversely

with the size of the *Shigella* inoculum. To identify the cellular source of gamma interferon, we infected SCID mice, T-cell-receptor-deficient mice, beige mice (a mouse strain deficient in natural killer [NK] cell activity), and mice depleted of NK cells using anti-asialo-GM_1. Each NK cell-deficient mouse strain exhibited a 10-fold-greater susceptibility to *Shigella* infection than immunocompetent mice. To test the protective effects of gamma interferon in vitro, survival of intracellular *Shigella* was examined in primary macrophages from wild-type mice, primary macrophages from gamma interferon-deficient mice, a macrophage cell line, and a fibroblast cell line. Following activation with gamma interferon, each cell type eradicated intracellular *Shigella*, while nonactivated macrophages fostered *Shigella* replication and nonactivated fibroblast cells fostered both *Shigella* replication and intercellular spread. Taken together, these data establish that NK cell-mediated gamma interferon is essential to resistance following primary *Shigella* infection."

107 Whitehouse CA, Balbo PB, Pesci EC, Cottle DL, Mirabito PM, Pickett CL. *Campylobacter jejuni* cytolethal distending toxin causes a G2-phase cell cycle block. Infect Immun 1998 May;66(5):1934-40. 36 ref, Eng. Department of Microbiology and Immunology, Chandler Medical Center, 800 Rose St., University of Kentucky, Lexington, KY 40536-0084, USA

108 Wu H-Y, Russell MW. Induction of mucosal and systemic immune responses by intranasal immunization using recombinant cholera toxin B subunit as an adjuvant. Vaccine 1998 Jan/Feb;16(2/3):286-92. 25 ref, Eng. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294-2170, USA

109 Yamaoka J-i, Yamasaki S, Kurazono H, Imamura S, Noda M, Miyai K, Takeda Y*. Loss of biological activity due to Glu_Arg mutation at residue 11 of the B subunit of cholera toxin. Microbial Pathogen 1997 Nov;23(5):297-302. 31 ref, Eng. *Research Institute, International Medical Center of Japan, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162, Japan

110 Yildiz FH, Schoolnik GK. Role of *rpoS* in stress survival and virulence of *Vibrio cholerae*. J Bacteriol 1998 Feb;180(4):773-84. 55 ref, Eng. Department of Microbiology and Immunology, Stanford University Medical School, Stanford, CA 94305-5428, USA

111 Ylitalo S, Uhari M, Rasi S, Pudas J, Leppäluoto J. Rotaviral antibodies in the treatment of acute rotaviral gastroenteritis. Acta Paediatr 1998 Mar;87(3):264-7. 29 ref, Eng. University of Oulu, Department of Paediatrics, FIN 90220, Oulu, Finland

"The efficacy of hyperimmune bovine colostrum received from cows immunized with simian rotavirus SA11 in the treatment of rotavirus gastroenteritis was compared in a randomized double-blind trial to colostrum and ordinary milk preparations. One hundred and thirty-five children aged 6-30 months with rotaviral gastroenteritis received either hyperimmune bovine colostrum (n=42), ordinary colostrum (n=42) or milk (n=41) as a 100 ml solution four times/d for 4 d. Even though the differences were in favour of hyperimmune bovine colostrum in all the variables evaluated [greater weight gain (403 vs 343 g), shorter duration of diarrhoea (3.1 vs 3.6 d), fewer stools during 6 d (11.5 vs 13.6) and fewer stools during the first 3 d (9.3 vs 11.3)], all the differences were statistically insignificant. Differences of this size are clinically unimportant in well-nourished immunocompetent children, but we suggest that the hyperimmune bovine colostrum tested in our trial had some effects in the treatment of acute rotaviral gastroenteritis and should be evaluated further."

112 Yoon SS, Katz J, Brendel K, West KP, Jr. Efficiency of EPI cluster sampling for assessing diarrhoea and dysentery prevalence. Bull WHO 1997;75(5):417-26. 22 ref, Eng. Epidemic Intelligence Service Officer, National Center for Environmental Health, Centers for Disease Control and Prevention, Mail Stop F-47,4770 Buford Highway NE, Atlanta, GA 30341-3724, USA

"This study examines the efficiency of EPI cluster sampling in assessing the prevalence of diarrhoea and dysentery. A computer was used to simulate fieldwork carried out by a survey taker. The bias and variance of prevalence estimates obtained using EPI cluster sampling were compared with those obtained using simple random sampling and cluster (stratified random) sampling. Efficiency ratios, calculated as the mean square error divided by total distance travelled, were used to compare EPI cluster sampling to simple random sampling and standard cluster sampling. EPI cluster sampling may be an appropriate low-cost tool for monitoring trends in the prevalence of diarrhoea and dysentery over time.

However, it should be used with caution when estimating the prevalence of diarrhoea at a single point in time because of the bias associated with this cluster sampling method."

113 Zimmerman RK. Risk factors for *Clostridium difficile* cytotoxin-positive diarrhea after control for horizontal transmission. Infect Cont Hosp Epidemiol 1991 Feb;12(2):96-100. 24 ref, Eng. Department of Family Practice, Box 381, University of Minnesota and Health Centre, 516 Delaware St., SE, Minneapolis, MN 55455, USA

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Molla AM, Sarker SA, Hossain M, Molla A, Greenough WB, III. Rice-powder electrolyte solution as oral therapy in diarrhoea due to *Vibrio cholerae* and *Escherichia coli*. *Lancet* 1982;1:1317-9.

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World Health Organization. Scientific Working Group. Rotavirus and other viral diarrhoeas. *Bull WHO* 1980;58:183-98.

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Mulligan ME, George WL, Roffe RD, Pinegold SM. Epidemiological aspects of *Clostridium difficile* induced diarrhoea and colitis. *Am J Clin Nutr* 1980;33(suppl II): 2533-8.

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(6) *Personal author(s)*

Walker-Smith J. Diseases of the small intestine in childhood. 2d ed. Kent: Pitman Medical, 1979:171-249.

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Vaughan VC, III, McKay RJ, Jr., Behrman RE, editors. Nelson textbook of pediatrics. 11th ed. Philadelphia: Saunders, 1979:1-9.

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Greenough WB, III. The use of antibiotics and other pharmaceutical agents in the treatment of diarrhoea - when are they necessary? *In*: Holme T, Holmgren J, Merson MH, Mollby R, editors. Acute enteric infections in children; new prospects for treatment and prevention. Amsterdam: Elsevier, 1981:333-9.

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Sack DA. Bacteriological and clinical variation of acute diarrheal disease. *In*: Mazumder DNG, Chakraborty AK, De S, Kumar AK, editors. Proceedings of the 8th National Conference on Communicable Diseases. Calcutta: All-India Institute of Hygiene and Public Health, 1980:89-93.

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Hamill PW. NCHS growth curves for children birth - 18 years - United States. Hyattsville, MD: National Center for Health Statistics, 1977. iv, 74 p. (DHEW publication no. (PHS) 78-1650) (Vital and health statistics, series 11, no. 165).

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