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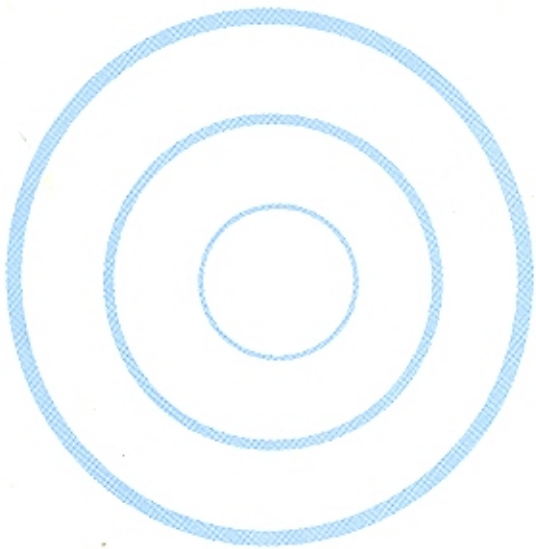

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EDITORIAL

Reporting to Our Contributors and Looking at the Future

With this issue the Journal of Diarrhoeal Diseases Research (JDDR) enters the fifteenth year of its existence. Published in a poor third-world country, the journal it addresses one of the major third-world health problems. From its inception it was meant to be a high-quality forum, first and foremost for third-world scientists, but it has greatly profited from the collaboration of scientists from more developed nations.

The authors who have collaborated to the JDDR over the years come from no less than 62 countries and all continents. They have submitted 684 papers, 434 of which were published. Not surprisingly, most of the latter submissions articles came from Asia (275 [63%]), followed in decreasing order by the Americas (North America 64 [15%]; Central and South America 14 [3%]), Africa (45 [10%]), Europe (30 [7%]), and Australia (6 [1.4%]). Thus, the Third World has contributed to about 75% of the JDDR's publications. This seems a satisfactory proportion. As could be expected, populous countries account for a major proportion of the contributions per continent: India (33%), Nigeria (47%) and the USA (78%). The last figure is very high (14% of the total) and reflects the historical close links between the JDDR's publisher, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), and academic centres in the USA. The multinational ICDDR,B staff have published 88 articles, the highest number for a single institution.

The journal has had an overall rejection rate of 37%; a low figure for a peer-reviewed medical journal. As could be expected, the rejection rate varies per continent or country, reflecting different levels in the development of medical research and personnel. Still, authors from the 22 Asian countries have about the same rejection rate as those from the 17 European ones.

The JDDR not only publishes papers. One of its special features is the '*Bibliography on Diarrhoeal Diseases.*' Fully indexed, it lists articles on diarrhoeal diseases published in as well the most prestigious international journals as in less-known third-world journals. In the past issues 6,085 articles have been cited, 3,178 with an abstract. It contains a wealth of well-focused information destined for all our readers, particularly those with no or limited access to bibliographic sources, such as Medline or Current Contents.

Electronic dissemination of information is quickly gaining in importance. The JDDR participates in this exciting new trend. The summaries of papers published in the JDDR's volume 14 are on the Internet at the website <http://www.icddrb.org.sg/journal.htm>. It is part of a more ambitious project, the PanAsia Networking, launched by the Canadian International Development Research Centre (IDRC). Since this year the full text of the JDDR (beginning Volume 11, No. 2, 1993 and onwards) is being disseminated on CD-ROM, as Asian Project [known as AHEAD (Asian Health, Environmental & Allied Databases)] participated by 8 Asian organisations including the ICDDR,B and funded by IDRC.

The Internet offers many opportunities. For third-world health care workers and medical scientists, the most promising is speed and ease of communication. In many countries of Africa and Asia postal mail is slow and unreliable while commercial courier services are too expensive. This applies to publishing in a scientific journal and particularly so when, like the JDDR, it is also a third-world journal. Submitted papers take a long time to arrive and letters of acknowledgement a long time to return. The JDDR's reviewers, the mainstay of any quality journal, frequently are from far away countries. Contacting them and receiving their opinion is again a slow process. When a paper requires changes – rare is the article that can be published 'as is' – it has to go back- and -forth from the editorial office to its author or authors. When it reaches its final form, galley-proofs have again to follow the same route. This cycle often leads to unacceptably long delays between submission and publication. Electronic mail (e-mail) can to a large extend make things easier.

When preparing to e-mail a paper to the JDDR, first send us a message indicating the following:

- Title of your paper (and number of illustrations).
- Your computer (PC or Mac) and its Operating System (e.g. Windows95)
- Your text-processing/spreadsheet software, with their version numbers.The JDDR uses PCs running under Windows95, MSWord 7.0 and Excel 5.0. If forewarned, we can usually read many other software, including Mac and MS-DOS based ones.
- The JDDR office will acknowledge your message within 2 working days in Dhaka (i.e. from Sunday to Thursday).
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- Technology permitting, we will further keep in touch by e-mail (reviewers' reports and editing). We will also let you know when we post any document, such as galleys.
- Please confirm receipt of our messages by return!
- Do not hesitate to contact us if you have problems or questions.

The JDDR invites its collaborators, authors and reviewers to use e-mail as much as possible. The editorial office accepts papers submitted by e-mail and uses it whenever possible to speed up communications. The box offers some suggestions to those interested in using the information highway. Electronic mail has its requirements. One needs a computer with appropriate software, a modem, a telephone line and an Internet provider. None of these are cheap and one must learn how to use them properly oneself. Relying on a secretary, however good, does not suffice. To state the obvious, scientists must be, and are, able to write by hand. Nowadays they also should be computer-literate. They should know how to use a text-processor or a spreadsheet, how to attach a file to an e-mail message and forward it. They also must know how to protect themselves and their correspondents again computer viruses – these can wreak havoc on any computer, and quickly discourage naïve users.

E-mail also has its limitations. An important one in the Third World is the quick ageing of the software. An older text-processor and a slow computer can still produce decent print-outs but may cause difficulties when its files are e-mailed to somebody with more recent equipment. The reverse situation is even more frustrating: unless due care is taken, recent text-processors do not communicate with older ones. In medical publishing, there are two other obstacles, illustrations and galleys. For these, postal mail is still necessary. Still, as to line drawings, such as line or bar charts, one should consider whether the data cannot be equally well presented as tables. Talking about tables, using the appropriate facilities offered by any decent text-processor is important. They may initially look complicated, but once mastered, they are much easier than trying to put plain text in tabular format, using tabs and spaces, as is still frequently done.

Whatever the means of communication, the JDDR will continue to strive for positive and constructive contacts with its collaborating authors. As most of them are not native English speakers, the editorial office will do whatever necessary to correct possible linguistic flaws. We will submit editorial corrections and suggestions related to both content and language to the authors, and discuss these with them. However, being as positive and constructive as possible does not imply that we are not as critical as required.

We welcome contributions from all disciplines interested in the many aspects of diarrhoeal diseases. These disciplines include epidemiology, ecology, clinical sciences, hygiene, microbiology, nutrition, preventive medicine and the social sciences. Given that economic and behavioural factors are at the root of the spread and the severity of most infectious diseases, including diarrhoea, they also hold the key to any successful prevention and treatment.

No journal can survive without the authors who publish in it. The JDDR is grateful to all its past contributors and looks forward to collaborating with new ones. Together with them, we intend to continue the fight against diarrhoeal diseases.

R Eeckels
Roger Eeckels
Editor-in-Chief

Vibrio cholerae O1 and O139 in Less Than Five Years Old Children Hospitalised for Watery Diarrhoea in Delhi, 1993

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ABSTRACT

In Delhi, patients with cholera-like illness are admitted to the Infectious Diseases Hospital. In 1993, rectal swabs from 836 such patients aged less than five years were examined for the presence of *Vibrio cholerae* O1 and O139. Of them, 232 (28%), 180 (22%), and 424 (51%) were found suffering from O1 cholera, O139 cholera, and non-cholera watery diarrhoea respectively. Twelve children (1.4%) excreted both *V. cholerae* O1 and O139. Both types of cholera were similarly distributed by age, with 19% of the cases occurring in infants. The findings indicate that cholera should be suspected in children aged less than two years and in infants with acute watery diarrhoea. For both serotypes, males were more represented than females; the differences were, however, not significant. Clinical features of patients with *V. cholerae* O139 and O1 were indistinguishable, except that a significantly higher percentage of the former had fever. Potential risk factors for cholera were almost equally prevalent in the families of children aged less than 5 years having either O1 or O139 cholera. The results suggest a similar mode of transmission of the two serotypes in children. By inference, the preventive and control measures are also likely to be similar.

***Key words: Cholera*;** *Vibrio cholerae;* Epidemiology

INTRODUCTION

Cholera affects more children than adults in endemic situations (1,2). At the same time, the disease may be very severe in children. Therefore, children constitute a special group in the epidemiology of cholera. Many investigators have dealt with the epidemiology of El Tor cholera in children in India (3-7), but there is limited information on the epidemiology of *Vibrio cholerae* O139 in paediatric age groups (8). We have studied the epidemiology of both O1 and O139 cholera in Delhi in 1993; some results have already been published (9). In this report, we further analyse data from children aged less than 5 years.

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PATIENTS AND METHODS

A laboratory-based sentinel surveillance system has been in effect in Delhi since 1965 to monitor the epidemiology of cholera. As a part of the system, patients with acute watery diarrhoea suspected to be cholera by clinicians are admitted to the Infectious Diseases Hospital (IDH). Rectal swabs from these patients are examined for the presence of *V. cholerae* by standard microbiological techniques. Immediately after a patient's admission, two rectal swabs are collected. One is put in Venkatraman-Ramakrishnan fluid and one in Cary-Blair medium, and both are transported within 24 hours to the laboratories of National Institute of Communicable Diseases (NICD), Delhi, about 5 kilometres 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. In 1993, all *V. cholerae* non-O1 isolates were also

tested for *V. cholerae* O139 by an antiserum prepared by Dr. Toshio Shimada, National Institute of Health, Tokyo, and supplied to NICD by Prof. Yoshifumi Takeda of Japan.

During June-September 1993, 2 trained investigators visited the IDH 2 to 3 times a week. They interviewed the parents of 264 of the 836 patients who were present in the ward at that time and asked about their education and occupation, patients' age, sex, clinical symptoms, treatment before hospitalisation, and presence of potential risk factors of cholera transmission in their families. The results of the rectal swabs were not available when these data were collected. The data were analysed for both types of cholera and non-cholera acute watery diarrhoea cases separately. Epi Info software version 5.01 was used for analysis. The data on the treatment before hospitalisation have been published (10).

RESULTS

Of the 836 patients with watery diarrhoea, 238 (28%) and 186 (22%) were excreting *V. cholerae* O1 and O139 respectively. However, twelve children (1.4%) excreted both *V. cholerae* O1 and O139. Thus, 412 patients (49%) were found suffering from cholera. As shown in Table I, *V. cholerae* O1 and O139 occurred with about the same frequency in infants. In the 1 to 4-year old children, *V. cholerae* O1 was significantly more frequent than *V. cholerae* O139.

Table I. Numbers of isolates of *V. cholerae* O1, *V. cholerae* O139, and of non-cholera cases by age. Row percentages are in parentheses and column percentages are in brackets and italics

Age (years)	No. of patients	*V. cholerae* O1	*V. cholerae* O139	Non-cholera	Total isolates*	Odds ratio**
<1	219	40 (18%) [*17%*]	41 (18%) [*22%*]	142 (64%) [*33%*]	223 (100%) [*26%*]	1.00
1 to 2	377	106 (28%) [*45%*]	73 (19%) [*39%*]	202 [*48%*]	(53%) [*48%*] [*45%*]	1.55
3 to 4	240	92 (38%) [*39%*]	72 (29%) [*39%*]	80 (33%) [*19%*]	244 (100%) [*29%*]	3.59
Total	836	238 (28%) [*100%*]	186 (22%) [*100%*]	424 (50%) [*100%*]	848 (100%) [*100%*]	

*4 cases in each age group excreted both *V. cholerae* O1 and O139.

**Chi-square for trend (both *V. cholerae* serotypes joined) = 45; df = 1; $p<10^{-6}$

Table I also shows the distribution by age of the patients. About 19% of each O1 and O139 cholera cases occurred in infants; the remaining 142 infants constituted 33% of all patients with non-cholera watery diarrhoea. With increasing age, the proportion of cholera isolates (both types combined) increased very significantly to 67% of all watery diarrhoea isolates (c^2 for trend = 45; df = 1; p<10-6).

Overall, as well as when stratified by age, cholera and non-cholera diarrhoea had an almost similar sex distribution, males being the more frequently represented (Table II). The differences were, however, not significant for the combined cholera cases (p>0.05) .

As Table III shows, cholera due to either serotypes had similar signs and symptoms, except that O139 cholera patients had significantly more often fever than O1 cholera patients (c 2 = 6.22; df = 1; p=0.013). Fever was defined as body temperature > 99 ° F or the patient feeling hot to the touch. Education and occupation of the parents did not differ significantly between O1 and O139 cholera and non-cholera diarrhoea cases. About 85% of the mothers and half of the fathers of the affected children were illiterate or educated up to a maximum of primary level. Another 12% of the mothers and 30% of the fathers had education up to matriculation level. Virtually all the mothers were housewives, whereas 40% of the fathers were labourers, and the remaining were either in service or involved in small business.

Table II. Distribution of isolates by age and sex; the percentages indicate the proportions of males and females for each of the three aetiological groups.

Age (years)	*V. cholerae* O1		*V. cholerae* O139		Non-cholera	
	Male	Female	Male	Female	Male	Female
<1*	27 (68%)	13 (32%)	20 (49%)	21 (51%)	84 (59%)	58 (41%)
1-2**	55 (52%)	51 (48%)	46 (63%)	27 (37%)	128 (63%)	74 (37%)
3-4**	50 (54%)	42 (46%)	42 (58%)	30 (42%)	47 (59%)	33 (41%)
Total	132 (55%)	106 (45%)	108 (58%)	78 (42%)	259 (61%)	165 (39%)

*2 males and females each excreted both V. cholerae O1 and O139
** 3 males and 1 female in each age group excreted both V. cholerae O1 and O139
Sex differential cholera morbidity p>0.05

Table III. Symptoms in cholera and non-cholera patients

Symptom	O1 cholera (n=107)	O139 cholera (n=46)	Non-cholera watery diarrhoea (n=111)
Diarrhoea	107 (100)	46 (100)	111 (100)
Vomiting	100 (100)	46 (100)	107 (96)
Abdominal pain	12 (11)	7 (15)	10 (9)
Fever	27 (25)*	21 (46)*	47 (42)
Mucus in stool	3 (3)	3 (7)	2 (2)
Blood in stool	2 (2)	4 (9)	3 (3)

*Difference is significant between these two groups (Chi-square = 6.22; df = 1; p = 0.013
Fever = Temperature > 99?F or hot to the touch
Figures in parentheses show percentages

Table IV. Frequency (in percentages) of potential risk factors in the affected families of under-five patients

Risk factor	Cholera cases both serotypes (n=153)	Non-cholera cases (n=111)
1. Using other than piped water for drinking.	57.5	54.1
2. Storing drinking water in wide-mouthed containers.	98.7	98.2
3. Drawing drinking water from containers by other than tap or ladle.	85.0	75.7
4. Using other than flush latrine.	66.7	68.5
5. Washing hands without using soap after defecation.	49.0	60.4
6. Washing hands with water alone before eating food.	95.4	97.3
7. Mothers illiterate or educated up to primary level	87.3*	86.1**

*n=102, **n=72

Risk behaviour occurring in the families of the patients are summarised in Table IV. Since the potential risk factors were equally present in the families of the children with O1 and O139 cholera, the two groups were joined. There was a high proportion of negative behaviour in the families of cholera as well as non-cholera diarrhoea patients, the risk factors being almost equally represented in these two groups.

DISCUSSION

V. cholerae O1 biotype El Tor and O139 entered Delhi for the first time in 1965 and 1993 respectively. In 1993, the two serotypes affected persons of different age groups. Most cases of O139 cholera were in adults, whereas significantly more children were affected by V. cholerae O1 (9). Since age is a very important variable in the epidemiology of cholera, we further analysed the data by smaller age groups to better understand the epidemiology of the disease in under-five children.

As Table I shows, V. cholerae O1 was more frequent than V. cholerae O139 in 1 to 4-year old children. Both serotypes increased with increasing age and the proportion of non-cholera watery diarrhoea decreased. Still, infants represented about 19% of both types of cholera in under-five children (Table I, column percentages). These results are at variance with the recommendations that "cholera

should be suspected when a patient older than five years develops severe dehydration from acute watery diarrhoea (usually with vomiting); or any patient older than two years has acute watery diarrhoea in an area where there is an outbreak of cholera" (11). That cholera is unusual in children aged less than two years is not in keeping with our data (12). They indicate that, during the cholera season in Delhi (April to September), cholera should be suspected in children aged less than two years, including infants, presenting with acute watery diarrhoea. Data from Calcutta and Bombay agree with our observations (4,7). Workers from Bangladesh have also shown higher attack rates of cholera in young children (2,13).

In general, female infants and children are less susceptible to infections than male children (14). Moreover, in northern India, as in many other cultures, there is a bias in favour of male children, especially in health care seeking behaviour. These factors may explain that we have seen more cases of cholera as well as of non-cholera diarrhoea in males than in females.

Cholera could not be distinguished from other sorts of acute watery diarrhoea on clinical grounds. Similarly, the clinical features of disease produced by V. cholerae O139 and O1 were indistinguishable except that a significantly higher proportion of V. cholerae O139 patients had fever. This difference was not apparent in older children and adults (9). It seems the disease produced by V. cholerae O139 is more frequently febrile in children than in adults. The potential risk factors for cholera were highly and almost equally present in the families of under-five patients having O1 or O139 cholera (Table IV). This is also true for adult patients (9). The results suggest a similar mode of transmission of two serotypes in all age groups. By inference, the preventive and control measures are also likely to be similar.

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Low Body Weight: A Simple Indicator of the Risk of Dehydration among Children with Diarrhoea

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ABSTRACT

The early identification of children at high risk of dehydration as a consequence of diarrhoea would be of great value for health care workers in developing countries. By comparing children aged less than two years with diarrhoea and moderate to severe dehydration with matched controls who had uncomplicated diarrhoea, a number of prognostic factors were assessed. Low body weight, regardless of age, was strongly associated with the risk of dehydration; using 7.0 kg as a cut-off, it had a sensitivity of 75% and a specificity of 68%. Low body weight was superior to more complex anthropometric indices, including weight for age, weight for length or length for age, and also to early signs and symptoms during the episode. By reflecting the effects of both young age and those of malnutrition, low body weight may prove to be a simple indicator for predicting dehydration among children with diarrhoea presenting at a health service.

***Keywords*:** Case-control study; Diarrhoea; Dehydration; Protein-energy malnutrition.

INTRODUCTION

Most diarrhoeal episodes in young children are self-limited. It is estimated that only 2 to 3% of these will lead to moderate or severe dehydration (1). The early identification of children with diarrhoea who are at a high risk of becoming dehydrated would allow doctors and health workers in developing countries to treat these children more intensively and to keep them under close, active surveillance.

Research carried out in Bangladesh by Black *et al.* (2) showed that the rate of stool output (in ml/kg per h) was inversely associated to the child's absolute weight, being about 30-40% higher among children weighing under 7 kg than among those weighing 7 kg or more. This finding led us to analyse the association between body weight and other anthropometric indices with the risk of dehydration among children presenting with diarrhoea. The study, therefore, was aimed at identifying prognostic factors for dehydration rather than risk factors for diarrhoea. Therefore, the control group had to include children with uncomplicated diarrhoea instead of children without diarrhoea. A case-control design was adopted, since it would be unethical to follow up prospectively children with diarrhoea without providing early appropriate treatment.

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MATERIALS AND METHODS

The study was carried out in the metropolitan area of Porto Alegre in Southern Brazil (population 2.5 million), a relatively developed area of the country. Access to health care is universal and there are many public health providers. The infant mortality rate in the city at the time of the study was approximately 30 per thousand, but there was substantial variation according to socio-economic status (3).

The cases were children aged 0 to 23 months presenting at the outpatient or emergency departments in the two largest paediatric hospitals in the city during the peak diarrhoea season. They had diarrhoea of less than eight days' duration, defined as three or more loose stools per 24 hours (for children aged less than three months, the mothers' opinion about diarrhoea was used for avoiding the misclassification of exclusively breast-fed infants who might have frequent loose stools). Cases were examined by an experienced paediatrician at admission according to a standardised protocol, and were only included if they had moderate or severe dehydration, defined as a persistent skinfold plus at least one other sign (sunken fontanelle, dry mouth and tongue, sunken eyes, reduced urinary output, weak pulse, sleepy or irritable condition) (4). Sixteen other children could not be examined at admission but were included since they presented a weight gain of 5% or more after rehydration (complete rehydration was defined as the disappearance of all signs of dehydration). Of the 200 children fulfilling the entry criteria, 192 were included in the study. Of the remaining eight children, the homes of six could not be located and there were two refusals.

One neighbourhood control was selected for each case. This was the child living nearest to the case's home, within the same broad age range (0-11 or 12-23 months), who had suffered a diarrhoea episode which started in the seven days preceding the interview. The definition of diarrhoea was the same as for the cases. The interviewers, who were university-trained nurses, examined the control children to ensure that they did not have signs of dehydration, using the same examination protocol applied to cases in the hospital. On seven occasions, it was not possible to include the first control child, and this was replaced by the next neighbourhood child. The parents of cases and controls were interviewed, and the children were weighed naked with a calibrated hanging scale (CMS Weighing Equipment, London, UK) and measured with a portable child length measurer (AHRTAG, London, UK). Cases were only weighed after complete rehydration.

Variables examined as possible prognostic factors for dehydration included weight and other anthropometric indices (weight for age, length for age and weight for length, according to the NCHS reference (5) as well as signs and symptoms reported by the mothers to have been present during the first 24 hours of the episode.

The study was sufficiently large for detecting an odds ratio of 1.5 with 80% power as significant at 5% level (two-tailed), with the prevalence of exposure among controls ranging from 20% to 65%.

The association between prognostic factors and the risk of dehydration was tested through conditional logistic regression. Odds ratios and 95% confidence intervals were calculated. The sensitivity of a prognostic factor was given by the proportion of cases with that factor while its specificity was the proportion of controls without the factor. No multivariate analysis was carried out since there was no interest in controlling confounding, but only in examining the predictive ability of each variable.

The study protocol was approved by the Medical Ethics Committees of the University of Pelotas and of the two hospitals involved.

RESULTS

A total of 192 cases and 192 controls were studied. In each group, 164 (85%) children were aged less than one year. A higher proportion of cases (16%) than controls (4%) were aged less than two months. Boys comprised 53% of the cases and 51% of the control infants. In addition to a persistent skinfold, cases presented the following signs: sunken fontanelle (70%), dry mouth and tongue (69%), sunken eyes (64%), sleepy (67%) or irritable (27%) condition, reduced urinary output (26%), and weak pulse (15%).

Table I shows that children with a post-rehydration weight of less than 5 kg were 70 times more likely to develop dehydration than those weighing 9 kg or more. A clear dose-response trend is observed.

Table I. Post-rehydration body weight and the risk of dehydration as a consequence of diarrhoea

Body weight	Cases	Controls	Odds ratio (95% C.L†)*
<5 kg	83	12	73.8 (20.7-263.4)
5 to 5.9 kg	29	23	13.8 (4.1-46.5)
6 to 6.9 kg	32	27	13.1 (4.0-42.9)
7 to 7.9 kg	19	49	3.9 (1.3-11.8)
8 to 8.9 kg	17	30	3.1 (1.1-9.4)
>=9 kg	12	51	1.0

*(p<0.001)
+(C.L =confidence limits)

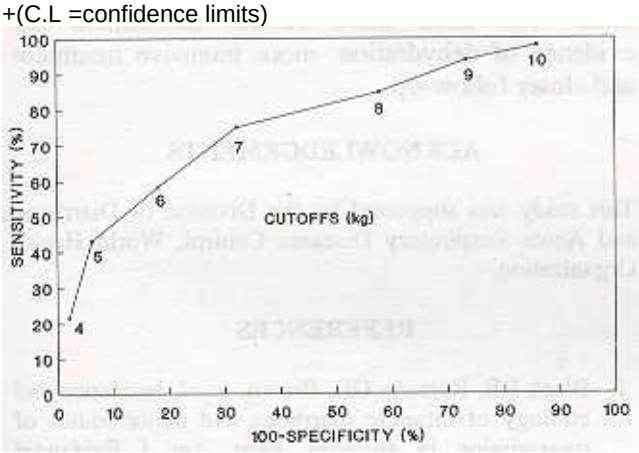

Fig. Sensitivity and specificity of post-rehydration body weight as an indicator of dehydration as a consequence of diarrhoea

Table II. Sensitivity and specificity of post-rehydration body weight and of reported signs and symptoms during the first 24 hours of diarrhoea, as indicating the risk of developing dehydration

Indicator	Sensitivity	Specificity
Post-rehydration body weight		
< 6 kg	58%	82%
< 7 kg	75%	68%
Weight for age (z-scores)		
<-2	41%	88%
<-1	78%	62%
Weight for length (z-scores)		
<-2	16%	95%
<-1	51%	85%
Length for age (z-scores)		
<-2	32%	86%
<-1	70%	58%
Signs and symptoms		
6+ stools	71%	45%
Reported fever	78%	60%
Vomiting	78%	58%
Reported fever or vomiting	66%	75%

The sensitivity and specificity of different cutoffs of body weight were calculated. The figure shows the ROC (receiver operator characteristic) curve of these combinations. Using 6 kg as a cut-off, weight has a sensitivity of 58% and a specificity of 82%, while the 7 kg cut-off yielded values of 75% and 68% respectively.

The analysis of the sensitivity and specificity of signs and symptoms presented by the children during the first 24 hours of the episode has been published elsewhere (6). Anthropometric indicators, including low weight for age, low height for age and low weight for height (7) were also associated with

the risk of dehydration. Table II shows the comparison of the sensitivity and specificity of absolute weight with those of traditional anthropometric indices and of selected clinical signs and symptoms.

The sensitivity and specificity of combinations of low weight with clinical signs and symptoms were also studied, but the performance of these combinations was not superior to that of isolated indicators. For example, the combination of a post-rehydration weight under 7 kg and vomiting had a sensitivity of 44% and a specificity of 52%, while the respective values for the combination of low weight and fever were 47% and 53%.

DISCUSSION

Children may have low body weight due to young age, to malnutrition or both. Young infants have a greater intestinal surface relative to body size, as well as a higher purging rate during diarrhoea than older children (2). Malnutrition, on the other hand, leads to impaired immune response (8). This may explain why small children are more likely to suffer dehydration as a consequence of diarrhoea. Other possible explanations may be the presence of specific aetiologic agents - such as rotavirus - which may be more common in young children and associated with both malnutrition and dehydration.

The results showed that absolute post-rehydration body weight was strongly related with the risk of dehydration as a consequence of diarrhoea. It compares well, in terms of specificity and sensitivity, with more complex anthropometric indices requiring calculations or use of a chart. It also compares positively with reported symptoms and signs.

The use of absolute body weight as an indicator of the risk of dehydrating diarrhoea has in its simplicity a clear advantage over other anthropometric indicators. It would be particularly helpful in health centres where the children could be weighed; on the other hand, the use of signs such as vomiting or fever, might be more advantageous at the household level. The latter, however, might present considerable cultural variability.

Low body weight may be associated with other socioeconomic and environmental factors which affect the risk of dehydration. The main objective of the present study, however, was to identify prognostic factors (or markers) for the risk of dehydrating diarrhoea. It is not necessary, therefore, to consider the possible confounding roles of age, socioeconomic status or other factors.

Some methodological aspects of the study deserve discussion. Cases were recruited from the outpatient and emergency departments of the two major hospitals in the study area. This may have led to selection bias. However, health facilities are widespread in the city, and the proportion of infant deaths taking place in a hospital is over 90%. When the study was carried out, ORT corners were not widespread. It is thus unlikely that many children with severe dehydration would not be seen at a hospital. The proportion of children with uncomplicated diarrhoea who were seen at a health facility was much smaller. For this reason, we have opted for selecting control children with mild diarrhoea in the community. Finally, the use of post-rehydration weight for the cases ensures that reverse causality (that is, dehydration leading to weight loss and a lower body weight, rather than low body weight increasing the risk of developing dehydration) did not affect the study results. In fact, post-rehydration weight is comparable to the weight that health workers would have measured, had these children been brought to their attention early in an episode of diarrhoea, before dehydration occurred.

One of the requirements for the diagnosis of dehydration was the presence of a persistent skinfold; this might lead to overdiagnosis among children of low weight, since this sign may also be associated with severe malnutrition. Despite the fact that the study protocol only required one other sign in addition to the persistent skinfold, all cases included had at least two other signs of dehydration, which makes such bias unlikely.

If these results are confirmed through research from other settings, they may provide health workers from developing countries with a simple way of identifying, among children with diarrhoea, those who need more careful assessment for evidence of dehydration, more intensive treatment and closer follow-up.

ACKNOWLEDGEMENTS

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RNA Profile and Structural Protein Analysis of Rotaviruses Isolated from Diarrhoeal Calves in India

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ABSTRACT

Two isolates of group A rotaviruses (CR129 and CR156) were isolated from faecal samples of diarrhoeal calves reared in two dairy farms at Hisar (Haryana, India) by using MA-104 cell lines. These isolates were compared with three standard reference bovine rotaviruses, UK, NCDV and B223, to reveal differences, if any, in their genome and protein migration profiles. The migration of RNA segment 4 of CR129 was slower than that of NCDV, but faster than that of UK. Segment 10 of CR156 moved faster than that of the reference viruses. The segments 2 and 3 co-migrated in CR129, but resolved separately in CR156. Five protein bands of size 116-120 KD (VP1), 95 KD (VP2), 90 KD (VP3/VP4), 44 KD (VP6) and 34 KD (VP7) were detected by protein analysis. No significant difference was observed in the protein profile of these two bovine rotavirus isolates by immunoblotting. However, VP1 was of approximately 116 KD size in the two isolates, compared to 120 KD in the reference strains. These findings indicate that these rotaviruses isolated from diarrhoeic Indian calves differed from the 3 reference strains.

Key Words: Rotaviruses; Diarrhoea; RNA; Viral Polyacrylamide Gel Electrophoresis

INTRODUCTION

Bovine rotaviruses (BRVs) are the major aetiological agents of neonatal calf diarrhoea worldwide. They cause economic losses to the cattle industry because of calf mortality, retarded physical growth, and treatment costs.

Rotavirus, a member of the family reoviridae, consists of 11 segments of double-stranded RNA (dsRNA) and six structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7). Morphologically, virus particles consist of three concentric proteinaceous layers. The innermost protein layer (viral core) is formed by VP2 molecules. A few copies of VP1, VP3 and 11 segments of dsRNA are internal to the VP2 layer. The middle protein layer is composed of VP6 protein (inner capsid). VP6 contributes to the group specificity. Based on it, 7 groups of rotaviruses (A through G) are recognised. The outer most protein layer (outer capsid) is composed of P or VP4 and G or VP7 proteins. Based on antigenic differences in the G and P proteins, group A rotaviruses have been classified into at least 14 different G types and 18 different P types (1).

There are only a few reports from India on isolation of BRVs by cell cultures (2, 3). Moreover, these BRV isolates have not been further characterised with respect to their antigenic structures and genome profiles. Studies on Indian BRVs have assumed added importance since bovine-like rotaviruses have been detected from faeces of asymptomatic human infants in India (4,5). We describe here two BRVs isolated from diarrhoeic calves in India and compare their RNA and protein profiles *vis-à-vis* standard strains of BRV.

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MATERIALS AND METHODS

Virus isolation and identification

Faeces samples of 1 to 30-day old calves collected from two local dairy farms during February 1994-January 1995 were analysed for the presence of rotavirus by RNA polyacrylamide gel electrophoresis (RNA-PAGE). RNA-PAGE-positive faeces samples, one from each farm, were diluted to a 20% suspension in phosphate-buffered saline (PBS), centrifuged at 1000 G for 20 minutes, and the supernatant was filtered (Millipore, 0.45 m membrane filter). After treating the samples with trypsin (20 m g/mL; Sigma, Porcine pancreas 1:250) for 30 minutes at 37 ^{o}C, these were inoculated in duplicate on washed MA-104 cell monolayers grown in milk dilution bottles. After adsorption for 1 hour at 37 ^{o}C, the inocula were removed and the cell monolayers washed with PBS before adding serum-free medium M199 (Sigma) containing 2 m g/mL of trypsin, 100 U/mL of penicillin and 100 m g/mL of streptomycin. The cultures were maintained at 37 ^{o}C and examined daily for cytopathic effects (CPE). After five days, the cells were frozen and thawed three times, and the lysate was passaged on fresh monolayers at least six times. The presence of rotavirus was confirmed by RNA-PAGE. Electron micrography of the cell culture supernatant was kindly done by Dr. M. Mathan, Department of Gastrointestinal Sciences, Christian Medical College and Hospital, Vellore (India). Virus stock was maintained at -20 ^{o}C until use.

Standard strains of BRVs, namely UK, NCDV and B223, were kindly provided by Dr. Campbell, Moredun Research Institute, Edinburgh (UK), and were grown in MA-104 cell cultures.

RNA extraction and RNA-PAGE

Rotavirus RNA was extracted from faeces sample or cell culture supernatant by phenol-chloroform extraction procedure as previously described (6). Electrophoresis was performed in 7.5% polyacrylamide slab gels with 4% stacking gel of 1 mm thickness using Laemmli's discontinuous buffer system at 100 V for 6-8 hours at room temperature in a vertical slab gel electrophoresis apparatus (Pharmacia). After that, viral RNAs were visualised by silver staining of polyacrylamide gels.

SDS-Polyacrylamide gel electrophoresis(SDS-PAGE)

Virus grown in MA-104 cells was clarified by centrifugation at 12,000 G for 15 minutes. The virus was pelleted from supernatant at 100,000 G for 2 hours (SW-28, Beckman) at 4 ^{o}C followed by ultracentrifugation through a 40% sucrose cushion (100,000 G, 2 hours, 4 ^{o}C). Similarly prepared mock-infected cell cultures served as negative controls. The pellets were dissolved in 0.05 M Tris-HC1, pH 7.5 containing 150 mM NaCl and 10 mM $CaCl_2$ (TNC). The viral proteins were denatured by boiling for 4 minutes in reducing sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% b -mercaptoethanol). Protein electrophoresis was carried out in slab gel, with 11% running and 4% stacking gel (7). The resolved proteins were stained with Coomassie brilliant blue-R250. Molecular weights of rotavirus proteins were estimated by comparing their relative mobility to those of marker proteins of known molecular weights, run on the same gel.

Immunoblotting

The proteins resolved by SDS-PAGE were electrophoretically transferred from the gel to a 0.45 m m pore-size nitro-cellulose membrane (Millipore) essentially according to the method of Towbin et al. (8). Following transfer, the membrane was blocked with 5% skimmed milk in PBS containing 0.05% Tween-20 for 2 hours. After washing, the membrane was incubated for 1 hour at 37 ^{o}C with a 1:1500 dilution of polyclonal serum against BRV strain 0510 (P5, G6) raised in guinea pigs, obtained from Dr. Y. Matsuda, Akita University Medical Centre, Japan. The membrane was then washed three times and incubated for 1 hour with horseradish peroxidase anti-guinea pig conjugate (Sigma) at a 1:3000 dilution. After washing four times, the membrane was developed with freshly prepared diamino benzidine (DAB) containing H_2O_2.

RESULTS

Rotavirus isolation

Of the 28 diarrhoeic faeces samples analysed, 10 (36%) were found positive by RNA-PAGE. Two of these samples were successfully adapted to grow in MA-104 cells. The adapted viruses produced CPE characterised by vacuolisation of cytoplasm, cell degeneration and detachment at sixth passage. CPE was observed on day 4 and 5 post-inoculation. After passage 10, complete CPE was observed on the second day. The presence of virus was confirmed by electron microscopy and RNA-PAGE (Fig. 1 and 2).

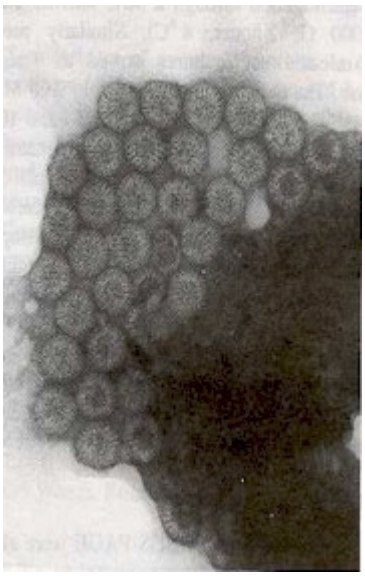


Fig. 1: Electron micrograph of rotavirus isolate CR129.The purified double-shelled particles, approximately 70 nm in diameter are shown (negative staining)

Electropherotyping

The arrangement of 11 RNA segments obtained from the two isolates by electrophoretic separation revealed a general migration pattern 4-2-3-2, similar to that found in the reference group A BRVs, UK, NCDV and B223 (Fig. 2). Electrophoretic patterns of both isolates slightly differed from the reference strains and from each other. Migration of segment 4 in case of CR129 was slower than that of strain NCDV, but faster than that of strain UK (Fig. 2.1). Further, segments 7 and 8 co-migrated and segment 9 moved as a separate band in both CR129 and CR156, whereas in case of strain NCDV, segment 7, 8 and 9 co-migrated

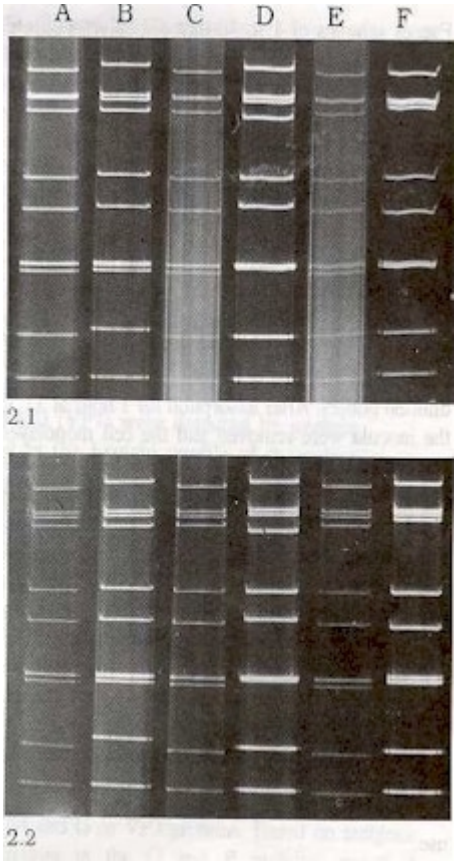
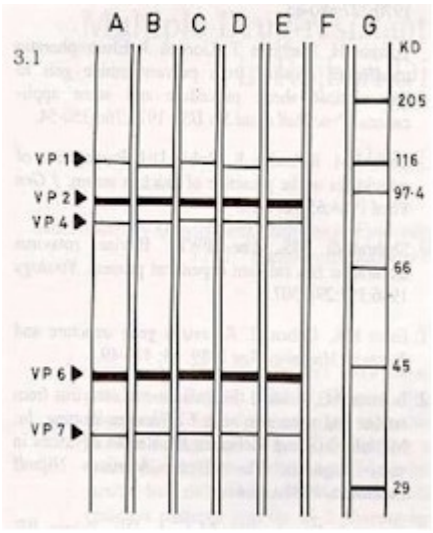


Fig.2. Comparative genome profile of Indian BRV isolates and other reference rotavviruses. (2.1) Lanes A, C and E: CR129;B: B223;D:NCDV;F:UK. (2.2) Lanes A,C and E: CR156; B:223; D:NCDV; F:UK

as a single band. On the other hand, segment 8 and 9 migrated closer in the UK strain (Fig. 2). Not only had segment 1 a higher molecular weight in strain B223, but also segment 10 of CR156 moved faster than that of the other BRVs. The two isolates differed from each other in mobility of segment 2 and 3 which were fused in CR129, but resolved separately in CR156.

SDS-PAGE and immunoblotting

Five protein bands of molecular weights 116 to 120 KD, 95 KD, 90 KD, 44 KD and 34 KD were observed in the semi-purified BRVs. These bands correspond to VP1, VP2, VP4, VP6 and VP7, respectively (Fig. 3.1). In both CR129 and CR156, VP1 was of 116 KD, compared to 120 KD in the reference strains. Interestingly, the bands of VP2 and VP6 were more intense than the other viral protein bands.



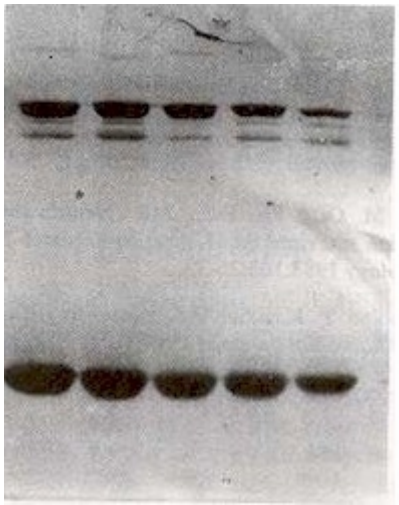


Fig. 3: Protein profile of Indian BRV isolates.(3.1) SDS-PAGE and (3.2) Western blot analysis using polyclonal serum against BRV strain 0510. Lanes A: UK; B: CR129; C: NCDV; D: CR156; E: B223; F: Cell control; G: Molecular weight marker

The viral polypeptides separated by SDS-PAGE were also transferred on to nitrocellulose membrane and probed with polyclonal serum raised against BRV strain 0510 (P5, G6). Four proteins of molecular weights 116 to 120 KD, 95 KD, 90 KD and 44 KD were detected representing VP1, VP2, VP4 and VP6, respectively (Fig. 3.2). One of the most intense bands was VP6, whereas VP1 gave only a faint reaction with immunoblotting.

DISCUSSION

Two of the ten faecal samples positive by RNA-PAGE were adapted to grow in MA-104 cell line. Although some of the factors which enhance the rate of isolation of rotaviruses have been identified, such as trypsin, chicken serum (9) and calcium (10), the isolation rate usually does not exceed fifty per cent.

The variation in electrophoretic patterns of these two isolates suggests that different electropherotypes of rotavirus may be circulating and causing diarrhoea in Indian calves. Although there is no definite correlation between RNA electropherotype and antigenic characteristics of the BRVs, the variation in RNA profile may be possibly due to differences in their G or P types (11).

The proteins of BRVs have been analysed by many investigators and the results are essentially similar with minor variations depending upon the viral strain employed, method of virus purification, protein

denaturation and polyacrylamide gel concentration used in such investigations (12-14). In this study, VP1 of the two isolates was of lower molecular weight than the reference BRVs. The protein band of 90 KD probably represents the two co-migrating proteins VP3 and VP4 as has been reported previously (15). Furthermore, VP6 of these isolates reacted strongly with the antiserum on immunoblotting. This is because VP6 is the most abundant viral protein and has strong antigenicity, reacting intensely with polyclonal sera against group A rotaviruses. VP1 gave a faint reaction; as it is a minor component of the viral core constituting about 2% of the virion mass (11). One polypeptide band of VP7 (34 KD) was missing in this immunoblot reaction. It might be because the antigenic epitopes on VP7 are conformational in nature (16), and such epitopes are likely to be lost during denaturing SDS-PAGE.

In the present study, BRV isolates differed in viral RNA electropherotypes. However, similar differences were not reflected in the protein profile and their antigenicity by immunoblotting. Additional molecular and immunological studies on these rotaviruses are now in progress to compare them with BRVs prevailing abroad as well as in other states of India.

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SHORT REPORT

Multiple Drug-resistant Shigella dysenteriae Type 1 in Rajbari District, Bangladesh

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ABSTRACT

Twenty-one *Shigellae* isolates were obtained from bloody faecal specimens of diarrhoeal patients at Rajbari District Hospital from January 1994 to June 1995, and serogrouped. Fourteen (67%) isolates belonged to the *Shigella dysenteriae* serogroup and 7 (33%) to *Shigella flexneri* serogroup. *Shigella dysenteriae* strains were further serotyped; all were *Shigella dysenteriae* 1. Each strain was tested for resistance to 6 common antimicrobial agents. The two strains had different antibiotic susceptibility patterns. The 7 *S. flexneri* showed 6 different resistant patterns and the 14 *S. dysenteriae* 1 isolates had 4 resistance patterns. One of the *S. dysenteriae* 1 isolates was resistant to all 6 antimicrobial agents; 10 to 5, and twice to a different combination of 4 antimicrobials. The 14 (100%) *S. dysenteriae* 1 strains were resistant to 3 major antimicrobial agents: ampicillin, tetracycline, and chloramphenicol; 13 (93%) were resistant to 5 agents: ampicillin, tetracycline, chloramphenicol, trimethoprim-sulphamethoxazole, and nalidixic acid. Ciprofloxacin was the only drug active against all 7 *S. flexneri* and 13 of the 14 (93%) *S. dysenteriae* 1 strains.

Key words: *Shigellae*; *Shigella dysenteriae*; *Shigella flexneri*; Drug resistance, Microbial

INTRODUCTION

Shigellosis is a major cause of diarrhoea-related morbidity and mortality among children in Bangladesh and other developing countries (1-3). It is highly endemic in Bangladesh and occasionally flares up into epidemics (4,5). The cause of bacillary dysentery may be any of the four Shigella species: *Shigella dysenteriae, S. flexneri, S. boydii*, and *S. sonnei*. The clinical spectrum of shigellosis can range from mild diarrhoea to severe dysentery (6). Severe and often life-threatening complications can occur, such as haemolytic-uraemic-syndrome, toxic megacolon, septicaemia, severe malnutrition, hyponatraemia, hypoglycaemia, and intestinal perforation (7,8).

Shigellae strains are particularly noted for their multiple drug resistance (9-11). Multiple drug resistance, identified in different areas and in different periods, is a major threat for the control of the disease. Ampicillin became widely available in the mid 1970s. Already in 1975, the first ampicillin-resistant strain of *S. dysenteriae* type 1 seen anywhere was isolated in Bangladesh (12). In 1980, 100% of *S. dysenteriae* type 1 were sensitive to co-trimoxazole, but in 1984 the situation changed abruptly. In a small study in Teknaf, Bangladesh, almost all strains were resistant to this drug (13). Although ampicillin and trimethoprim-sulphamethoxazole remained the main drugs used in Bangladesh for the treatment of shigella infections (14), further studies reported that they were no longer useful for treating shigellosis (11). The susceptibility of all *Shigellae* to nalidixic acid decreased from 99.7% in 1986 to 79.8% in 1990 (11). The present study was performed to identify the prevalent serogroups of *Shigella* organisms in Rajbari district and to determine their resistance to 6 major antimicrobials.

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MATERIALS AND METHODS

Study Area: Rajbari District Hospital, Bangladesh.

Study Period: From January 1994 to June 1995.

Collection of specimens

Rectal swabs (RS) were collected from 54 patients who attended Rajbari district hospital out-patient department with complaints of bloody diarrhoea.

Isolation and identification

The specimens were inoculated on MacConkey and Salmonella-Shigella agar media, and incubated at 37 ° C. in air for 18 hours. Suspected colonies growing overnight were further inoculated to Kliegler's iron agar and motility indole urea media to identify the strains biochemically. Biochemically identified strains were then serogrouped by the slide agglutination test using polyvalent antisera. Only *S. dysenteriae* strains were subsequently tested with specific monovalent antisera (Murex Diagnostic Ltd, England) for serotype detection.

In vitro antibiotic sensitivity testing

Antimicrobial sensitivity tests were performed by the standard disc diffusion method (15). The antibiotic discs (Bio Mérieux, France) were ampicillin (Amp, 10 m g), tetracycline (Te, 30 m g), chloramphenicol (Clm, 10 m g), trimethoprim-sulphamethoxazole (SXT, 25 m g =1.25 m g +23.75 m g), nalidixic acid (NA, 30 m g), and ciprofloxacin (Cipro, 30 m g).

The control organism was *Escherichia coli* ATCC25922, sensitive to the 6 antibiotics used.

Storage and quality control

The strains were identified biochemically and serologically in Rajbari, stored in nutrient agar slope tubes and kept in the refrigerator at 4 ° C., and finally transported to the Microbiology Laboratory of Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, for cross checking.

RESULTS

Age of the patients

Of the 54 RSs of the bloody diarrhoeal cases tested, 21 were found positive for *Shigella* organisms. Therefore, the isolation rate of *Shigellae* organisms was 39%. Of the 21 shigellosis cases, 6 were aged less than five years and 12 were less than 15 years. This confirms that children are at special risk for *Shigella*-related gastroenteritis, as found by several researchers (1,16). The distribution of *Shigellae* spp. among age groups is shown in Table I.

Table I. *Shigella* spp. distribution among different age group of shigellosis cases

Age (years)	*S. flexneri* (n=7)	*S. dysenteriae 1* (n=14)
0 to <1	1	2
1 to <5	2	1
5 to <10	0	3
10 to <15	0	3
15 to <20	1	1
20 and above	3	4

Table II. Antibiotic resistance patterns of 21 *Shigellae* isolates

Shigella spp.	Resistance patterns*	Total
S. dysenteriae 1 (n=14)	Amp Te Clm NA	1
	Amp Te Clm SXT	1
	Amp Te Clm SXT NA	10
	Amp Te Clm SXT NA Cipro	1
S. flexneri (n=7)	None	2
	Te	1
	Te Clm	1
	Amp Te Clm	1
	Amp Te Clm NA	1
	Amp Te Clm SXT	1

*Amp = ampicillin; Te = tetracycline;
Clm = chloramphenicol; Na = nalidixic acid;
Cipro = ciproflaxacin; SXT = trimethoprim-sulphamethoxazole

Serogroup and serotype

A total of 21 *Shigellae* strains of group A and B were isolated. Fourteen (67%) were *S. dysenteriae* 1 and 7 (33%) *S. flexneri*.

Antibiotic sensitivity patterns

The antibiotic resistance profile appeared different for both the strains (Table II). Six resistance profiles were found among the 7 *S. flexneri* strains (Table II). Five (71%) were resistant to tetracycline. Six (86%) were sensitive to both nalidixic acid and trimethoprim-sulphamethoxazole, and all 7 (100%) to ciprofloxacin (Table III). On the other hand, multiple drug resistance patterns were detected among the *S. dysenteriae* 1spp. (Table II). The most

prevalent antibiotic resistance pattern, found in 10 (77%) *S. dysenteriae* 1, was Amp Te Clm SXT NA. All 14 (100%), *S. dysenteriae* 1 strains were resistant to Amp, Te, Clm; 13 (93%) to 5 antimicrobials (Amp, Te, Clm, NA and SXT). Only 1 (7%) strain was resistant to ciprofloxacin (Table III, Fig.). All other organisms were sensitive to ciprofloxacin.

Table III. Percentage of *Shigellae* isolates resistance to antimicrobial agents

Antimicrobial	*S. dysenteriae* 1 (n=14)	*S. flexneri* (n=7)
Ampicillin	14 (100%)	2 (29%)
Tetracycline	14 (100%)	5 (71%)
Cloramphenicol	14 (100%)	4 (57%)
Trimethoprim-sulfa-methoxazole	13 (93%)	1 (14%)
Nalidixic acid	13 (93%)	1 (14%)
Ciprofloxacin	1 (7%)	0

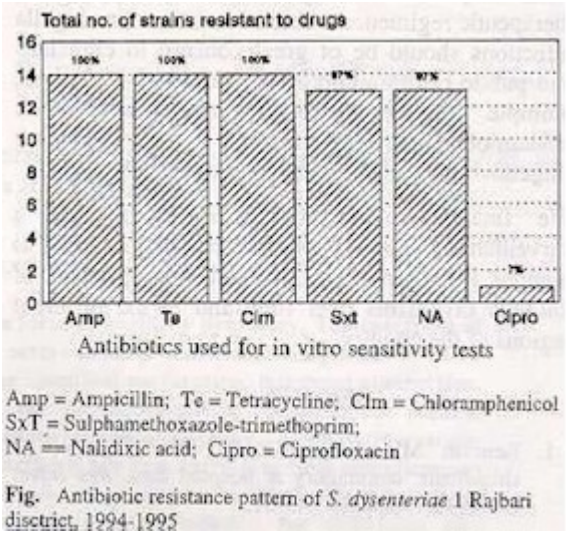

Fig. Antibiotic resistance pattern of *S. dysenteriae* 1 Rajbari disctrict, 1994-1995

DISCUSSION

Shigellae species play a major role in bloody dysentery in Bangladesh and their antibiotic sensitivity patterns are of importance to shigellosis control. The isolation rate of *Shigella* species among bloody diarrhoeal cases was 39%, this figure is almost similar to that of the observation of Stoll BJ *et al.* (17) which was reported in Bangladesh in 1982. The study reveals that *S. dysenteriae* 1 is the predominant species in the aetiology of shigellosis in that particular period (Jan 1994 - June 1995) in Rajbari district. Keusch and Bennish (18) also identified the same organisms as the main aetiological agent causing clinical dysentery in developing countries.

Tetracycline resistance (74%) among *S. flexneri* strains was an important finding in this study. Nalidixic acid and trimethoprim-sulphamethoxazole still are drugs of choice for shigellosis caused by *S. flexneri.* However, multiple drug resistance profile to 5 and even to all 6 antimicrobial agents among *S. dysenteriae* 1 spp. is an alarming finding. Ampicillin and tetracycline are not reliable for either strains. Resistance to ampicillin and trimethoprim-sulphamethoxazole has been observed in family outbreaks of *S. dysenteriae* 1 in rural Bangladesh (13,14,19). Nalidixic acid resistance among *Shigellae* in Bangladesh was also reported by Bennish *et al.* (11). Above all ciprofloxacin resistance in one of the *S. dysenteriae* 1 strain of Rajbari district is probably the first and enticing information in this study. Data on ciprofloxacin resistance to *S. dysenteriae* 1 have not yet been reported from Bangladesh. The zone diameter of inhibition for the ciprofloxacin disc was 20 mm (control range = 30-40 mm). However, inadequate facility was our constraint to perform MIC of that disc to that strain. Although ciprofloxacin was active against any of these strains, unfortunately it is not indicated in children.

The study observed dissimilar antibiotic resistance pattern among the 2 *Shigella* species, highlighting the necessity of identification serogroups along with resistance patterns for treating bacillary dysentery. Multiple drug resistance among the *Shigella* spp. studied underscores the need of sensitivity tests to determine, whenever possible, the appropriate therapeutic regimen. Antibiotic resistance in shigella infections should be of great concern to clinicians and public health authorities. Additional data about multiple drug resistance of *Shigella* strains are epidemiologically significant for planning shigellosis control strategies in Bangladesh.

We finally would recommend establishing a surveillance system throughout Bangladesh to monitor the distribution and resistance patterns of *Shigella* organisms over time and in the different regions of the country.

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CASE REPORT

Reactive Arthritis Associated with Shigella dysenteriae type 1 Infection

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SUMMARY

Shigella dysenteriae type 1 causes the most severe form of bacillary dysentery. The spectrum of illness ranges from mild watery diarrhoea to severe bloody diarrhoea. Shigellosis is often associated with intestinal complications, including intestinal perforation, intestinal obstruction, toxic dilatation of the colon, and prolapse of the rectum; systemic complications include septicaemia, hyponatraemia, hypoglycaemia, seizure, encephalopathy, haemolytic-uraemic syndrome, and malnutrition. Arthritis and conjunctivitis are rare extra-intestinal complications of shigellosis. Annually, about 110,000 patients receive treatment in the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh for diarrhoea and diarrhoea-associated illnesses, of which 11% are due to shigellosis. However, arthritis associated with shigellosis has not been reported from this population. Arthritis has been reported in association with infection due to *S. flexneri* and *S. sonnei* from other places. We are unaware of any reported case of arthritis in association with *S. dysenteriae* type 1 infections. In this report, we describe the clinical and laboratory features of a young woman who developed arthritis following *S. dysenteriae* type 1 infection.

Key words: Dysentery, Bacillary; Arthritis; *Shigella dysenteriae; Shigella flexneri; Shigella sonnei*

CASE REPORT

A woman aged 16 years was admitted to the Dhaka Hospital of the ICDDR,B with a history of bloody and mucoid diarrhoea, fever, and anorexia for 15 days. She had noticed a progressive swelling of her left elbow joint 12 days after the onset of her illness (3 days before hospitalisation). On admission, she was not dehydrated and was mildly febrile (oral temperature 38 ° C). Her systemic examination did not reveal any abnormality, except a swollen left elbow joint that was red, hot, and painful, with both active and passive movements being restricted. Before reporting to the hospital, she had been treated with inadequate doses of ampicillin, amoxycillin and furazolidone. The day following admission, she developed a painful swelling of her right ankle joint with limitations of movements. On the 4th hospital day, her left knee was similarly affected (Fig. 1 and 2). She did not have any history of joint swelling or joint pain before.

After admission, patient was treated with oral ampicillin (500 mg every 6 hours) for 48 hours. Within 48 hours of her admission *S. dysenteriae* type 1 was isolated from her stool specimen. The isolate was susceptible to nalidixic acid and mecillinam, but resistant to ampicillin, amoxycillin, furazolidone, and trimethoprim-sulphamethoxazole, as determined by disc diffusion method (1). Accordingly, ampicillin was discontinued and nalidixic acid was started (1 g every 6 hours). In addition, acetyl salicylic acid was started orally in a dose of 300 mg every 6 hours. After 5 days of therapy the swelling of the affected joints regressed and the mobility improved.

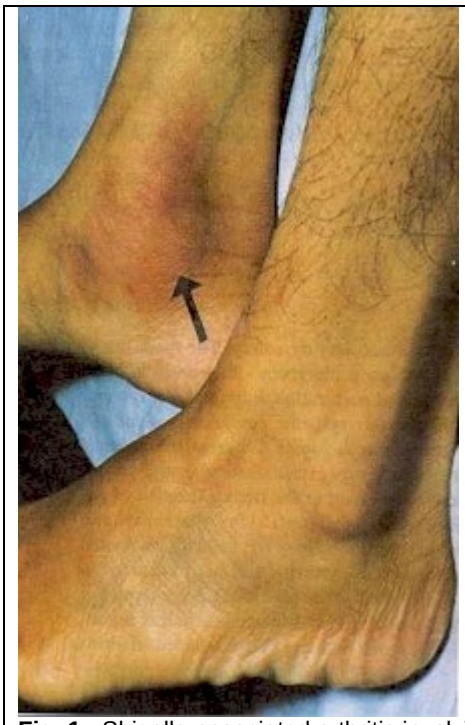

Fig. 1: Shigella-associated arthritis involving right ankle joint

Table: Results of the laboratory tests

Test	Result
Stool	
Nos. pus cells / HPF *	15-20
Nos. RBC / HPF	20-30
Ova, Parasite	Not found
Culture	*S. dysenteriae* 1
Haematology	
Haematocrit (%)	33
Total WBC/mm^3	14,000
Neutrophil	88 %
Lymphocyte	10%
Eosinophil	1%
Monocyte	1%
ESR (Wintrobe's) in 1st hour	18 mm
Serum	
ASO titre, IU	50
VDRL	Negative
Rheumatoid factor	Negative
Antinuclear antibody	Negative
Glucose (Random), mmol/L	6.3
Urea, mmol/L	1.4
Creatinine, mol/L	65
Protein, g/L	67
Na^+, mmol/L	140
Cl^-, mmol/L	102
K^+, mmol/L	3.8
HCO_3; mmol/L	26
Urine	
Protein, g/L	Trace
Nos. pus cell / HPF	8-10
Nos. RBC / HPF	1-2
Culture	No growth
Joint aspirate	
Pus cell/HPF	Occasional
Protein, g/L	39.4
Glucose, mmol/L	3.22
Culture	No growth

*HPF = high power field

Microscopic examination of three consecutive stool specimens did not reveal any ova or parasites. Other than mild anaemia, the haematological values were within normal limits (Table). The erythrocyte sedimentation rate (Wintrobe's method) was 18 mm in the first hour. The serum antistreptolysin O titre (ASO) was 50 IU, and rheumatoid arthritis (RA) factor and antinuclear antibody and VDRL tests were negative. Plasma glucose, and serum concentrations urea, creatinine, total protein, sodium, potassium, chloride, and bicarbonate were within normal limits. Trace amounts of albumin, a few pus cells, and erythrocytes were found on microscopic examination of the urine, but urine culture did not grow any organism. Chest radiograph was normal, and radiographs of her involved joints did not disclose any abnormality except soft tissue swelling. A left knee joint aspirate was straw-coloured, with normal protein and glucose concentrations. Microscopic examination of the joint aspirate showed occasional pus cells, and culture of the fluid did not grow any organisms. Colonoscopy demonstrated extensive exudation, focal areas of haemorrhages, and marked oedema of the colonic mucosa (Fig. 3) (2).

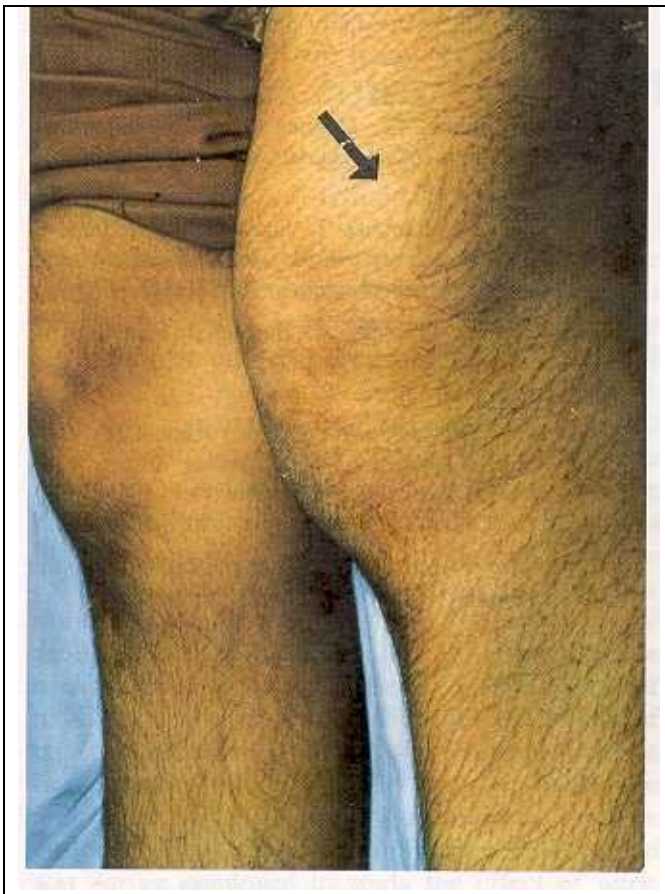

Fig. 2: Shigella-associated arthritis involving left knee joint. Notes the swelling (arrow)

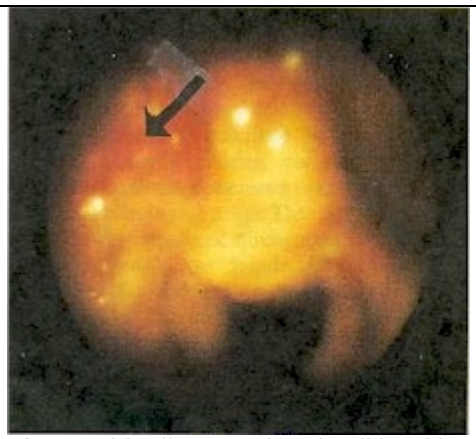

Fig. 3: Shigella-associated colitis with marked oedema and erythema (arrow)

DISCUSSION

Shigellosis is a major public health problem in developing countries (3). Among the diarrhoeal diseases, shigellosis is associated with a high death rate and is an important cause of malnutrition (4). Moreover, it is also associated with many complications that are not usually seen in other diarrhoeal diseases. Complications occur frequently with S. dysentery type 1 infection (5). Reactive arthritis, a rare complication of shigellosis, has been reported in association with S. flexneri and S. sonnei infections (6,7). This complication usually occurs on the second week of illness. However, to our knowledge this is the first case of arthritis seen in association with S. dysentery type 1 infection. The monoarticular or migratory arthritis in shigellosis usually affects large joints. It is characterised by sudden onset painful swelling of one or several joints. There is, however, no local redness or rise of temperature. The articular fluid is straw-coloured, without signs of bacterial infection. The joint fluid of this patient was similar to that described earlier with S. sonnei and S. flexneri infections. The affected joints in this patient were swollen, erythematous with a local rise in temperature. Reiter's syndrome may present with migratory joint pain, but conjunctivitis and urethritis are usually present (8). Both these signs were absent in this patient. Although the pathogenesis of arthritis in shigellosis remains unclear, it is considered to be reactive in nature. Why arthritis is seen with some serotypes of Shigella (arthritogenic strains) and not with others is unclear. It has been suggested that structural similarities between host cells and S. species may trigger the process of reactive arthritis (9). This may also be true for S. dysenteriae type 1. It has also been suggested that Shigella endotoxin may trigger prostaglandin-E mediated synovial inflammation (10).

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LETTER TO THE EDITOR

Does Nitric Oxide Induce Diarrhoea in Mice?

Sir:

It has recently been demonstrated that castor oil may cause purgation by release of nitric oxide. Agents known to inhibit the synthesis of nitric oxide significantly reduced the diarrhoeagenic response of castor oil (1). A role for nitric oxide in diarrhoea caused by magnesium sulphate has been suggested (2). However, attempts to evaluate the direct effects of nitric oxide on intestine produced conflicting results (3). Studies of stripped sheets of colonic and ileal mucosa in Ussing chamber experiments indicated a pro-secretory or antiabsorptive role of nitric oxide, while in ligated jejunal loops of anaesthetised rats in vivo a clear pro-absorptive role of nitric oxide was demonstrated (3). Such a role of nitric oxide is supported by the promotion of intestinal secretion in vivo (4) by inhibitors of nitric oxide synthase. To resolve this issue, we resorted to a different way of generation of nitric oxide within the colon by acidified sodium nitrite. This method was earlier employed to study the effect of nitric oxide on sheep urethra (5).

Details of mice, the method of scoring diarrhoea, preparation of acidified sodium nitrite, procedure of cannulation are described elsewhere (5,6). Male Swiss albino mice weighing 25-30 g were used for this study. They were fitted with intra-caecal cannulae using polyethylene tubing under light anaesthesia as described by Yagi *et al.* (7). Briefly, a small incision on the left ventral abdominal wall one centemeter below and parallel to the costal margin was made. The tip of a fine curved scissors was introduced through the incision which was extended to the length of 1 cm by blunt dissection. The tail end of the underlying caecum was identified with the help of sharply focused light. The caecum was lifted through the incision with a pair of blunt forceps. A few drops of normal saline were poured over the caecum to keep it moist. A stab wound was made with the help of a thick sharp needle in the tip of the tail of the caecum. A polyethylene tube (PE50, Clay Adams, ID 0.58 mm and O.D. 0.95 mm) of six cm length was taken. One end of the tube was gently pressed on the surface of a hot plate a few times. This procedure allowed the tip of the tube to enlarge and fold back on itself forming a collar. The collared portion of the polyethylene tube was then inserted into the stab-wound made in the tail end of the caecum and secured with a silk suture (Size O, Terd K) to prevent the removal of the tube by the mouse. This area was thoroughly cleaned with saline to eliminate the caecal contents adhering to the exterior. The caecum was replaced into the abdominal cavity along with the attached tube. The skin on the nape of the neck was then pierced with a thin sharp needle measuring eight cm in length. The needle was then passed subcutaneously upto the ventral abdominal incision and the free end of the polyethylene tube was firmly fixed onto the tip of the needle. The needle with the attached tube was then withdrawn gently so that the free end of the tube travelling subcutaneously came out of the opening at the nape of the neck. A tiny polyethylene cap was used for closing the open end for preventing leakage of the caecal contents. The incisions on the skin and muscle layers were closed, with a single proline (6/0) suture. The skin wound was then washed with cotton soaked in the antiseptic betadine. The cap at the outer tip of the tube was removed and 0.2 mL of normal saline was introduced through the cannula to wash out the caecal content present inside the tube. The cap was then replaced.

The animals were used for experimentation a week after recovery. Acidified sodium nitrite solution was prepared afresh before the experiment by dissolving the compound in concentrated hydrochloric acid. The volume was adjusted to a final concentration of 66 mM and the pH was adjusted to 2.0. Non-acidified sodium nitrite solution (pH 7.4) and dilute hydrochloric acid (pH 2.0) served as controls. After the administration of drugs the animals were placed in individual cages with floors lined by blotting paper. The time taken for the onset of passage of stool, in minutes, was noted in each animals. On the basis of consistency of stool, a numerical score was assigned as follows: 1 = normal stool, 2 = semi-solid stool, 3 = watery stool. The faecal output index (FOI) was defined as the sum of the consistency scores of all the motions passed within the observation period of 3 hours. An animal passing a stool of score 2 at least once was counted as suffering from diarrhoea. Results shown in Table I indicate that sodium nitroprusside did not induce diarrhoea in mice. This finding is similar to the observation of a lack of

secretory response in jejunal loops of rats (3). While dilute hydrochloric acid at pH 2.0 or a solution of sodium nitrite at pH 7.4 failed to elicit a diarrhoeal response, acidified sodium nitrite (pH 2.0) was able to do so. Hence diarrhoeagenic response can be attributed to nitric oxide released by acidified sodium nitrite. Ability of methylene blue or cystamine to block the diarrhoeal response indicates that it is possibly mediated through guanylate cyclase which generates cGMP, the second messenger as in the case of castor oil (6). Anthracene-9-carboxylic acid, the chloride channel blocker (8), was able to reduce the diarrhoea by 27% which was statistically significant. Hence the opening of chloride channel accounts for the diarrhoeagenic response of acidified sodium nitrite to this extent.

Table I. Effects of various treatments on induction of diarrhoea

Chemical	Stool Onset in minutes	FOI
Sodium nitroprusside	No stool	No stool
Sodium nitrite solution	No stool	No stool
Dilute hydrochloric acid	No stool	No stool
Acidified sodium nitrite	8±1	22±3
Methylene blue* + acidified sodium nitrite	No stool	No stool
Cystamine* + acidified sodium nitrite	No stool	No stool
Indomethacin* + acidified sodium nitrite	No stool	No stool
Anthracene-9-carboxylic acid* + acidified sodium nitrite	9±2	16±1

All values are expressed as mean?SEM of 6 experiments in each group. All agents were administered through intracaecal route in the following dosages in a volume of 0.2 mL. Sodium nitroprusside 2.6 mg/kg, indomethacin 120 ?moles/kg, methylene blue 0.65 ?moles/kg, cystamine 2.72 ?moles/kg, anthracene-9-carboxylic acid 6.75 ?moles/kg, dilute hydrochloric acid (pH 2), sodium nitrite solution 66 mM, acidified sodium nitrite 66 mM (pH 2). Drugs marked * were given 30 minutes prior to administration of acidified sodium nitrite.

In our experiments, indomethacin was able to completely block the diarrhoea induced by acidified sodium nitrite. In studies reporting a secretory effect of sodium nitroprusside, cyclooxygenase inhibitors were able to block such response (3). This indicates the possibility that prostaglandins may be involved in the diarrhoeal response to nitric oxide. Tissue damage associated with the preparation of sheets of intestine used in vitro was held responsible for the release of prostaglandins (3). In our studies, the acid introduced is likely to cause some tissue damage in the colon. In fact, acetic acid has been employed to produce colitis in experimental animals (9). These findings suggest but do not prove that the interaction of nitric oxide and tissue damage/production of prostaglandins is necessary for the diarrhoeal response. Interestingly, prostaglandin release and tissue injury in the intestine seem to occur in the diarrhoea induced by castor oil (10). However, the possibility of some other species of ions produced by acidified nitrite being responsible for induction of diarrhoea cannot be excluded.

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"The emergence of *Vibrio cholerae* O139 Bengal as the second aetiologic agent of epidemic cholera in October 1992 in the south Indian coastal city of Madras has shattered the long-held notion that only *V. cholerae* belonging to serogroup O1 are capable of causing epidemic (and pandemic) cholera. Within months of its appearance in Madras, *V. cholerae* O139 engulfed the entire Indian subcontinent in a series of outbreaks of cholera. It also spread to several neighbouring countries in Asia. Several western countries also reported imported cholera cases due to this organism. In the regions of the Indian subcontinent where cholera due to *V. cholerae* O1 is endemic, children are mostly susceptible because adults would have acquired at least some immunity due to earlier exposure. However, when *V. cholerae* O139 struck people in these areas, even though all age groups were affected, the disease was more prevalent in adults, which suggested that the disease is new in this population. As with O1 cholera, water and food seemed to be the vehicles of infection. Many family contacts of index cases of O139 cholera were found to be infected with *V. cholerae* O139, and in many of them, the infection was asymptomatic which is reminiscent of O1 ElTor infection. Again as with O1 ElTor infection, individuals of blood group O were more susceptible to O139 infection than those with other blood groups. In its molecular aspects, O139 vibrio resembles O1 ElTor vibrio. The virulence genes encoding cholera toxin, zonula occludens toxin, accessory cholera enterotoxin and core-encoded pilin are present in a 4.5 kb "virulence cassette" region of the chromosome as in ElTor vibrios and the expression of these virulence factors, toxin coregulated pilus (TCP) and several outer membrane proteins are found to be under the control of the master regulator ToxR as in ElTor vibrios. The iron-regulated genes involved in virulence are also found in the same locus as in ElTor vibrios. However the genes involved in the somatic antigen synthesis in O1 vibrios are found to be deleted in O139 vibrios and are replaced by a new region of chromosome which encodes the new surface antigen synthesis in O139 vibrios. When *V. cholerae* O139 emerged and caused outbreaks, the prevailing O1 ElTor vibrios virtually disappeared from most of the areas. The disappearance of ElTor vibrios, the rapid spread of O139 vibrios and the resemblance of O139 vibrios to ElTor vibrios seemed to suggest that O139 vibrios might be the causative agent of the "eighth" pandemic of cholera. However, after a year of its appearance, O139 vibrios are on the wane and O1 ElTor vibrios have re-emerged as the predominant organism, in the Indian subcontinent. Thus the immediate threat of a new cholera pandemic posed by *V. cholerae* O139 may not be as large as it first seemed. However, whether it will follow the pattern of ElTor vibrio which took approximately 60 years since its first isolation before emerging as the seventh pandemic strain of cholera, is not clear. The factor(s) contributing to the diminished isolation of O139 vibrios and the re-emergence of O1 ElTor vibrios is not understood. The vibrios might have undergone changes that would have affected their ability to survive and compete in the environment."

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"Antibody responses to the lipopolysaccharide (LPS) of shigellae were compared between children with uncomplicated and complicated *Shigella dysenteriae* 1 infection. One hundred fifteen children between 12 and 60 months of age with *S. dysenteriae* 1 infection were studied. Of these children, 42 had complications (leukemoid reaction and/or hemolytic-uremic syndrome [complicated shigellosis]) and 73 had no complications (uncomplicated shigellosis). Antibodies to the LPS of *S. dysenteriae* 1 and *Shigella flexneri* Y were measured in plasma and stools, as were total immunoglobulin A (IgA) and IgG concentrations in plasma and the total IgA concentration in stool, on enrollment and 3 to 5 days later. In the plasma, the concentrations of homologous (IgG) and heterologous (IgA) LPS antibodies on enrollment were higher in children with complicated shigellosis than in those with uncomplicated shigellosis. In stool, the concentrations on enrollment were similar between the two groups of children. There was a rise in antibody concentrations in the plasma (homologous and heterologous) and stool (homologous) between the day of enrollment and 3 to 5 days later in children with uncomplicated shigellosis but not in those with complicated shigellosis. These findings suggest that systemic stimulation is more marked in children with complications, so that a subsequent rise in plasma antibody concentrations does not occur in these children. In contrast, the lack of a rise in stool antibody concentrations in children with complicated shigellosis is suggestive of a lower-level mucosal response. Because the duration of diarrhea before enrollment influenced the homologous antibody concentrations, children were further divided into three subgroups (short [3 to 5 days], medium [6 to 9 days], and long [>9 days] diarrhea durations before enrollment). Comparisons of homologous antibody concentrations between the two groups of children following such subdivisions showed that in children with complicated shigellosis, antibody concentrations were higher in the plasma of children in the short diarrhea duration subgroup but lower in the stool of children in the medium diarrhea duration subgroup. No differences in antibody concentrations were observed in children in the other diarrhea duration subgroups. Thus, complications in shigellosis are associated with an early and strong systemic stimulation without a concomitant stimulation of the mucosal antibody response."

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"*Shigella flexneri* was the most common *Shigella* serogroup isolated in Turkey. Recently, an increase in the number of *Shigella sonnei* isolates was noticed. A retrospective analysis of 2,710 isolates, obtained from stools of Turkish children between January 1980 and September 1994, revealed that, between 1980 and 1987, *S. flexneri* was the most common subgroup. The isolation rate of *S. sonnei* increased steadily from 1987 to 1994 reaching to a peak of 78% of all isolates in 1991. The antibiotic susceptibility of 206 strains isolated in 1994 was also studied. A marked difference between the two species was observed for chloramphenicol (98% susceptibility in *S. sonnei* versus 20% in *S. flexneri,* ampicillin (90% vs. 18%), ampicillin-sulbactam (98% vs. 53%), and tetracycline (46% vs. 18%) ($p<0.001$). Susceptibility to trimethoprim-sulphamethoxazole was similar between the two groups (42% vs. 38%). All isolates were susceptible to ciprofloxacin and ceftriaxone. Comparing our results with resistance rates in 1989, a marked increase in ampicillin (from 44.1% to 82%), chloramphenicol (from 36.7% to 56%) and trimethoprim-sulphamethoxazole (from 35.8% to 62%) resistance was observed."

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"Plasma zinc, selenium, and vitamin A concentrations in 25 children with persistent diarrhoea (PD) and acute diarrhoea (AD) were determined and compared with 25 age-matched control children. Plasma retinol concentrations (PRC) and plasma zinc concentrations (PZC) (3.92 µg/dL and 79.4 µg/dL respectively) were found to be significantly lower ($p<0.05$) in children with PD. PZCs were also significantly reduced ($p<0.05$) in children with AD. However, reduction in PZC was more in PD than that in AD. There was no significant difference in PSC in children with either types of diarrhoea as compared with the control group. The results of the study showed that there was deficiency of vitamin A and zinc in diarrhoeas which needs to be correlated for proper nutritional management."

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"We prospectively compared the clinical features of cholera due to *Vibrio cholerae* O1 and *V. cholerae* O139 in 242 men 18-60 years of age, with a history of diarrhoea of 24 h or less, and moderate or severe dehydration. The antimicrobial susceptibility of all of the *V. cholerae* strains isolated from these patients was determined, and *in vitro* cholera toxin production determined for 68 isolates. On admission, the 110 patients infected with *V. cholerae* O1 significantly more often had body temperature <36^{o}C (85% vs. 66%, p=0.05), faecal leucocyte count >50/high power microscope field (40% vs. 12%), and lower mean faecal chloride content (94 vs. 103 mmol/L) than did the 132 patients infected with *V. cholerae* O139. Patients infected with *V. cholerae* O1 also initially had significantly higher median volumes of stool (13 vs. 11 mL per kg body weight per h), vomitus (1 mL/kg/h vs. nil), and intravenous fluid requirements (23 vs. 21 mL/kg/h). All *V. cholerae* O1 and O139 isolates were susceptible to ciprofloxacin, all but one were susceptible to doxycycline and erythromycin, and the majority of both serogroups were resistant to co-trimoxazole (95% and 97%, respectively). *V. cholerae* O1 and O139 susceptibilities differed for tetracycline (58% vs. 100%) and furazolidone (27% vs. 93%) (P<0.001 in both cases). The amount of cholera toxin produced *in vitro* by strains of *V. cholerae* O1 and O139 was similar, and did not correlate with stool volume. The results demonstrated that *V. cholerae* O139 does not cause more severe, or more invasive, disease than *V. cholerae* O1, as had been previously suggested, but that clinically important differences in antimicrobial susceptibility do exist among strains isolated in Bangladesh."

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"The effects of the energy density of gruels on energy and protein intake and the nutrition status of 148 hospitalized malnourished children age 3 to 24 months were determined under standardized conditions. Severely malnourished children consumed significantly more high- or low-energy-density gruels than children less affected. The feeding of high-energy-density (1.0 kcal/ml) versus low-energy-density gruels (0.5 kcal/ml) allows for a significant increase in mean energy and protein intakes from gruels: 29.4 ± 2.1 (SE) versus 18.9 ± 1.4 kcal/kg/day ($p<0.001$), and 1.10 ± 0.07 versus 0.75 ± 0.05 g/kg/day ($p<0.001$). The mean serum albumin concentration tended to increase more for children consuming high-energy-density gruels. These data emphasize the interest of high-energy-density gruels in the rehabilitation of severely malnourished children."

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"This study reports the bactericidal activity of potash alum when added to water, against various epidemic causing enteric pathogens like *Vibrio cholerae* O1, *V. cholerae* 0139 and *Shigella dysenteriae* 1 by lowering the pH of water (from 6.0 to 4.0). Growth of the enteric pathogens was monitored *in vitro* by inoculating broth cultures of the different organisms in distilled water containing increasing concentrations of potash alum and quantitatively determining the concentration of viable organisms over a 48 h period by the standard plate count method. Controls constituted cultures of each organism grown in the absence of potash alum. The pH of alum administered water was measured in each test tube before inoculation of organisms. Potash alum was found to inhibit growth (10^5 viable count per ml) of most of the organisms examined, particularly *V. cholerae* 01 and *V. cholerae* 0139 in a dose dependent fashion. Reduction of colony forming units was observed in presence of 0.25 g/dl of alum after 5 h and no growth was noticed after 24 h."

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"In an epidemiological study of human rotavirus (HRV) infections in metro Jeddah, Saudi Arabia, the relationships among subgroups, serotypes, and RNA electropherotypes of the rotavirus isolates were investigated. Of the 523 rotavirus-positive stool specimens, 245 were examined for subgroup, serotype, and electropherotype. Of these, 84 isolates were analyzed for their subgroup and RNA electropherotype specificities, 12 (14.3%) were of subgroup 1, 69 (82.1%) were of subgroup II, and 3(3.6%) were a mixture of subgroup I and II. Of the subgroup I specimens, 5 (41.7%) showed long electrophoretic migration

patterns and 7 (58.3%) showed short patterns. In subgroup II specimens, 66 (95.7%) were of long patterns and 3 (4.3%) of short patterns. The relationship between HRVS serotypes and electropherotypes was also determined for the same 245 rotavirus specimens. Of these, 36 (14.7%) exhibited short RNA patterns and 209 (85.3%) exhibited long patterns. The short pattern specimens consisted of serotype 1 (8.3%), serotype 2 (63.9%), serotype 3 and serotype 4 (2.8%) each. The long pattern specimens consisted of serotype 1 (60.3%), serotype 2 (1.4%), serotype 3 (7.2%) and serotype 4 (17.7%). Among the previous 245 specimens, subgroup specificities were available for 51 specimens. All subgroup I were of serotype 2, and all subgroup II were of serotype 1, 3 or 4. RNAs of either subgroup showed both long and short electropherotypes. No relationship could be established between subgroups or serotypes and a particular electropherotype. It seems unlikely that electropherotyping of human rotavirus (HRV) can be used for identifying the subgroups or serotypes of strains."

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"At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) Dhaka we studied the trends in cholera for the period January 1992 to May 1995. *Vibrio cholerae* 0139 Bengal emerged as a second aetiologic agent of cholera in Dhaka in January 1993. In 1993, the majority of cholera cases was due to *V. cholerae* O139, with *V. cholerae* O1 accounting for a small proportion of cases. During the latter part of the study period (Jan 1994-May 1995), *V. cholerae* O1 re-emerged as the predominant cholera strain. The predominant age group affected in endemic cholera due to *V. cholerae* O1 was children 2-9 years old, and the organism was isolated from more females than from males at all ages. In contrast, cholera due to *V. cholerae* O139 caused disease mostly in adults 15 years and older, which indicated that this organism was new in this population. As with *V. cholerae* O1, *V. cholerae* O139 was isolated from more females than males. The initial rapid emergence and predominance of *V. cholerae* 0139 was considered possibly to herald the start of the eighth pandemic of cholera. However, just after a year, the prevalence of *V. cholerae* O139 decreased dramatically with *V. cholerae* O1 resuming the role of the dominant cholera strain. The factor(s) contributing to the dramatic decline in prevalence of *V. cholerae* O139 is not well understood."

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"Based on studies showing improved absorption of hypo-osmolar oral rehydration solutions (ORS) with reduced glucose and sodium concentration, a hypo-osmolar ORS with sucrose replacing glucose (sodium 60, potassium 15, chloride 50, citrate 5, sucrose 58 $mmoll^{-1}$, calculated osmolality 198 $mOsm\ kg^{1}$) was compared with mildly hyperosmolar glucose ORS (WHO) in 46 children aged 6-30 months with acute diarrhoea and dehydration. In the hypo-osmolar sucrose ORS group (n=18) faecal output was less by 30% during the initial 24 and 48 h compared with controls, suggesting better absorption. Sucrose may be a suitable alternative to glucose in an absorption-efficient hypo-osmolar."

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"In a controlled clinical trial conducted in 34 adults with severe cholera diarrhoea, the use of a relatively dilute oral rehydration salt (ORS) solution (sodium 67, potassium 20, chloride 66, citrate 7, glucose 89 mmol/l, osmolality 249 mOsmol/kg) caused a 29% (p=0.003) reduction in stool output over the first 24 h and a 37% (p=0.001) reduction over the first 48 h compared with 29 controls who received the hyperosmolar WHO/UNICEF ORS. No controls but 3 study-group patients had marked but asymptomatic hyponatraemia (sodium <125 mmol/l) at 24 h. Twenty-four % of controls and 12% of patients receiving the dilute ORS needed unscheduled intravenous therapy for recurrence of dehydration. The ORS intake was twice the 48 h stool volume in controls and 3 times in the study group. The test ORS with a reduced glucose and sodium concentration is more efficient than the WHO/UNICEF ORS in preserving net intestinal fluid balance in severe cholera."

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047. Ghosh A, Thungapathra M, Ghosh RK. Strategies for production of a potential candidate vaccine for cholera. Indian J Med Res 1996 Jul;104:60-75. 111 ref, Eng. Institute of Microbial Technology, Sector 39-A, Chandigarh 160026, India

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"Young infants admitted to hospital for diarrhoea were studied to identify and understand the reasons for early complementary feeding and to examine its effect on nutritional status. Of 132 infants, 71 per cent were being breastfed, 24 per cent had already stopped, and 5 per cent had never been breastfed.

Complementary feeds were started by the mothers when infants' median age (range) was 27 (1-180) days. Mothers' perceptions regarding breastmilk being insufficient (53 per cent) or causing diarrhoea (19 per cent), were the major reasons for complementary feeding. The mean weight-for-age of the infants given complementary feeds before the age of 2 months was 72 per cent of the National Centre for Health statistics (NCHS) standards, compared to 82 per cent in those starting after 2 months of age (p=0.01). Similarly, the mean weight-for-length in these two groups were 86 and 91 per cent, respectively (p=0.04). Initiation of early complementary feeding is associated with infant malnutrition and this alarming trend should be strongly discouraged."

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"Inadequate handwashing after defecation and anal cleaning practices in the Indian subcontinent is an important source of faeco-oral transmission of enteric diseases. To better understand the process as traditionally practised, 90 women in semi-rural Bangladesh were observed washing hands after defecation. Several components of handwashing practices were identified: the cleaning agent, using left or both hands; frequency of rubbing hands, type and amount of water used to wash, and the drying of hands on the wearer's clothes. A subsequent experiment was conducted to assess the effect of currently practised handwashing and drying according to standardised procedure on faecal coliform count of hands. As a rubbing agent, soil was commonly used (40%); soap was used by 19% and was reported unaffordable by about 81% of the non-users. Good handwashing behaviour was positively associated with better social and economic indicators including education of the women observed. Both hands were unacceptably contaminated after traditional handwashing (the geometric mean count of left was 1,995 and right hand was 1,318 faecal coliform units/hand). After standardising the observed components of handwashing procedures the use of any rubbing agent, i.e. soil, ash or soap, produced similar acceptable cleaning. Use of a rubbing agent (e.g. soil, ash or soap), more rubbing (i.e. six times), rinsing with safer water (e.g. 2 litres of tubewell water) and drying with a clean cloth or in the air produced acceptable bacteriological results. Components of traditional handwashing practices were defined through careful observation, and experiments on handwashing with standardised components showed that efficient and affordable options for handwashing can be developed; this knowledge should be helpful in disease control programmes."

053. Hoque BA, Juncker T, Sack RB, Ali M, Aziz KMA. Sustainability of a water, sanitation and hygiene education project in rural Bangladesh: a 5-year follow-up. Bull WHO 1996;74(4):431-7. 23 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"An integrated water supply, sanitation and hygiene (WSH) education intervention project was run by the International Centre for Diarrhoeal Disease Research, Bangladesh, over the period 1983-87. In the intervention area the project provided handpumps, pit latrines, and hygiene education to about 800 households. The control population did not receive any interventions, but had access to the usual government and private WSH facilities. After 1987 no external support was provided to maintain these provisions. A cross-sectional follow-up survey, which was carried out in 1992, involved about 500 randomly selected households from the intervention and control areas. In 1992 about 82% of the pumps were still in good functional condition and of these, 94% had been functioning well in 1987. Fewer latrines were functional in 1992 (64%) than at the end of 1987 (93%). In the former intervention area about 84% of the adults were using sanitary latrines in 1992 compared with only 7% in the control area. Knowledge related to disease transmission, however, was poor and similar in both areas. People claimed that they used the WSH facilities to improve the quality of their lives. The prevalence of diarrhoeal diseases in the 1992 survey among the control population was about twice that among those in the intervention area."

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"In the present study, 36 samples positive for rotavirus by ELISA were subgrouped using cloned cDNA of gene segment 6 from SA11 (subgroup 1) and Wa (subgroup II) by dot blot hybridization and subsequently G genotyped by RT-PCR and hybridization with serotype-specific (serotypes) 1 to 4) oligonucleotide radiolabelled probes. Out of 36 samples, 7 belonged to subgroup I of which 5 were typed as G2 and two were untypeable. Twenty four samples were characterized as subgroup II of which 15 were typed as G1, 5 as G3, 2 as G4, 1 as G3 plus G4, and 1 was G untypeable. Four samples were typed as non-subgroup -I and -II, 2 of them were typed as G2 and 2 remained G untypeable. One sample which had dual (I+II) subgroup specificities was typed as G4. Further analysis revealed that subgroup II specificity was associated with G1, G3 and G4 as well as long electropherotype, whereas subgroup I specificity, with G2 and short electropherotype. There was one unusual sample which was subgroup I, G untypeable but had long electropherotype, all other samples had usual correlation of subgroup, serotype and electropherotype."

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056. Islam S, Mahalanabis D, Chowdhury AKA, Sarker SA, Wahed A, Rahman ASMH. Intestinal transport of water, sodium & glucose from an electrolyte solution with & without bicarbonate. Indian J Med Res 1996 Oct;104:254-6. 16 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"In this study we have evaluated the role of bicarbonate on water and sodium transport in normal and secreting ilea of rabbits as controversy exists regarding the inclusion of bicarbonate in oral rehydration solution (ORS). In anaesthetized rabbits 10 cm closed ileal loops were constructed and filled with 5 ml of an electrolyte solution with and without bicarbonate, which contained polyethylene glycol (PEG; mol wt 4,000) as a non-absorbable marker. The fluid was withdrawn after an hour and analyzed for PEG, sodium and glucose. Similar studies were carried out in loops one hour after exposure to 1 µg/ml of purified cholera toxin. Body temperature was maintained at 37^{o}C during the experimental by using a lamp. The mean ± SE of water and sodium absorption, with bicarbonate versus without bicarbonate, was -1.4 ± 0.1

vs -1.1 ± 0.3 ml/h/10 cm, and -340.8 ± 23.0 vs -308.4 ± 35.6 mM/h/10 cm, respectively from secreting rabbit ilea. A similar effect was observed in normal ilea. It is concluded that bicarbonate containing electrolyte solution has no additional promoting effect on water and sodium absorption in normal or secreting ilea of rabbits."

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059. José MV, Bobadilla JR, Bishop RF. Oscillatory fluctuations in the incidence of rotavirus infections by serotypes 1, 2, 3, and 4. J Diarrhoeal Dis Res 1996 Sep;14(3):194-200. 50 ref, Eng. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. Apartado Postal 70228, Mexico.

"The statistical evidence for regularity in the epidemic cycles of rotavirus infection for serotypes 1, 2, 3, and 4 was examined. Hospitalization longitudinal data of the monthly incidence of rotavirus infections from the city of Melbourne, Australia during 1977-1993 were used. Periodograms were used for exploring seasonal and longer-term cycles (interepidemic periods) of rotavirus infection. There was a satisfactory agreement between the interepidemic period estimated by means of periodograms with the one predicted by theoretical epidemiological studies. Thus, there is a clear evidence of a biennial peak in the epidemiology of rotavirus. Results of the study show an evidence of the likely existence of an interepidemic cycle of 4.6-5.2 years of duration. The finding of this interepidemic cycle was unexpected, and does not arise from the alternating incidence of the 4 serotypes since this peak appears in the periodogram of each serotype."

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061. Khan WA, Bennish ML, Seas C, Khan EH, Ronan A, Dhar U, Busch W, Salam MA. Randomised controlled comparison of single dose ciprofloxacin and doxycycline for cholera caused by *Vibrio cholerae* 01 or 0139. Lancet 1996 Aug 3;348(9023):296-300. 25 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"**Background** Effective antimicrobial therapy can reduce the duration and volume of cholera diarrhoea by half. However, such treatment is currently limited by *Vibrio cholerae* resistance to the drugs commonly prescribed for cholera, and by the difficulties involved in the administration of multi-drug doses under field conditions. Because of its favourable pharmacokinetics we thought it likely that single-dose ciprofloxacin would be effective in the treatment of cholera. **Methods** In this double-blind study treatment was either a single 1 g oral dose of ciprofloxacin plux doxycycline placebo, or a single 300 mg oral dose of doxycycline plus ciprofloxacine placebo. 130 moderately or severely dehydrated men infected with *V. cholerae* O1 and 130 infection with *V. cholerae* O139 were randomly assigned treatment. Patients stayed in hospital for 5 days. We measured fluid intake and stool volume every 6 h, and a sample of stool for culture was obtained daily. The primary outcome measures were clinical success - the cessation of watery stool within 48 h; and bacteriological success - absence of *V. cholerae* from cultures of stool after study day 2. **Findings** Among patients infected with *V. cholerae* O1, treatment was clinically successful in 62 (94%) of

66 patients who received ciprofloxacin and in 47 (73%) of 64 who receive doxycycline (difference 21% [95% CI 8-33]); the corresponding proportions with bacteriological success were 63 (95%) and 44 (69%) (27% [14-39]). Among patients infected with *V. cholerae* O139, treatment was clinically successful in 54 (92%) of 59 patients who received ciprofloxacin and in 65 (92%) of 71 who received doxycycline (<1% [-9 to 9]), and bacteriologically successful in 58 (98%) and 56 (79%), respectively (19% [9-30]). Total volume of watery stool did not differ significantly between ciprofloxacin-group and doxycycline-group patients infected with either *V. cholerae* O1 or O139. All but one of the *V. cholerae* O1 and all of the O139 isolates were susceptible in vitro to doxycycline, whereas 48 (37%) of the *V. cholerae* O1 isolates and none of the O139 isolates were resistant to tetracycline. Treatment clinically failed in 14 (52%) of 27 doxycycline-treated patients infected with a tetracycline-resistant *V. cholerae* O1 strain, compared with three (8%) of 37 patients infected with a tetracycline-susceptible strain (44%) [23-65]). **Interpretation** Single-dose ciprofloxacin is effective in the treatment of cholera caused by *V. cholerae* O1 or O139 and is better than single-dose dosycycline in the eradication of *V. cholerae* from stool. Single-dose ciprofloxacin may also be the preferred treatment in areas were tetracycline-resistant *V. cholerae* are common. In *V. cholerae*, in-vitro doxycycline susceptibilities are not a useful indicator of the in-vivo efficacy of the drug."

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"***Background***. Rotavirus (RV) diarrhea is an important cause of childhood morbidity and mortality in Bangladesh and is responsible for 24% of hospital admissions for diarrhea in children from 3 months to 2 years of age. However, the prevalence of neonatal RV infections and characteristics of RV strains infecting neonates have not been explored in Bangladesh. ***Methods***. We investigated neonates at six hospitals in Bangladesh to determine the prevalence of neonatal RV infection, to identify risk factors for infection and to characterize neonatal RV strains by reverse transcription-polymerase chain reaction. ***Results***. Of 381 neonates screened at 6 hospitals, 61 of 146 infants (42%) at 2 hospitals in Dhaka were RV-positive. Of these 62% were detected within the first 5 days of life. We found an increased risk for neonatal RV infection among infants whose mothers reported no handwashing during care of the neonate (P=0.03). Analaysis of RV strains in enzyme-linked immunosorbent assay-positive specimens identified P[6]G4 and P[6]G1 genotypes to be most common; 7% (2 of 27) of strains were nontypable. A concurrent analysis of RV strains circulating in Bangladesh suggested that RV genotypes infecting neonates had a distinct P genotype, because most community strains were P-nontypable compared with neonatal strains, which carried the P[6] genotype. ***Conclusions***. Hospitalized neonates in Dhaka have increased risk for infection with RV as early as the first week of life with strains having the unusual P[6] genotype. Our findings confirm studies in India showing that neonatal RV infection can be common and may occur with strains distinct from those circulating in the community. Neonatal RV infections could alter a child's response to the RV vaccine as well as the calculation of RV vaccine efficacy in these populations."

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"*Background*. The gastric acid barrier, an important host defence against small bowel infection, may be compromised by infection with *Helicobacter pylori*. In developing countries, *H. pylori* infection occurs early in life and prevalence of hypochlorhydria is high particularly in the malnourished, which may predispose a child to repeated gastrointestinal infection and diarrhoea. Diarrhoea being a leading cause of childhood mortality and morbidity in developing countries, we investigated the prevalence of *H. pylori* infection in children in a poor Bangladeshi community and explored its asscoiation with socieconomic and nutritional status. *Methods*. The study was conducted in a poor periurban community among 469 children aged 1-99 months. Parents were interviewed using a questionnaire. To detect active infection with *H. pylori* a ^{13}C-urea breath test was performed and weight was recorded on a beam balance with a sensitivity of 20 g. *Results*. In all, 61% of 36 infants aged 1-3 months were positive for *H. pylori* this rate dropped steadily with increasing age and was 33% in 10-15 month old children and then rose to 84% in 6-9 year olds. Overall *H. pylori* infection had no association with nutritional state of the child, or family income but the infection rate was 2.5 times higher in children of mothers with no schooling. *Conclusions*. The *H. pylori* infection rate is very high in early infancy in a poor periurban community of Bangladesh. The reason for a drop in the infection rate in late infancy is unclear but could be due to initial clearance of the infection by the body's defence mechanisms but with possible alteration of the gastric mucosa which sustains infection. Maternal education may be protective and may operate through some unidentified proximate behavioural determinants. The rate of *H. pylori* infection in infants and young children may predispose them to repeated gastrointestinal infection and diarrhoea."

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069. Martinez H, Habicht J-P, Garza C, Mota F. Clinical trial of a rice-powder oral rehydration beverage. Food Nutr Bull 1996 Jun;17(2):129-37. 65 ref, Eng. Instituto Nacional de la Nutricion, Subdireccion de Nutricion de Comunidad in Mexico City, Mexico

"A clinical trial was conducted to assess the efficacy of a rice-based gruel as a rehydration solution, identified in a previous study to be widely used by mothers during diarrhoeal episodes in their children. Seventy children under five years of age, admitted to a paediatric hospital with clinical dehydration secondary to acute diarrhoea, were randomly assigned to receive rice-based gruel (n=37) or oral rehydration salts (ORS) (n=33). The hydration status was measured on admission and hourly until discharge, for a maximum of 8 hours, after which treatment was considered to have failed in children who had not been rehydrated. Successful rehydration was achieved in 92% of the patients receiving rice-based gruel and 91% of those receiving ORS. Over 50% of all patients were rehydrated 4 to 5 hours after treatment was initiated; at these times the faecal output was statistically significantly lower in patients receiving rice-based gruel than in those receiving ORS. More patients were discharged from the study with hypernatraemia in the group receiving ORS than in the group receiving rice-based gruel, whereas the number of patients with hyponatraemia on discharge was similar in both groups. Faecal sodium concentrations were similar on admission in both groups but were statistically significantly lower at

discharge in the group receiving rice-based gruel. We concluded that rice-based gruel could be safely used as an oral rehydration solution at the community level."

070. Martinez H, Bojalil R, Guiscafre H. Promotion of oral rehydration therapy comparing a home-made rice-powder gruel and oral rehydration salts. Food Nutr Bull 1996 Jun;17(2):138-4. 44 ref, Eng. Instituto Nacional de la Nutricion, Subdireccion de Nutricion de Comunidad in Mexico City, Mexico

"Characteristics of the use of two oral rehydration solutions [rice-based gruel (RBG)] or oral rehydration salts (ORS)] were assessed in 162 mothers of children under five years of age who presented a first episode of diarrhoea during the study period, in 12 rural villages of Central Mexico. Eighty-six mothers lived in six villages randomly assigned to receive the RBG promotion and 76 lived in six villages assigned to receive the ORS promotion. The intervention strategy, relying on face-to face contact by health auxiliaries who teach mothers about the dangers of dehydration, how to recognize it, and how to prepare and feed an oral rehydration solution, closely resembled that used by the National Program for the Control of Diarrheal Diseases. Before the intervention, 42% of all mothers used RBG and 58% used ORS; 8% of mothers who used RBG and 18% of those who used ORS used the beverage for rehydration purposes. After the intervention, in the villages where RBG was promoted, 57 (66%) of the mothers used RBG and 14 (16%) used ORS. In the villages where ORS was promoted, 9 (12%) of the mothers used RBG and 58 (76%) used ORS. In both groups, all mothers used at least one other beverage (usually herbal tea) during diarrhoea, but the promoted beverages were used first. The use of the promoted beverage was higher when mothers had used it before the intervention. Eighty-six percent of mothers who prepared RBG used the promoted concentration of ingredients, whereas all mothers who prepared ORS correctly diluted one package in 1 L of water. After the intervention, 54% and 67% of mothers said they used RBG and ORS specifically to prevent dehydration."

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"An ethnographic study was conducted in 12 villages located in the central highland plateau of Mexico to explore mothers' practices for the management of acute diarrhoea, in order to identify a fluid that could be safely used as a rehydration solution for the sick child. Teas were reported to be used by 90% of 142 mothers interviewed, while rice-based beverages, which included rice water and rice gruel, were used by 77% of them; another 19% said they would accept using rice-based beverages if advised to do so. A key difference was that teas were mainly used as a treatment to stop diarrhoea, while rice-based gruels were used as a palliative to soothe the child. Rice-based beverages were selected to be further tested as a likely rehydration solution, as mothers reported using them longer than teas during the diarrhoeal episode. Their use was not subject to a complicated system of specific remedies for particular types of disease, as was the case with herbal teas. They were widely accepted and compliance was expected to be greater than with teas, as there was no expectation that rice-based beverages would shorten the duration of diarrhoea. The preparation of rice-based beverages was assessed in those mothers who mentioned using it. Fifty-nine percent of mothers used a concentration of rice between 40 and 70 g/L, which was deemed to be safe, as previous studies had successfully used 50 g/L for oral rehydration solutions."

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074. Mondal SK, Gupta PGS, Gupta DN, Ghosh S, Sikder SN, Rajendran K, Saha MR, Sircar BK, Bhattacharya SK. Occurrence of diarrhoeal diseases in relation to infant feeding practices in a rural community in West Bengal, India. Acta Paediatr 1996 Oct;85(10):1159-62. 16 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33 CIT Road Scheme XM, Calcutta 700010, India

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076. Mukhopadhyay AK, Garg S, Mitra R, Basu A, Rajendran K, Dutta D, Bhattacharya S, Shimada T, Takeda T, Takeda Y, Nair GB. Temporal shifts in traits of *Vibrio cholerae* strains isolated from hospitalized patients in Calcutta: a 3-year (1993 to 1995) analysis. J Clin Microbiol 1996 Oct;34(10):2537-43. 41 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

"This study presents results of a surveillance on cholera conducted with hospitalized patients admitted to the Infectious Diseases Hospital, Calcutta, India, from January 1993 to December 1995. The 0139 serogroup of *Vibrio cholerae* dominated in 1993 but was replaced by 01 as the dominant serogroup in 1994 and 1995. The isolation rate of *V. cholerae* non-01 non-0139 did not exceed 4.9% throughout the study period, while the isolation rate of the 0139 serogroup in 1994 and 1995 was below 9%. No temporal clustering of any non-01 non-0139 serogroup was observed. With the exception of 1 strain, none of the 64 strains belonging to the non-01 non-0139 serogroup hybridized with *ctx, zot*, and *ace* gene probes, while 97.3 and 97.7% of the 0139 and 01 strains, respectively, hybridized with all the three probes. Multiplex PCR studies revealed that all the 01 strains belonged to the ElTor biotype. There was a progressive increase in the cytotoxic response on CHO and HeLa cells evoked by culture supernatants of strains of *V. cholerae* non-01 non-0139 isolated during 1994 and 1995 compared with the response evoked by those isolated in 1993. Dramatic shifts in patterns of resistance to antibiotics between strains of *V. cholerae* belonging to different serogroups and within strains of a serogroup isolated during different time periods were observed. There was a discernible increase in the incidence of multi-drug-resistant strains of *V. cholerae* 01 isolated in 1994 and 1995 compared with that in 1993. On the basis of the results of this study, we predict the possibility of newer variants of *V. cholerae* emerging in the future."

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078. Nagamune K, Yamamoto K, Naka A, Matsuyama J, Miwatani T, Honda T. In vitro proteolytic processing and activation of the recombinant precursor of El Tor cytolysin hemolysin (Pro-HlyA) of *Vibrio cholerae* by soluble hemagglutinin/protease of *V. cholerae*, trypsin, and other proteases. Infect Immun 1996 Nov;64(11):4655-8. 21 ref, Eng. Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita, Osaka 565, Japan

079. Naik TN, Krishnan T. Rotavirus vaccine: current status & future prospects. Indian J Med Res 1996 Jul;104:76-85. 98 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

080. Nair GB. Molecular insights into the evolution & epidemiology of *Vibrio cholerae*. Indian J Med Res 1996 Jul;104:5-13. 73 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

081. Nandy RK, Albert MJ, Ghose AC. Serum antibacterial and antitoxin responses in clinical cholera caused by *Vibrio cholerae* 0139 Bengal and evaluation of their importance in protection. Vaccine 1996 Aug;14(12):1137-42. 37 ref, Eng. Department of Microbiology, Bose Institute, P-1/12, CIT Scheme VII-M, Calcutta 700054, India

"*Vibrio cholerae* 0139 Bengal strain was the causative agent of the recent epidemics of cholera in India and Bangladesh. We studied antibacterial and antitoxin immune responses in acute and convalescent phase paired sera collected from seven of these cholera patients. Significant rise in the levels of both antibacterial and antitoxin antibodies was demonstrable in the sera of convalescent cholera patients. Antibacterial antibodies, directed primarily against 0139 lipopolysaccharides (LPS), belonged to IgM class, while antitoxin antibodies were of IgG and IgA class and neutralized cholera toxin. The convalescent sera, however, showed no increase in the reactivity towards *V. cholerae* O1 whole cells or their LPS preparation. Immunoblotting experiments revealed that the convalescent, but not the acute, phase serum recognized the truncated form of LPS characteristics of 0139 strains. Convalescent serum also induced definite protection against 0139, but not 01, challenge in experimental animal model. Further studies showed that such protection was probably mediated by antibodies inhibiting intestinal colonization of 0139 organisms. These results suggest that critical difference(s) exists between the immunogenic somatic components of *V. cholerae* 01 and 0139 organisms that are of considerable importance in protection against cholera."

082. Nizami SQ, Bhutta ZA, Molla AM. Efficacy of traditional rice-lentil-yogurt diet, lactose free milk protein-based formula and soy protein formula in management of secondary lactose intolerance with acute childhood diarrhoea. J Trop Pediatr 1996 Jun;42(3):133-7. 20 ref, Eng. Department of Paediatrics, The Aga Khan University, Stadium Road, PO Box 3500, Karachi, Pakistan

"Secondary lactose intolerance is often a cause of prolongation of diarrhoeal episodes. As appropriate management of lactose intolerance is elimination of lactose from diet, expansive lactose free formulae are often prescribed in acute childhood diarrhoea without establishing diagnosis of lactose intolerance. Since cheap weaning diets made from locally available cereals have been found effective in management of persistent diarrhoea, we postulated that same weaning diet made of rice lentil and yogurt (K-Y diet) could be effectively used in management of acute childhood diarrhoea associated with secondary lactose intolerance. We compared this K-Y diet with milk protein-based lactose free and soy-protein formula. Thirty children between 3-18 months of age completed dietary trial for 72 h. Of these nine children received K-Y diet (Group A), four children received milk protein-based formula (Group B) and 11 children received soy protein formula (Group C). Stool frequency was significantly reduced in children in Group A (13 ± 6 on day 1 to 6 ± 5 on day 3) and in Group B (13 ± 5 on day 1 to 7 ± 4 on day 3), but not in Group C (13 ± 4 on day 1 to 10 ± 8 on day 3). No significant difference was observed in intake of diet, total calories intake, and fluid intake among the three groups. It is concluded that cheap weaning diet made of locally available cereals and yogurt can be used effectively in management of secondary lactose intolerance associated with acute childhood diarrhoea."

083. Oyofo BA, Mohran ZS, El-Etr SH, Wasfy MO, Peruski LF, Jr. Detection of enterotoxigenic *Escherichia coli*, *Shigella* and *Campylobacter* spp. by multiplex PCR assay. J Diarrhoeal Dis Res 1996 Sep;14(3):207-10. 10 ref, Eng. Enteric Microbiology Branch, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

"Three oligonucleotide primers were used in a polymerase chain reaction (PCR) assay for the simultaneous amplification of regions of the invasive plasmid antigen (*ipaH*) of *Shigella* spp., flagellin gene (*flaA*) of *Campylobacter* spp., and heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* (ETEC). The multiplex assay was performed using DNA extracted by a chaotropic method directly from diarrhoeal stools. The diagnostic efficacy of the assay was analyzed by agarose gel electrophoresis. This assay shows a novel approach for the diagnosis of diarrhoea caused by *Shigella* spp., ETEC, and *Campylobacter* spp."

084. Pal S, Sengupta K, Manna B, Sarkar S, Bhattacharya S, Das P. Comparative evaluation of somatic & excretory-secretory antigens of *Entamoeba histolytica* in serodiagnosis of human amoebiasis by ELISA. Indian J Med Res 1996 Jul;104:152-6. 30 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

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086. Pinfold JV, Horan NJ. Measuring the effect of a hygiene behaviour intervention by indicators of behaviour and diarrhoeal disease. Trans R Soc Trop Med Hyg 1996 Jul-Aug;90(4):366-71. 13 ref, Eng. Punjab Rural Water Supply and Sanitation Project, PO Box 1449, Lahore 540000, Pakistan

"A social marketing approach used both qualitative and quantitative methods to develop a hygiene behaviour intervention in rural north-east Thailand. Behaviours were preselected from a previous study and the intervention was designed to promote hand washing, especially before feeding a baby, cooking, eating, and after defaecation or cleaning a baby's bottom, and dish washing immediately after eating. A bacteriological indicator (enumerating faecal streptococci using a finger impression technique) was developed to measure changes in hand washing behaviour and observation (spot checks) of dirty dishes to indicate dish washing practice. There was a significance improvement in both behaviours and a significant reduction in diarrhoeal disease as a result of the intervention. However, receiving and being able to recall the intervention messages was not necessarily sufficient to ensure behaviour change, as some adults found it difficult to change old habits. Villages showing the greatest improvement tended to have a stronger sense of community than others and to have more people actively involved in the intervention."

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"A monoclonal antibody, ICT11, specific for the toxin of enterotoxigenic *Bacteroides fragilis* (ETBF) neutralized the cytotoxic effect of the toxin on human colonic cell line HT-29/C_1. In an evaluation using 115 diarrheal stool specimens and culture as the "gold standard," the assay showed a sensitivity of 85% and a specificity of 100%. An ICT11-based sandwich enzyme-linked immunosorbent assay showed a

sensitivity of 100% and a specificity of 98% for direct detection of toxin from stool samples compared with those of culture. Thus, ICT11-based assays will be useful for screening for ETBF."

089. Quick RE, Gerber ML, Palacios AM, Beingolea L, Vargas R, Mujica O, Moreno D, Seminario L, Smithwick EB, Tauxe RV. Using a knowledge, attitudes and practices survey to supplement findings of an outbreak investigation: cholera prevention measures during the 1991 epidemic in Peru. Int J Epidemiol 1996 Aug;25(4):872-8. 12 ref, Eng. Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

090. Rahman I, Shahamat M, Chowdhury MAR, Colwell RR. Potential virulence of viable but nonculturable *Shigella dysenteriae* type 1. Appl Environ Microbiol 1996 Jan;62(1):115-20. 34 ref, Eng. University of Maryland Biotechnology Institute, 4321 Hartwick Rd., Ste. 550, College Park, MD 20740, USA

"We examined a virulent strain of *Shigella dysenteriae* type 1 after induction into the viable but nonculturable (VBNC) state for its ability to (i) maintain the Shiga toxin (*stx*) gene: (ii) maintain biologically active Shiga toxin (ShT); and (iii) adhere to intestinal epithelial cells (Henle 407 cell line). PCR was used to amplify the *stx* gene from VBNC cells of *S. dysenteriae* type 1, thereby establishing its presence even when cells are in the VBNC state. VBNC *S. dysenteriae* type 1 ShT was monitored by the enzyme-linked immunosorbent assay with mouse monoclonal antibodies against the B subunit of ShT and affinity-purified rabbit polyclonal antibodies against ShT. We used the Henle 407 cell line to study the adhesive property of VBNC *S. dysenteriae* type 1 cells in a series of tissue culture experiments. Results showed that VBNC *S. dysenteriae* type 1 not only maintained the *stx* gene and biologically active ShT but also remained capable of adhering to Henle 407 cells. However, *S. dysenteriae* type 1 cells lost the ability to invade Henle 407 cells after entering the VBNC state. From results of the study, we conclude that VBNC cells of *S. dysenteriae* type 1 retain several virulence factors and remain potentially virulent, possing a public health problem."

091. Ramakrishna BS. Colonic fluid handling in health & acute diarrhoeal diseases. Indian J Med Res 1996 Jul;104:52-9. 47 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

092. Ramamurthy T, Bhattacharya SK, Nair GB, Takeda Y. Comparison of the sensitivity & specificity of a polyclonal versus monoclonal capture antibody based Bead ELISA for direct detection of cholera toxin from stool specimens. Indian J Med Res 1996 Jul;104:125-8. 11 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

093. Rennels MB. Influence of breast-feeding and oral poliovirus vaccine on the immunogenicity and efficacy of rotavirus vaccines. J Infect Dis 1996 Sep;174(1 Suppl):S107-11. 35 ref, Eng. Department of Pediatrics, University Hospital, Room N5W70, 22 S. Greene St., Baltimore, MD 21201, USA

094. Rhoads JM, Gomez GG, Chen W, Goforth R, Argenzio RA, Neylan MJ. Can a super oral rehydration solution stimulate intestinal repair in acute viral enteritis? J Diarrhoeal Dis Res 1996 Sep;14(3):175-81. 25 ref, Eng. Department of Pediatrics, Division of Gastroenterology, School of Medicine, University of North Carolina at Chapel Hill, Campus Box 7220, Chapel Hill, NC 27599-7220, USA

"This study was designed to screen several treatments for their effects on mucosal repair in an established model of piglet rotavirus enteritis. Six ingredients selected to facilitate repair were added to the oral rehydration solution (ORS) and subsequently to the diet: L-glutamine (GLN); rice solids; a soluble fiber (carboxymethylcellulose); nucleotides; polyamines; and fructooligosaccharides. Rotavirus infection

consistently induced a watery diarrhoea lasting 5 to 10 days and produced a jejunal mucosal lesion which was maximal at 3 days, post-inoculation (manifested by a reduction of villus surface area to 30% to 50% of normal). By 7 to 10 day post-inoculation, the villus surface area returned to 50% to 80% of normal. None of the supplemental ingredients added to the ORS had a significant effect in either shortening the clinical illness or in stimulating recovery of the affected mucosa. It is concluded that several types of "Super ORS" are ineffective in enhancing repair in viral enteritis in neonatal colostrum-deprived piglets. These results do not rule out beneficial effects of the additives tested in subjects with more extensive intestinal damage, in those who receive breast milk, or in those with bacterial enteritis."

095. Ross-Degnan D, Soumerai SB, Goel PK, Bates J, Makhulo J, Dondi N, Sutoto, Adi D, Ferraz-Tabor L, Hogan R. The impact of face-to-face educational outreach on diarrhoea treatment in pharmacies. Health Pol Plann 1996 Sep;11(3):308-18. 35 ref, Eng. Department of Ambulatory Care and Prevention, Harvard Medical School, 126 Brookline Avenue, Suite 203, Boston, MA 02215, USA

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097. Sarkar BL, Chakrabarti AK, Koley H, Chakrabarti MK, De SP. Biological activity & interaction of *Vibrio cholerae* bacteriophages in rabbit ileal loop. Indian J Med Res 1996 Jul;104:139-41. 6 ref, Eng. National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

098. Schmidt H, Karch H. Enterohemolytic phenotypes and genotypes of Shiga toxin-producing *Escherichia coli* 0111 strains from patients with diarrhea and hemolytic-uremic syndrome. J Clin Microbiol 1996 Oct;34(10):2364-7. 16 ref, Eng. Institut für Hygiene und Mikrobiologie der Universitat Würzburg, D-97080 Würzburg, Germany

099. Singh J, Bora D, Khanna KK, Jain DC, Sachdeva V, Sharma RS, Verghese T. Epidemiology and transmission of *V. cholerae* O1 and *V. cholerae* O139 infections in Delhi in 1993. J Diarrhoeal Dis Res 1996 Sep;14(3):182-6. 15 ref, Eng. Institute of Communicable Diseases, 22-Shamnath Marg, Delhi 110054, India.

"In 1993, rectal swabs from clinically suspected cases of cholera admitted to the Infectious Diseases Hospital (IDH), Delhi were examined for of *Vibrio cholerae* O1 and O139. Epidemiological data of 396 cholera cases were collected before the patients' discharge from IDH. Of the 1528 laboratory-confirmed cholera cases, 46% and 54% were caused by serotype 01 and 0139 respectively. Both serotypes appeared and disappeared simultaneously, and peaked during the same time for the year. However, the two serotypes affected persons of different age groups; about 65% of the 01 cases occurred in children aged less than 10 years, whereas this age group accounted for 40% of the cases due to *V. cholerae* 0139. Although there were some focal outbreaks due to serotype 0139, both serotypes had almost similar geographical distributions. Important risk factors for transmission of cholera were almost equally prevalent in the majority of both types of cholera cases. Since the seasonality, geographical distribution, and risk factors for transmission were similar for both serotypes, the study indicates that the preventive and control measures are also likely to be similar. The study also shows that the emergence of *V. cholerae* 0139 in 1993 did not affect the incidence, seasonality, and epidemiology of endemic *V. cholerae* 01 El Tor strains in Delhi."

100. Sodemann M, Jakobsen MS, Molbak K, Martins C, Aaby P. Maternal perception of cause, signs and severity of diarrhoea in a suburban West African community. Acta Paediatr 1996 Sep;85(9):1062-9. 16 ref, Eng. Department of Epidemiology and Social Medicine, University of Aarhus, Norrebrogade 44, Building 2C, DK-8000 Aarhus C, Denmark

"With the aim of investigating perceived morbidity and care seeking behaviour, a household survey was conducted, where 319 episodes of diarrhoea were followed by interviews every second day. The chance of consultation increased with the number of symptoms reported by the mother. The appearance of the eyes and how the child breastfed were early warnings that mothers recognized best. By contrast, there was an 80% reduction in the likelihood of seeking consultation when the mother perceived the diarrhoea as caused by teeth eruption (n=96). Children with "teething diarrhoea" were, however, just as likely to develop signs of dehydration as children with non-teething diarrhoea. We conclude that health education concerning diarrhoea should emphasize early signs of dehydration and the discouragement of "teething" as an explanation of natural diarrhoea."

101. Sreedharan A, Jayshree RS, Sridhar H. Cryptosporidiosis among cancer patients: an observation. J Diarrhoeal Dis Res 1996 Sep;14(3):211-3. 14 ref, Eng. Department of Microbiology, Kidwai Memorial Institute of Oncology, Hosur Road, Bangalore 560029, India

"The incidence of cryptosporidiosis was studied in 560 cancer patients presenting with symptoms of diarrhoea. *Cryptosporidium* oocysts were detected in the stool specimens by modified acid fast staining. Blood was examined for HIV antibodies from patients whose stool specimens were positive for *Cryptosporidium* oocysts. Oocysts were detected in 7 of the 560 (1.3%) patients who were receiving chemotherapy and had diarrhoea; in five of them the symptoms were acute, while two patients had chronic diarrhoea. Of these seven patients, five had cancer of haemopoetic system, one had cancer of cervix and one had uterine sarcoma. None of the patients positive for oocysts were positive for HIV antibodies, ruling out the possibility of an opportunistic infection due to HIV infection."

102. Steinbach G, Lupton J, Reddy BS, Lee JJ, Kral JG, Holt PR. Calcium carbonate treatment of diarrhoea in intestinal bypass patients. Eur J Gastroenterol Hepatol 1996 Jun;8(6):559-62. 31 ref, Eng. The University of Texas Md Anderson Cancer Center, 1515 Holcombe Blvd, Houston, Texas 77030, USA

103. Tamura K, Sakazaki R, Murase M, Kosako Y. Serotyping and categorisation of *Escherichia coli* strains isolated between 1958 and 1992 from diarrhoeal diseases in Asia. J Med Microbiol 1996 Nov;45(5):353-8. 42 ref, Eng. Department of Bacteriology, National Institute of Health, Toyama, Shinjuku-ku, Tokyo 162, Japan

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105. Taylor DN, Echeverria P. Enteric infections including travelers' diarrhea. Curr Opin Infect Dis 1996 Oct;9(5):340-5. 56 ref, Eng. Armed Forces Research Institute of Medical Sciences, 315/8 Rajvithi Road, Bangkok 10400, Thailand

106. Teka T, Faruque ASG, Fuchs GJ. Risk factors for deaths in under-age-five children attending a diarrhoea treatment centre. Acta Paediatr 1996 Sep;85(9):1070-5. 31 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"Few case-control studies have examined possible risk factors for diarrhoeal deaths in under-age-five children in the developing countries. We analysed data from the surveillance system of our diarrhoea

treatment centre/hospital for the period 1990-94 on 928 children less than 5 years of age. In univariate analysis, 11 factors were significantly associated with death: lack of breastfeeding, severe malnutrition, complicated diarrhoea, pneumonia, xerophthalmia, duration of diarrhoea 7-14 days, moderate or severe dehydration, recent history of measles, *Shigella flexneri* infection, maternal illiteracy, and very low household income. Rotavirus diarrhoea was negatively associated with fatal outcome. In the assessment of severe malnutrition, weight-for-height measurement discriminated mortality risk better than weight-for-age or height-for-age indices. Only two factors retained their significance, severe malnutrition and non-breastfeeding in the multivariate analysis with adjusted odds ratio (95% confidence interval) of 84.2 (9.1, 775.9) and 4.2 (1.3, 13.2) respectively."

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109. Treem WR, Ahsan N, Kastoff G, Hyams JS. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. J Pediatr Gastroenterol Nutr 1996 Oct;23(4):280-6. 39 ref, Eng. Division of Pediatric Gastroenterology and Nutrition, Duke University Medical Center, PO Box 3009, Durham, North Carolina 27710, USA

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infections. N Engl J Med 1996 Oct 3;335(12):1022-8. 44 ref, Eng. Department of Infectious Diseases, Instituto Nacional de la Nutricion, Vasco de Quiroga no. 15, Delegacion Tlalpan, Mexico City 14000, DF, Mexico

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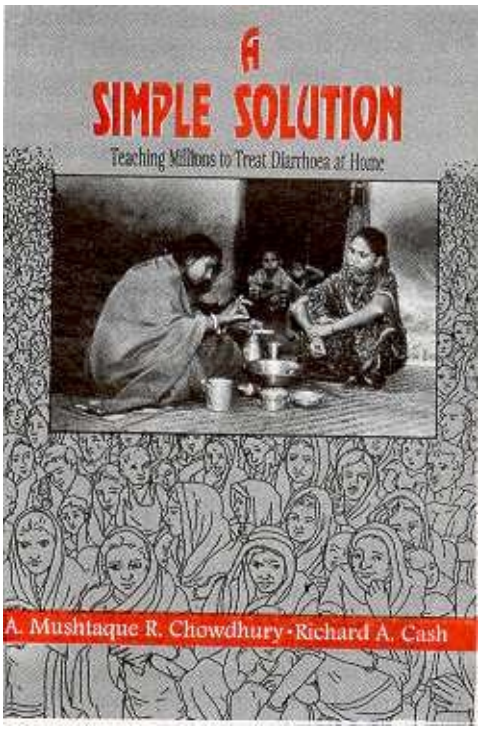
BOOK REVIEW

A Simple Solution: Teaching Millions to Treat Diarrhoea at Home

by A Mushtaque R Chowdhury and Richard A Cash

University Press Ltd, Dhaka, Bangladesh, 1996. xxxiii + 149 p.
Price: Tk 225.00 (hard cover)



Diffusion of an innovation, whether technological or philosophical, and whether originating from within the masses or from the result of research and further development, undergoes a long social process before it is widely adopted. So did oral rehydration solution (ORS), a simple substitute to the intravenous saline injection for patients with cholera and other severe diarrhoeal diseases.

The discovery of ORS, often termed "the miracle solution." has been described in 1978 by *The Lancet* as "potentially the most important medical advance [of] this century." ORS, if properly used, can save over one million lives and billions of dollars every year. Still, to fulfil its potential, it must be accepted by countless mothers and fathers, or, as Chowdhury and Cash aptly put it: it must become part of the culture. How this aim has been pursued by a national non-governmental organisation in one of the poorest countries of Asia is the subject of A Simple Solution: Teaching Millions to Treat Diarrhoea at Home. This fascinating book first briefly describes the earliest clinical applications of oral rehydration therapy (ORT) in Bangladesh and in Calcutta. Then it documents in detail the various phases of the diffusion of ORT in Bangladesh by one non-governmental organisation – the Bangladesh Rural Advancement Committee (BRAC).

A special feature of the book is that both authors have been directly involved in the events and experiences they describe, unlike those authors who have to use second-hand information. Dr. Mushtaque Chowdhury has been working with the Bangladesh Rural Advancement Committee (BRAC) since 1977, and is its Director of Research since 1992. He was the evaluation manager of BRAC's Oral Therapy Extension Programme (OTEP) and thus has been instrumental in the diffusion of ORT throughout his country. Dr. Richard Cash, with his colleagues, conducted the first successful clinical trials of oral rehydration therapy in 1967-68 at the then Cholera Research Laboratory in Dhaka. Now a lecturer at the Harvard School of Public Health, Dr. Cash is still actively involved in the efforts to expand the use of ORS worldwide.

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and its predecessor – the Cholera Research Laboratory – have received more credit for the clinical use of ORS than for any of their work. BRAC, now internationally acclaimed, has gone an important step further. Against many odds, BRAC realised the importance of the diffusion of ORS throughout the whole of Bangladesh, with its 115 million inhabitants. The story of this truly remarkable achievement is aptly described in the book. It is specially interesting that the readers can follow BRAC's thinking process, its constant self-criticism and in-house evaluation, its adaptability to local culture and customs – all key elements to its success. No less important were the hiring and training of young female field workers, their constant supervision, and the

considerable attention given to staff development. In 1979 BRAC started to teach mothers (how to use ORT) from 245 households in Sulla, a remote low-lying area in north-east Bangladesh. By 1990 it had reached 12,000,000 households, most of them being extended families, covering almost the whole country and its population.

Much that goes well beyond oral rehydration therapy can be learned from this book. One example

is cost-consciousness in health education and health care delivery: BRAC's oral therapy extension programme, at the height of its campaign, with close monitoring of all activities, used no more than 3 jeeps and 12 motorbikes. Empowerment of women was one of the main aims. Self-reliance and self-evaluation is another message. So is collaboration with all tiers of the Government and, at the local level, village elders. Listening to mothers and trusting their ability to learn has clearly been a keystone in achieving success.

The presentation is simple and factual, with ample illustrations, vivid case stories, appendices, 93 well-chosen references and an index. The book will be of value to medical and social researchers, medical practitioners, paramedics and to all interested in the promotion of health status in developing countries.

MA Rahim and R Eeckels

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