



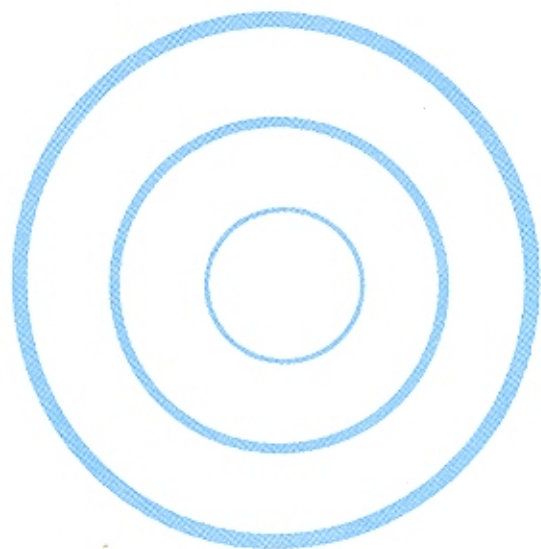
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Persistent Diarrhoea in Nigerian Children Aged Less Than Five Years: A Hospital-Based Study

Olugbemiro Sodeinde¹, Adebowale A Adeyemo¹, Rasheed A Gbadegesin¹, and
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ABSTRACT

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

Key words: Diarrhoea, Persistent; Diarrhoea, Infantile; Diarrhoea, Acute; Risk factors; Prospective studies; Child nutritional status; Child nutrition disorders

INTRODUCTION

Persistent diarrhoea remains an important clinical and epidemiological entity, because it carries a higher risk of mortality and has a greater impact on nutritional status than acute diarrhoea (1). Risk factors for persistent diarrhoea include poor nutritional status, prior recent diarrhoeal illness, and impaired immunocompetence (1-8). Although estimates indicate that 3-23% of acute diarrhoea episodes become persistent (1), the incidence of persistent diarrhoea from various studies ranges from 7 to 10 episodes per 100 child-years (1,4).

There are very few studies of persistent diarrhoea from Africa. Two studies have noted the relatively low frequency of persistent diarrhoea in Nigerian children. Oyejide *et al.* (9) found that of the 420 children in four village clusters in Oyo State followed up for six months, only 4.7% of all episodes of diarrhoea became persistent. In another study, Ekanem *et al.* (10) found that persistent diarrhoea accounted for only 2.4% of 251 episodes of diarrhoea observed in 628 children over a three-and-a-half-month period. The reasons for this low proportion of cases of diarrhoea that become persistent are currently unknown. To investigate persistent diarrhoea in Nigeria further, we undertook a prospective hospital-based study to

describe the characteristics of children with persistent diarrhoea and to compare these characteristics with children presenting with acute diarrhoea.

METHODS AND MATERIALS

This study was carried out between July 1993 and April 1994. The patients recruited into the study consisted of 307 children aged 6-60 months who presented with acute diarrhoea to the outpatient clinic at the University College Hospital, Ibadan and who were followed up for at least 15 days from the onset of diarrhoea. Diarrhoea was defined as 3 or more loose bowel motions in the preceding 24 hours (11). Information was obtained from each child's mother at presentation and included the age (in completed months) and sex of the child, the duration of diarrhoea, the presence of blood or mucus in the stools, and the presence of any other symptoms, e.g. fever and vomiting. Information was also sought on any treatment given at home prior to presentation in hospital, including use of oral rehydration therapy, drugs and herbal preparations. Information was further obtained on the mode of feeding, specifically whether the child was on breast feeding, bottle feeding, weaning or adult diet or a combination of any of the above. On presentation, a full clinical examination was done. Rectal temperature was measured on admission using a mercury clinical thermometer. Each child's weight, in kilogram, height in centimetres and mid-upper arm circumference in centimetres were measured using standard methods. For those patients who were dehydrated on presentation, anthropometry was done after rehydration. Nutritional status was assessed in three ways: using the Wellcome classification (12), the NCHS standards (13), and the mid-upper arm circumference. Each child's weight and height (length for children aged 6-24 months) were transformed into z scores of the indices: weight-for-height, height-for-age and weight-for-age of the NCHS reference population using Epi Info version 6 (14). In addition, the Wellcome classification was used for classifying each child into 'normal,' 'underweight,' 'marasmus,' 'kwashiorkor' and 'marasmic kwashiorkor' using the criteria of weight-for-age and presence of peripheral oedema (12). All subjects were screened for malaria parasitaemia by means of Giemsa-stained thick peripheral blood films. All the children were followed up in the outpatients until the resolution of diarrhoea. Those children whose diarrhoeal illness lasted more than 14 days were described as having *persistent diarrhoea*, while those whose illnesses lasted up to 14 days were described as having *acute diarrhoea* (11). Data entry and analysis were done using Epi Info 6 (14). Associations between categorical variables were explored by means of the chi square test with Yates' correction, or Fisher's exact test. Differences between the means of continuous variables were analysed using Student's *t* test. We have assumed statistical difference at a probability (*p*) of ≤ 0.05 .

RESULTS

Of the 307 children, 36 (11.7%) had persistent diarrhoea, while the remaining 271 (88.3%) had acute diarrhoea. The age and sex distribution of the children are shown in Table I. About one half of all the children were aged 6-12 months. There was a male preponderance in both groups, although there was no statistically significant association between persistent diarrhoea and male gender. Similar proportions of both groups (51% of the persistent diarrhoea group and 55% of the acute diarrhoea group) were fully immunised for their ages at the time of presentation. Among those children aged 6-18 months, persistent diarrhoea was observed in similar proportions of those being currently breastfed and those not being breastfed.

Exposure to individuals with diarrhoea

The exposure of the children to other individuals with diarrhoea in the two-week period preceding the onset of diarrhoea is shown in Table II. Overall, exposure to other individuals with diarrhoea was low. Although children with persistent diarrhoea had been more frequently exposed to older children with diarrhoea (8.3%) than those with acute diarrhoea (0.7%), two of the three children so exposed in the persistent diarrhoea group were siblings. Regarding these two siblings as one case and re-analysing the data did not yield a significant difference as above. A previous history of persistent diarrhoea was obtained in 3 (8.3%) of children in the persistent diarrhoea group compared with 11 (4.1%) of those in the acute diarrhoea group, although odds ratio was 2.15, the difference was not significant (95% confidence interval 0.45-8.94).

Table I. Age and sex distribution of 307 children presenting with diarrhoea				
	Persistent diarrhoea (n=36) (%)	Acute diarrhoea (n=271) (%)	χ^2	Statistics p
Age (months)				
6-12	19 (52.8)	133 (49.1)		
13-24	15 (41.7)	106 (39.2)		
25-36	2 (5.5)	21 (7.7)		
37-48	0 (0.0)	5 (1.8)		
49-60	0 (0.0)	6 (2.2)	1.187*	0.276
Sex				
Male	23 (63.9)	150 (55.4)		
Female	13 (36.1)	121 (44.6)	0.63	0.429
Immunisation status				
Fully immunised	18 (50.0)	149 (55.0)		
Not fully immunised	18 (50.0)	122 (45.0)	0.15	0.699
Currently breast feeding (6-18-month olds only)				
Yes	15 (60.0)	136 (71.2)		
No	10 (40.0)	55 (28.8)	0.84	0.359

* χ^2 for linear trend, ages 25 to 60 joined

Table II. Exposure to people with diarrhoea in the two-week period prior to onset of diarrhoea among 307 children presenting with diarrhoea			
Individual with diarrhoea	Persistent diarrhoea (n=36) (%)	Acute diarrhoea (n=271) (%)	Statistics Fisher's exact test (p)
Mother	1 (2.8)	5 (1.8)	0.529
Adult other than mother	0 (0.0)	5 (1.8)	1.000
Child (aged: <5 years)	1 (2.8)	2 (0.7)	0.313
Older child	3 (8.3)	2 (0.7)	0.013*

* Significant at p<0.05

Treatment before coming to hospital

Treatment given at other hospitals, dispensaries and at home before presenting in our Outpatient Department included oral rehydration therapy, antibiotics, mixture kaolin and herbal remedies given with similar frequencies in both groups (Table 3). However, children in the persistent diarrhoea group had been more frequently given antimalarial drugs than those in the acute diarrhoea group (p=0.037).

Clinical features on presentation

Children in the persistent diarrhoea group had a higher frequency of blood in the stools when compared with the acute diarrhoea group. Fever was more common in the acute diarrhoea group while a history of decreased urinary output occurred only in the acute diarrhoea group (Table IV). Other clinical features such as vomiting, anorexia, restlessness and a change in sensorium occurred in both groups with similar frequencies. Dehydration, an elevated temperature ($>38^{\circ}\text{C}$) and malaria parasitaemia were more common in the acute diarrhoea group than in the persistent diarrhoea group (Table IV).

Nutritional status

Only 43% of the persistent diarrhoea group and 59% of the acute diarrhoea group could be classified as normal according to the Wellcome classification of nutritional status (Table V). Both marasmus and kwashiorkor were more common in the persistent diarrhoea group. Being underweight (weight-for-age z score < -2.00) was also more common in the persistent diarrhoea group, although most malnourished children in both groups fell into the 'underweight' category. When the severely malnourished children (marasmus, kwashiorkor and marasmic kwashiorkor) were grouped together there was a significant difference between the persistent diarrhoea and acute diarrhoea groups (chi-square for trend = 4.84, $p=0.028$). The mean z scores for weight-for-height (WHZ) and weight-for-age (WAZ) of the persistent diarrhoea group were significantly lower than those of the acute diarrhoea group ($p<0.03$). In children aged 12-60 months, the mean mid-upper arm circumference was also significantly lower for the persistent diarrhoea group when compared with the acute diarrhoea group ($p<0.02$). Thus, the overall picture is that those with persistent diarrhoea had worse nutritional indices than those who had acute diarrhoea.

Treatment	Persistent diarrhoea (n=36) (%)	Acute diarrhoea (n=271) (%)	Statistics	
			χ^2	p
Oral rehydration therapy	11 (30.6)	77 (28.4)	0.01	0.943
Antibiotics	10 (27.8)	60 (22.1)	0.30	0.585
Mist kaolin	6 (16.7)	37 (13.7)	0.05	0.815
Herbal remedies	0 (0.0)	4 (1.5)	0.00	0.961
Antimalarial drugs	16 (44.4)	71 (26.2)	4.35	0.037*
Antipyretic drugs	23 (63.9)	170 (62.7)	0.00	0.961

* Significant at $p<0.05$

Clinical feature	Persistent diarrhoea (n=36)	Acute diarrhoea (n=271)	Statistics	
			χ^2	p*
History				
Blood in stools	5 (13.9)	19 (7.0)	1.24	0.265
Mucus in stools	24 (66.7)	130 (48.0)	3.73	0.054
Fever	20 (55.6)	203 (74.9)	5.05	0.025
Vomiting	15 (41.7)	137 (50.6)	0.68	0.410
Anorexia	23 (63.9)	142 (52.4)	1.26	0.262
Decreased urine volume	0 (0.0)	17 (6.3)	1.34	0.247
Restlessness	7 (19.4)	44 (16.2)	0.06	0.804
Change in sensorium	13 (36.1)	91 (33.6)	0.01	0.909
Findings				
Admission temperature				
$\geq 38^{\circ}\text{C}$	9 (25.0)	123 (45.3)	4.59	0.032
Dehydration	1 (2.8)	31 (11.4)	1.71	0.191
Malaria parasitaemia+	1 (2.8)	41 (15.5)	3.26	0.071

*Significant at $p<0.05$. Percentages are given in parentheses

+ 6 children with acute diarrhoea did not have blood film for malaria parasites done

DISCUSSION

This study has described the characteristics of children seen with persistent diarrhoea in an urban hospital setting. A hospital-based design was justified in view of the reported low incidence of persistent diarrhoea in previous community-based studies in Nigeria (9,10).

Factors found to be associated with persistent diarrhoea in this study included exposure to an older child with diarrhoea in the two weeks preceding the onset of the illness and poor nutritional status. Poor nutritional status is widely recognised as one of the major risk factors for persistent diarrhoea and has been reported from a number of studies (2-4, 7-8). However, it should be noted that the children suffering from persistent diarrhoea were more wasted than stunted. This implies that short-term malnutrition (as indicated by wasting) is more important than long-term malnutrition (as indicated by stunting). Most other studies used only one index of nutritional status (usually weight-for-height). One study, which did consider other measures, found that although there was little difference in the mean duration of diarrhoea between those who were stunted and those of normal nutritional status, the difference between the wasted and normal groups was quite marked. The reason for this is uncertain. It is logical to expect that the two factors commonly invoked for the greater duration of diarrhoea in malnutrition (poor healing capacity of the intestinal epithelium and impaired immunity with inability of the body to eliminate the causative organisms) should be present in both wasted and stunted children. However, endocrine and genetic factors which may affect growth were not ruled out in this study. Further investigation of these issues seems warranted.

A history of fever and a rectal temperature greater than 38 ° C were more common in children with acute diarrhoea compared with those with persistent diarrhoea. This may be related to the higher frequency of malarial parasitaemia observed in children with acute diarrhoea which, in turn, may be due to the lower frequency of pre-hospital treatment with antimalarial drugs. The age distribution observed in our patients with persistent diarrhoea did not differ significantly from that observed in those with acute diarrhoea. This contrasts with the findings of other studies (2,4,7,8) that reported young age to be a risk factor for persistent diarrhoea. Remarkably, dehydration was far less common in children with persistent diarrhoea despite their longer duration of diarrhoea and similar pre-hospital frequencies of administration of oral rehydration therapy. This may be accounted for by differences in absolute stool volumes (which we did not measure) rather than the differences in frequency of vomiting between the two groups which was not significant (Table IV).

Table V. Nutritional status in relation to type of diarrhoea among 307 Nigerian children				
Nutritional status	Persistent diarrhoea (n=36)	Acute diarrhoea (n=271)	Statistics χ^2 /student's <i>t</i> test	
Wellcome classification. No (%)				
Normal	16 (44.4)	160 (59.0)		
Undernutrition	15 (41.7)	98 (36.2)		
Marasmus	3 (8.3)	9 (3.3)		
Kwashiorkor	2 (5.6)	3 (1.1)		
Marasmic kwashiorkor	0 (0.0)	1 (0.4)	$\chi^2=4.84^*$	p=0.028
*chi-square for trend; 3 last rows collapsed				
Comparison with NCHS reference Mean (SD) of z scores				
Weight-for-height (WHZ)*	- 1.609 (1.387)	- 1.044 (1.416)	t=2.22	p=0.026
Weight-for-age (WAZ)*	- 2.276 (1.379)	- 1.803 (1.178)	t=2.21	p=0.026
Height-for-age (HAZ)	- 1.566 (1.926)	- 1.410 (1.670)	t=0.51	p=0.618
Mid-upper arm circumference in CM Mean (SD)				
All patients	12.7 (1.4)	13.4 (2.3)	t=1.18	p=0.070
Patients aged 12-60 months*	12.6 (1.3)	13.5 (1.4)	t=2.77	p=0.006

*Significant at p<0.05

Although contact with an older child with diarrhoea was commoner in persistent diarrhoea, this finding cannot be given much weight since the actual numbers were small and two of the three children were siblings. The latter fact suggests a common source of infection or similar host factors predisposing both siblings to diarrhoea (which became persistent) from a specific infective agent. These non-random events preclude any generalisation of the finding.

In conclusion, we have shown that poor nutritional status is the major risk factor for persistent diarrhoea in a group of Nigerian children. The implication of these findings is that reduction in the prevalence of malnutrition may be associated with reduction in the proportion of acute diarrhoeal episodes that eventually progress to persistent diarrhoea. Further studies are needed to understand the association between persistent diarrhoea and host factors, especially nutritional status and age, and to identify factors that may be amenable to intervention.

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Breast-feeding Promotion in a Diarrhoea Programme in Rural Communities

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ABSTRACT

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

Keywords: Breast feeding; Child survival; Diarrhoea, Acute; Primary health care; Health education.

INTRODUCTION

Breast feeding is an important child survival strategy. It protects the infant from the morbidity and mortality of diarrhoea in the first few months of life (1-5). Exclusive breast feeding (breast milk only) offers the greatest protection (1,2,4,6), and child survival programmes promote breast milk only for the first 6 months of life (7-9). However, studies on child feeding practices show that, world-wide, exclusive breast feeding is rare, as breast milk is supplemented early in the child's life with water, other milks, juices, teas, and other foods and fluids (3,10-12).

Diarrhoea is a major cause of infant and child morbidity and mortality in Nigeria (13) as in most of the developing world. Although virtually all Nigerian mothers breastfeed their infants, exclusive breast feeding lacks local credibility (14,15) and less than 2% of infants are fed breast milk exclusively at 2 months of age (16).

The present study was carried out to promote exclusive breast feeding in rural communities in Nigeria where the prevalence of infant and childhood diarrhoea is especially high. The main objective was to assess the impact of a focused breast-feeding counselling programme targeting mothers of infants currently suffering from diarrhoea. The main test hypothesis is that the intervention will have no significant effect on the prevalence of exclusive breast feeding. Previous work promoting breast feeding in the same rural communities have recorded increased prevalence of early breast-feeding initiation and of full (i.e. exclusive plus predominant) breast feeding (15,17) but not of exclusive breast feeding. The results of the present study indicate that focused breast feeding counselling can significantly increase the prevalence of exclusive breast-feeding in these communities and reduce diarrhoeal morbidity.

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MATERIALS AND METHODS

Location

The study was carried out in Ife South Local Government (ISLG) area (district) of Osun State, Nigeria, between November 1995 and March 1996. ISLG has an estimated population (in 1996) of about 153,000 persons, living in about 450 villages and smaller settlements that are mainly Yoruba farming communities. Its health facilities consist of health centres, health clinics, and health posts, manned by community health workers (CHW). There is one doctor at the State health centre at Ifetedo, the ISLG headquarters. Sanitation in ISLG communities is generally poor, and only about 15% of households have access to safe water.

At the time of the study, ISLG was actively promoting breast feeding in all its health facilities, which prominently displayed promotional posters and distributed handbills.

Samples

Mothers who had brought their young (≤ 3 months) infants to a primary health care facility for the treatment of diarrhoea were recruited into the study. To qualify for inclusion, the infant's diarrhoea must be uncomplicated (not bloody; dehydration mild or less; duration less than one week; no overt infections), must be manageable by oral rehydration at home and not require referral to a secondary facility. After giving informed consent, selected mother-infant pairs were randomised into one of two groups – controls and those to receive an intervention – following a sequence indicated from a table of random numbers.

Intervention

The intervention consisted of three sessions of individual breast-feeding counselling, lactation management and problem-solving for the mothers. On day 0 (presentation), mothers in the control and intervention groups were given advice on diarrhoea, focusing on home management with salt and sugar solution, discontinuation of anti-diarrhoeal agents (including antibiotics), continuation of breast feeding, avoidance of the use of feeding bottles, breast milk only, and recognition of the signs of dehydration. In addition, each of the mothers in the intervention group was given a breast-feeding and lactation management session lasting about 20 minutes on the average, at which lactation problems were discussed and solved. The counselling and problem-solving sessions were repeated at home visits to the mothers in the intervention group only on days 2 and 7, each of these sessions lasting about 30 minutes.

The breast-feeding counselling to the intervention group included messages that (a) exclusive breast feeding, without supplements or water, prevents diarrhoea in the young infant; (b) it promotes recovery from diarrhoea; (c) the breastfed infant can survive well on breast milk only for the first 6 months of its life, and does not require supplementary water and teas, all of which promote diarrhoea; (d) intensive breast feeding on demand, as increased suckling promotes the production of breast milk and improves the baby's nutrition; and (e) the mother should be assured that, with frequent suckling and correct positioning and attachment of the infant at the breast, she can produce all the breast milk that the

infant requires. In addition, the mothers were given demonstrations on, and assistance with, correct positioning and attachment of the baby to the breast, and hand expression of breast milk.

On days 7 and 21, home visits were made to the mothers in both groups, during which a structured questionnaire on the infant's feeding (24-hour recall) was completed. This included what was being fed to the infant (water, teas, juices, breast milk, formula, other baby foods, semi-solids), how and how frequently the child was being fed, and the use and care of feeding bottles. Morbidity data were also collected. For the intervention group, the data collection on day 7 preceded the counselling session, but both were done during the same home visit.

Five CHWs and two research field assistants had received specific training in breast-feeding and lactation management to assist in the intervention and data collection. The training had been adapted from the WHO/UNICEF 18-hour breast-feeding course manual (17,18), appropriately modified to suit non hospital-based primary care and rural settings. They also had specific training in breast-feeding counselling skills [19], specifically aimed at addressing mothers' perceptions of lactation insufficiency, following the WHO guidelines.

The same field assistants that implemented the intervention counselling had also collected the post-intervention data on feeding practices. This can lead to unreliable data from observer bias or misinformation (especially from the intervention group) or both, and is an important limitation to the design of the study.

Data analysis

The outcome variables in this study were breast-feeding mode (exclusive, predominant, partial) at days 7 and 21, and the re-occurrence of diarrhoea in the infant by day 21. Analysis of results included comparisons between intervention and control groups. Statistical analysis was performed calculating mean, standard deviation, standard error of the mean and chi-square test, using the software SPSS for Windows, version 6.1.00. A 5% level of significance was chosen.

Sample size considerations were based on the assumption that the intervention would increase the prevalence of exclusive breast feeding (24-hour recall) from 5% to 25%. Given a level of significance of 5% and a 90% power of detection, 67 mother-infant pairs were required for each group.

RESULTS

Data were complete for 79 of the 85 mother-infant pairs in the control group and 82 of 84 in the intervention group. Maternal age, parity, educational level, and family characteristics were comparable in both groups (Table I). The characteristics of the infants at presentation were also comparable (Table II). In particular, the proportions of the infants that were being fed breast milk only were similar.

Table III shows the values for the outcome variables for the control and study groups. At day 7, 6% of the control mothers and 49% of the intervention group were feeding their infants breast milk exclusively. The difference in proportions of 43% (95% CL: 25%, 56%) is significant ($\chi^2=37.72$, $p<0.00001$). At day 21, the corresponding proportions of exclusively breast feeding were 8% (control) and 46% (intervention). Again, the difference in proportions of 38% (95% CL: 25%, 53%) is significant ($\chi^2=28.32$, $p<0.00001$).

Characteristic	Controls (n=79)	Intervention (n=82)
Age (years), % (N)		
15-19	12.7 (10)	11.0 (9)
20-24	32.9 (26)	30.5 (25)
25-29	16.5 (13)	20.7 (17)
30-34	21.5 (17)	20.7 (17)
35-39	8.9 (7)	8.5 (7)
40-44	7.6 (6)	8.5 (7)
Level of education, % (N)		
None	35.4 (28)	34.2 (28)
Primary	38.0 (30)	41.5 (34)
Secondary	26.6 (21)	24.4 (20)
Religion, % (N)		
Christian	41.8 (33)	53.7 (44)
Islam	58.2 (46)	46.3 (38)
Family characteristics % (N)		
Married	97.5 (77)	91.5 (75)
Polygamous	40.5 (32)	46.3 (38)
First time motherhood	20.3 (16)	18.3 (15)
Number of children, mean (SD)	3.5 (1.9)	3.5 (2.0)
Ethnic group % (N)		
Yoruba	84.8 (67)	86.6 (71)
Igbo	8.9 (7)	11.0 (9)
Hausa	1.3 (1)	2.4 (2)
Others	5.1 (4)	

Also, at day 21, more mothers in the control (14%) than in the intervention group (5%) were using feeding bottles to give supplements to their infants. The commonest supplements given in both groups were *ogi* (a watery corn gruel), herbal teas, and soft drinks. None of the mothers had used commercial breast-milk substitutes. The reasons most frequently given by the mothers in the intervention group for introducing supplements were that the baby was hungry and cried for more food, that breast milk alone did not satisfy the baby, and that it was thirsty, hiccuping, and cried for water.

By day 21, a new episode of diarrhoea had occurred in 22% of the infants in the control group and 12% in the intervention group. This difference is not significant. The 10 children in the intervention group who had a recurrence of diarrhoea included 2 of 38 (5%) who were fed breast milk only and 8 of 44 (18%) who were receiving supplements. In the control group, none of the 6 who were fed breast milk only had a recurrence. For both groups joined, 2 of 44 (5%) who were fed breast milk only and 25 of 117 (21%) who were not had had a re-occurrence of diarrhoea by day 21.

Table II. Distribution of the infants by selected sociodemographic characteristics

Characteristic	Controls (n=79)	Intervention (n=82)
Age in months, % (N)		
0-1	13.9 (11)	12.2 (10)
1-2	40.5 (32)	46.3 (38)
2-3	45.6 (36)	41.5 (34)
Mean age (SD) days	55.6 (22.0)	52.9 (21.4)
Males, % (N)	54.4 (43)	57.3 (47)
Mean duration of diarrhoea at presentation, days (SD)	3.8 (1.8)	3.9 (1.8)
Infant feeding:		
Exclusive breast feeding	8.9 (7)	11.0 (9)
Predominant breast feeding	50.6 (40)	50.0 (41)
Partial breast feeding	40.5 (32)	39.0 (32)

Table III. Values of outcome variables for the control and intervention groups

Variable	Controls (n=79)	Intervention (n=82)
Exclusive breast feeding, % (N)		
Day 0	8.9 (7)	11.0 (9)
Day 7	6.3 (5)	48.8 (40)
Day 21	7.6 (6)	46.3 (38)
Use of feeding bottles, % (N)	13.9 (11)	4.9 (4)
Re-occurrence of	21.5 (17)	12.2 (10)

DISCUSSION

We had set out in this study to assess the impact of a focused, individual breast-feeding counselling to mothers of infants with a current episode of diarrhoea on the prevalence of exclusive breast feeding. The results show that this type of breast-feeding promotion intervention has been associated with a marked increase in exclusive breast feeding. In addition, the intervention has been associated with lower diarrhoea prevalence and lower use of infant-feeding bottles. These results are largely in agreement with those from a similar study in a specialist diarrhoea hospital in Bangladesh by Haider and co-workers (20).

Research studies promoting breast feeding have recorded increased prevalence of exclusive breast feeding (21,22). We have earlier reported increased breast-feeding rates associated with two different promotion approaches in these rural communities: prenatal and postnatal breast-feeding counselling and support of rural mothers was associated with a sharp increase in early breast feeding and a four-fold increase in full breast feeding at 4 months (14). Also, the training of extension health workers was associated with increased early breast-feeding initiation and markedly improved breast-feeding recommendations to mothers (17). The present results complement and extend the findings of our previous studies. They suggest that specific, intensive training of health workers in breast-feeding and lactation management, and the use of each contact with the mother of the child as an opportunity to give her breast-feeding counselling and support, together with lactation support from the community, is an effective approach to the promotion of exclusive breast feeding in these rural communities.

Breast feeding of infants is universally practised in our communities. However, exclusive breast feeding is rare: there is widespread perception of lactation insufficiency, and exclusive breast feeding is seen as dangerous (15). Complementary foods are, therefore, introduced typically in the infant's second month of life. The breast-feeding promotion approach of this study seems to have reversed, at least for the short duration of the study, the behavioural effects of local knowledge that strongly deprecates exclusive breast feeding. In particular, the approach included messages supporting breast milk only and increased suckling for the avoidance and resolution of diarrhoea, and for optimum child nutrition, growth and development. One factor that may have contributed to the results of the study is that ISLG was actively promoting breast feeding at the time of this study. Our intervention messages may have acted to reinforce and extend those that were contained in the posters and handbills that were prominently displayed at the health facilities and other places. Furthermore, this time we dealt with anxious mothers of children who were ill, and, therefore, were likely to be immediately receptive to advice. There was also the factor of ready availability of lactation support and problem-solving.

Huttly and co-workers found that non-breastfed Nigerian children aged 3 months and below were 10 times more likely than exclusive breastfed ones to have diarrhoea (23). In this short study, the prevalence of a new episode of diarrhoea was lower in infants fed breast milk only than those who also had supplements. However, no definitive conclusions on this aspect can be derived from the present data and a purposely-designed study is needed to confirm this association.

In our rural communities, where most mothers and their children live, CHWs are the predominant prenatal and postnatal source of contact with the health-care system for mothers and their children. CHWs give antenatal and postnatal care to mothers, deliver them, immunise their babies and take care of them when they are ill. They can, therefore, play a crucial role in breast-feeding counselling, support, and problem-solving, as the results of this study suggest. They should be specifically trained to give breast-feeding counselling and support, and be encouraged to use every contact with the infant and its mother to do this.

As earlier stated, the same research personnel implemented the intervention and collected the postintervention data, and were not blinded to the respondents' experimental grouping (control or intervention). This could have led to unreliable data. Our close supervision of the field assistants was designed to minimise possible errors from their bias. Also, we collected some observational data to validate study participants' responses and control for any possible misreporting of feeding practices. However, a longer period of observation than that obtainable in this study is necessary to reduce such reporting errors to an absolute minimum. Moreover, data from 24-hour recall can lead to feeding group misclassifications. However, such misclassification should be equally distributed in the two experimental groups. In addition, such misclassification usually leads to an underestimation of exclusive breast-feeding rate (24), and it cannot explain the increased rates in the intervention group recorded in this study. Even so, these results should be cautiously interpreted and need to be confirmed from studies in which data have been collected by independent observers that are blinded to respondents' experimental groups.

Breast feeding is cost-effective, and it is important that promotion schemes preserve its cost effectiveness. The scheme indicated here consists of health worker training in counselling and lactation support in the community. The training of CHWs can be achieved quite economically in our rural communities (14). It can, however, be argued that the extra work involved in home visiting and follow-up for lactation support can overburden the health system in these rural communities. We suggest that this need not be the case; with adequate encouragement and incentives, CHWs can accommodate the extra work in their existing schedules. In addition, lactation support groups in the communities, involving civic and opinion leaders and women's groups, can provide complementary support and can be self-sustaining. The scheme can, therefore, remain cost-effective without also overloading the local health system. Besides, cost-savings will accrue to the community from consequential decrease in diarrhoeal morbidity.

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Grandma Ahead of Her Time: Traditional Ways of Diarrhoea Management in Lesotho

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ABSTRACT

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

Key words: *Diarrhoea; Diarrhoea, Infantile; Infant foods; Oral rehydration therapy*

INTRODUCTION

Global Policy to Reduce Diarrhoeal Mortality

Global mortality due to diarrhoeal diseases among children aged less than five years is measured in millions (1). Efforts to reduce this level of mortality have focused on the prevention and treatment of dehydration through oral rehydration therapy (ORT) with a solution containing oral rehydration salts (ORS) or home-prepared sugar and salt solutions (SSS). While used successfully in clinical settings, both technologies encountered difficulties at the household level (2). Problems with acceptability, access and preparation spurred an interest in home-based alternatives (3). At the same time there was a growing awareness of the potential of home-based fluids and foods for prevention of dehydration.

In 1987, WHO issued a guide that would assist managers of Control of Diarrhoeal Diseases (CDD) programmes in selecting fluids already available in the home (4). The view at the time was that home fluids should be as "ORS-like" as possible and the guide specified rigorous criteria for testing the composition of candidate fluids. Soon thereafter, however, WHO began shifting emphasis away from fluid composition as the key selection criterion. In 1990, a new list of criteria for selecting home fluids (5) was proposed. On this list "composition" had been shifted from first to last place, while "acceptability" was moved to the top of the list. A revised selection guide reflecting this shift from efficacy to effectiveness was published in 1993 (6). The revised guide considers both fluids and foods, divided into salt and non-

salt containing ones, and recommends the selection of "one or more" of each type. Actual testing of fluid composition is not prescribed.

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National Policy in Lesotho to Reduce Diarrhoeal Mortality

In Lesotho, government efforts targeted to diarrhoeal disease control began in 1984 when the Combating Childhood Communicable Diseases (CCCD) Project was launched (7). In 1986, the Ministry of Health adopted a five-year plan to control diarrhoeal diseases. The communication strategy for the programme was developed by the Communication and Marketing for Child Survival Project (HEALTHCOM). In 1987, the Ministry of Health formed an Ad Hoc Committee on ORT to review government policy which, until then, had favoured the use of SSS. According to the revised policy, SSS would still be promoted, but ORS was declared to be the "superior and ideal solution" (8). Moreover, to prevent dehydration in the home, mothers and other family members would be "informed and trained to recognise diarrhoea in infants and children as an illness requiring early treatment" (8). The educational message, "dehydration kills," was expected to motivate mothers to follow the action message: "As soon as your child has more than one watery stool or vomits more than once in a day, start giving ORS or sugar-salt-water mixture" (9).

OBJECTIVE

The objective of our research in Lesotho was to obtain information on home management of infant and young child diarrhoea. The research was designed in response to a request from the Ministry of Health for a study that would enable the Ministry to issue specific recommendations regarding home fluids (10).

In line with the policy shift that had taken place prior to the study, our search for suitable home fluids focused on effectiveness rather than on efficacy. We were in other words more concerned about how well a fluid, as actually used, might prevent or treat dehydration than how well it might do this when properly prepared and administered. In community settings, multiple factors will influence actual use. Besides its composition, the effectiveness of a given home fluid in a certain setting depends on the extent to which it is actually given to children during diarrhoea, and this depends on a number of factors. These include availability and cost of ingredients; convenience of preparation; and caregivers' perception of the appropriateness of a fluid for children with diarrhoea. The latter may be influenced by health professionals' recommendations.

METHODS

Qualitative data were obtained through focus groups and individual interviews with mothers, grandmothers and nurses. These interviews were conducted in three geographically different locations during two phases of data collection, in 1991 and in 1992 respectively. During the first phase we carried out 9 focus groups and 19 individual interviews, and during the second round 10 focus groups and 24 individual interviews.

For practical reasons nurses were interviewed individually, usually at their workplaces. Mothers and grandmothers were interviewed mainly through focus groups near their homes. These homogeneous groups of mothers or grandmothers were encouraged by a moderator to share their experiences and perspectives regarding home care of children with diarrhoea. We aimed for a group size of 6-8 people,

but occasionally had slightly larger groups. For both focus groups and individual interviews, a question guide served to remind the moderator / interviewer of topics to be covered. The focus groups with mothers and grandmothers were conducted in Sesotho and tape recorded. Individual interviews with nurses were conducted in English and recorded through written notes.

The typed interview notes and translated tape transcripts were coded according to themes which emerged from these qualitative data. Thereafter the coded statements were sorted into new computer files by theme and respondent category. Theme by theme we then compared the statements made by the mothers, the grandmothers and the nurses. We found that their statements consistently converged into one coherent picture of how the children were fed and cared for during episodes of diarrhoea.

RESULTS

Feeding the Healthy Infant and Young Child

To have a point of reference, we enquired first about foods and fluids given to healthy infants and young children and then about what was given during episodes of diarrhoea. We found breast feeding to be nearly universal and commonly continued into and beyond the second year. While breast feeding continues, supplements of water become part of the diet of most infants during the early months. This may be plain water, or water with sugar or with sugar and salt added. As a result there is little exclusive breast feeding. According to most respondents, complementary feeding should begin around four months of age with gruel or soft porridges and other liquid or semi-solid foods, such as milk, soups, mashed vegetables, and soft-boiled eggs. Traditionally there are two main types of gruel; regular unfermented gruel, called "*lesheleshele*," and fermented gruel, called "*motoho*." For both, the preferred grain is sorghum. "*Papa*", a stiff maize porridge, the staple dish for adults, could be given to young children if softened with milk or vegetable broth.

Feeding the Infant and Young Child during Diarrhoea

Nurses' recommendations

For a child with diarrhoea, many nurses first advised home-prepared sugar and salt solution – SSS –, to be prepared "as soon as the diarrhoea starts." While nurses themselves said they explained that this fluid was to replace fluids and salts lost, a common misunderstanding seemed to be that SSS should be given to stop the diarrhoea.

Continued feeding was another aspect of diarrhoea management that several nurses emphasised, a number of them adding that, in the past, the opposite had been recommended. "*Previously we used to tell them to stop all the feeds*," one nurse informed us. Mothers and grandmothers had clearly heard nurses stress the importance of SSS, but they did not seem to have noticed any change in nurses' recommendations regarding feeding. One group of mothers, for example, said, "*We are advised at the clinic to stop all foods except breast feeding and use only SSS or ORS until the diarrhoea is better*." Grandmothers were very concerned that nurses "*say the children should just be given SSS when they have diarrhoea and not any food*," and wondered, "*How can a child survive?*" Grandmothers who themselves had always been feeding their children during episodes of diarrhoea explained: "*You see, since we did not go to the clinics, we did not know of these things. We were our own clinics in the villages*."

Grandmothers' views and practices

Grandmothers scorned the nurses' advice to give SSS which in their opinion *"makes the children worse."* The grandmothers' strategy was to *"ignore the SSS when the child has diarrhoea and give them food and herbs. Then the diarrhoea stops. Our medicines stop diarrhoea. The SSS tastes so bad and children don't like it. I tried to taste it once and it was so bad."*

Grandmothers were adamant about keeping both healthy and sick children well-fed: *"A child has to be fed all the time, sick or not sick. We want a fit child who is strong even when he is sick."* As if they had heard this very statement, a group of mothers said: *"Grandmothers say we should feed the child all the time, when he is sick or not, we should feed them the same way."*

According to the grandmothers, a child's poor appetite during diarrhoea required the mother to *"coax the child into eating,"* and pay attention to *"what feeds make the child worse when he has diarrhoea, so that in the next bout you do not feed it to the child."* While grandmothers might, thus, adjust the diet in the individual case, they did not believe there was a need to change the diet just because a child had diarrhoea. They emphasised that they continued giving *"all the things that the child has been eating,"* and that there was *"no change at all."* How strongly grandmothers felt about feeding during diarrhoea is illustrated by an instance when the interviewer challenged a group of grandmothers by stating, falsely, that other grandmothers had said they would not feed a child with diarrhoea. The challenged group answered incredulously: *"No, they should feed the child. Maybe they have adopted these things today. Or they wanted to impress you so that you will think they are doing modern things."* Grandmothers' main motivation for feeding children during diarrhoea was to keep the children strong, but they had other reasons too. One grandmother explained that she *"gave the child food so that it can stabilise the bowels."* Describing the grandmothers' practices, a group of mothers said, *"Sometimes they give the child juice from the Sesotho indigenous beans or from maize or potatoes to constipate the child."* Among foods that grandmothers gave during diarrhoea, gruel seemed to play an especially prominent role. *"Definitely, during the diarrhoea episode we give [gruel],"* one group said in response to the interviewer's question of how feeding during diarrhoea compared to the feeding when children were healthy. One reason for giving gruel was that *"When you give [gruel] the diarrhoea stops at once."* Mothers also knew that: *"Grandmothers say that [gruel] will also stop the diarrhoea."* Studies showing that faecal losses are smaller with food-based solutions than with the standard glucose-based oral rehydration solution (11), indicate that there may be a scientific basis for the grandmothers' observations.

Mothers' practices

Mothers received advice both from nurses at the clinics and from the grandmothers. These advisors often contradicted each other, and spoke derisively of each other's advice. The mothers' strategy was to make use of both sources of advice, because as one mother explained: *"When my child is sick and I am scared, anything that works I use. When I have money I go to the hospital, while at the same time using what my mother gave me."* Whose advice she would choose at a given time *"just depends on who scolds you more...you use the advice from the one who scolds you more."*

Like the grandmothers, mothers explained that they continued feeding children during episodes of diarrhoea and had ignored the nurses' advice to stop feeding. One mother explained to the interviewer *"You know, we don't listen to the clinic when they say we should not feed."* Mothers also expressed great concern about children's poor appetites during, and after, an episode of diarrhoea. This poor appetite had to be overcome by *"coaxing a child into eating,"* by giving *"nice things"* or *"those things that always bring back the appetite like the juice of an orange,"* or any fruit juice or fruit drink. One group explained: *"When children have diarrhoea they do not eat well. Their appetites are so low you have to coax the child into eating more by also giving them food that brings back the appetite like fruit juices. Even sometimes when the diarrhoea has stopped, this child may not have a good appetite. So you have to continue coaxing him into eating more food."*

Nurses' own practices

During the first phase of data collection, a number of nurses indicated that their previous advice to withhold food during episodes of diarrhoea had been followed by mothers. However, we found the mothers' and the grandmothers' assertions, that they had not followed the advice, but made great efforts to feed children during episodes of diarrhoea, more convincing.

When queried about this during the second phase, the nurses' responses were in closer agreement with the mothers' and the grandmothers' descriptions. For example, one nurse recalled when she had worked in the paediatric ward how *"At night mothers used to steal food from the cupboard to give to their children, even milk."*

We also wondered about what nurses themselves had done when their own children had diarrhoea. A number of nurses then told us that they had continued feeding. *"You feed,"* stated one nurse. *"I feed a child, that's all,"* said another. Several of them described how, as mothers, they would become anxious when their children were ill. *"It's the maternal instinct. When your child is ill, you panic. You've got to do something,"* explained one nurse. Another nurse remembered how, when her children had diarrhoea, *"as a mother that's terrible. You keep trying to give food."* She and her colleague agreed that a child's appetite could be poor, but emphasised that *"you, as a parent, don't stop"* feeding. Regarding breast feeding, one of the nurses said, *"If they cry you find yourself breast feeding."*

Two nurses, upon being asked whether it was not difficult as a nurse to recommend one thing and as a mother to do another, seemed to feel it was not. With a shrug of her shoulder, one of them said, *"Sometimes you are forced by the policy. You follow the policy without questioning it."*

A few nurses did say that they had tried to withhold food when their children had had diarrhoea. One of them related that, when she tried to do this, her mother had taken over and started feeding the children. This nurse remarked that being a nurse was *"pretend-like."* Her comment, by reminding us of the multiple roles most of us play, helped us reconcile the nurses' seemingly contradictory responses. We realised that, during the first phase, we had approached the nurses as professionals only. During the second phase, we also asked about their views as mothers.

DISCUSSION

Mothers Continue Feeding During Diarrhoea

The most salient feature of home management of diarrhoea as described by the mothers and grandmothers in this study in Lesotho was the continuation of feeding during episodes of diarrhoea. In this regard, the mothers and the grandmothers had actually been ahead of the health professionals who in the past had recommended that feeding be stopped.

Studies in Bangladesh (12), India (13), Kenya (14), Mexico (15), Nigeria (16), Pakistan (17), Peru (16, 18, 19), Saudi Arabia (20), and Swaziland (21) show that, in these countries, too, mothers continue feeding during diarrhoea. Like the mothers and grandmothers in our study, traditional healers in Swaziland (21) stated that feeding was important to maintain the child's strength. The Swazi healers and the Basotho grandmothers shared the view that food, and in particular gruel, was useful for stopping diarrhoea.

Both the mothers and the grandmothers we interviewed expressed great concern about children's poor appetites during diarrhoea, and described how they took great effort to coax a child to eat. Mothers in other countries appear to share this concern about children's poor appetite during diarrhoea. Nigerian mothers said that they were force-feeding more, while Peruvian mothers said that they were breastfeeding more during diarrhoea, due to children's poor appetite (16). Bentley found in India that "some mothers shifted the diet during diarrhoea towards foods that they perceive as 'helpful'" (19), and in Peru that mothers said children could eat "whatever they wanted" during diarrhoea (16).

To understand the dynamics between mothers and grandmothers in relation to child care and feeding practices, it is essential to understand the power relations between them. In the Sesotho culture, grandmothers are thought of as wise and as custodians of culture, particularly as it relates to child care. Their superior status and their experience of bringing up children give them the authority to provide advice and make decisions regarding the care of their grandchildren.

Feeding is Beneficial for Nutrition and Hydration

Gruel is commonly given to Basotho children during diarrhoea. For the main varieties, there is little variation regarding the type of grain preferred, and some variation with respect to the addition of salt and sugar. Yet, we would be reluctant to single out one particular gruel as especially suitable. We no longer believe that the selection of a small number of home fluids is a useful approach, although this was the initial motivation for our study. This conviction grew out of a critical review of the role fluids and foods available in the household have played in home management of diarrhoea (2).

A single formula is practical in clinical settings where procedures are standardised. In community settings, characterised by variation among households and even within the same household over time, a broad selection of fluids and foods is more appropriate. It may even be harmful to limit the number of fluids or foods recommended, as this would imply that those not recommended are not useful.

Limitation is unnecessary since a wide range of home-based fluids and foods could be used for preventing dehydration. This is because most foods contain carbohydrates and protein, which enhance intestinal fluid absorption and thereby aid in the prevention of dehydration (22). In other words, almost any food with sufficient amounts of water could help prevent dehydration. This means that the mothers' efforts to feed their children during diarrhoea should be seen as beneficial not only for nutrition but also for hydration.

Protection and Support before Promotion

The idea that protection and support of beneficial existing practices should precede the promotion of new ones is a useful concept which deserves to be extended beyond the context of breast feeding programmes (23). Data presented above suggest that mothers in varied geographical settings wisely have insisted on continued feeding. In such cases beneficial practices should be protected and supported rather than new ones promoted. An approach which fails to take into account existing knowledge and practices also runs a greater risk of producing harmful effects. Existing practices which are disregarded may be destroyed (24) and new potentially harmful practices may unintentionally be encouraged.

CONCLUSION

This study provides evidence that beneficial diarrhoea management practices have existed traditionally among women in Lesotho. Mothers' and grandmothers' efforts to continue feeding children during episodes of diarrhoea will help both to maintain nutritional status and to prevent dehydration. Before planning an intervention, existing practices should be assessed, so that whenever possible such helpful practices can be protected and supported. Then there is less need to promote new practices and less risk of causing harm.

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Isolation of *Escherichia coli* to Detect Faecal Contamination of Infants and Their Mothers in West Bengal

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ABSTRACT

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

Key words: *Escherichia coli*; Diarrhoea, Infantile; Hygiene; Prospective studies

INTRODUCTION

Diarrhoeal diseases still hold the second rank in the causes of global mortality and morbidity amongst infants in the developing world. Children aged less than one year are the most vulnerable group. A higher risk of diarrhoea occurs when babies are on complementary feeding (1,2). Complementary feeding includes formula and traditional food given to infants along with breast feeding. Lack of personal hygiene and improper environmental sanitation increase the risk of contamination and cross-contamination. There are previous reports about weaning diarrhoea, bacteriological studies of weaning foods and drinking water (3-6), conducted in various geographical areas. Early introduction of artificial foods occurs in urban areas of Calcutta (7). The present study aims at quantifying sources of faecal contamination of infants in rural Bengal by isolating *Escherichia coli* as an indicator organism.

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MATERIALS AND METHODS

Study area and population

This prospective, community-based study was carried out from June 1993 to August 1995 in a rural community, 10 km from the National Institute of Cholera and Enteric Diseases, Calcutta, India. We worked in three villages, with a total population of about 9,800, living in 1,938 families. The study design has been fully described earlier (15). The study families were visited twice a week by an epidemiological team of the Institute. A total of 148 infants, born at term, and their mothers were included. At inclusion the children were aged between 0 and 2 months. Children completing one year of age during the study were dropped and replaced by other infants of 0 to 2 months of age. A questionnaire, including information about infant feeding practices, was administered during each visit to the family.

Sample collection

Seven hundred and twenty-five samples were collected from the study families, irrespective of history of diarrhoea. They included hand rinsing from the study children (HRC) and from their mothers (HRM), swabs from feeding utensils, leftover food, milk and other liquids. Twenty mL of sterile peptone water was used for hand (fingertip) rinsing. Feeding utensils included cups and spoons, bottles and teats. They also were rinsed with peptone water.

Samples were collected at least once from each of the study family. Of the 725 samples collected, 37 samples of fingertip rinses were from exclusively breastfed infants and their mothers.

Bacteriology

All samples were processed within one hour of receipt in the microbiology laboratory of our institute following standard published techniques (8). We used MacConkey agar (Difco), Hektoen enteric agar (Difco) and T.C.B.S. agar (thiosulphate citrate bromothymol blue and sucrose, Difco).

Since *E. coli* was the indicator bacteria (9) for faecal pollution, particular attention was given to the MacConkey agar plate showing lactose-fermenting colonies to select the colonies into various groups according to their shape and colour. Colonies were inoculated into tubes containing freshly prepared tryptone water, single strength MacConkey broth and citrate medium. After incubation at 44 ° C for 24 hours, isolates were identified as *E. coli* by their ability to produce indole from tryptophan and gas from lactose and their inability to use citrate (10).

Statistical analysis was done by the chi-square test.

RESULTS

Among the families studied, more than 50% of the mothers were illiterate; others had followed primary level education. The monthly income was Rs 1,000 (about US\$ 30) or less in 48% of the families. Water sources were from hand pumps for drinking and cooking, and from ponds for bathing and washing utensils and clothes. Infant-rearing practices, more particularly infant-feeding habits, were similar throughout the families. Early complementary feeding of infants was common. In an earlier report on the same community (15), we found that of the 78 infants who were exclusively breastfed from birth on, only 20 (25%) were still fully breastfed at 4 months of age.

Table I shows the percentage distribution of *E. coli* isolated from the various samples, with a total isolation rate of 30%. Faecal contamination of fingertip rinsing from exclusively breastfed and partially breastfed infants and their mothers are compared in Fig. 1. Fig. 2 shows the seasonal pattern of isolation, the highest proportion being during the rainy season (46%).

Type of samples	Samples examined (n)	Samples positive for <i>E. coli</i>
HRM	196	78 (40)
HRC	199	34 (17)
Feeding* utensils	301	90 (30)
Leftovers foods/drinks	29	17 (59)
Total samples	725	219 (30)

*Cups and spoons, bottles and teats
Figures in parentheses indicate percentages

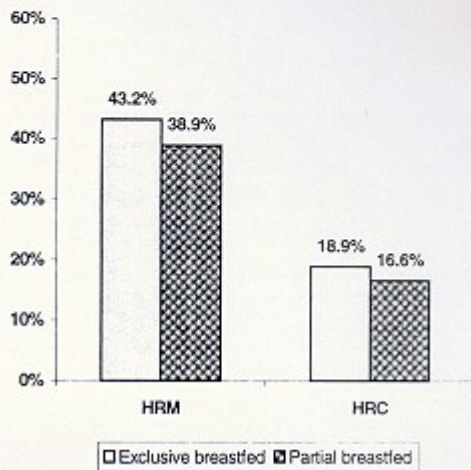
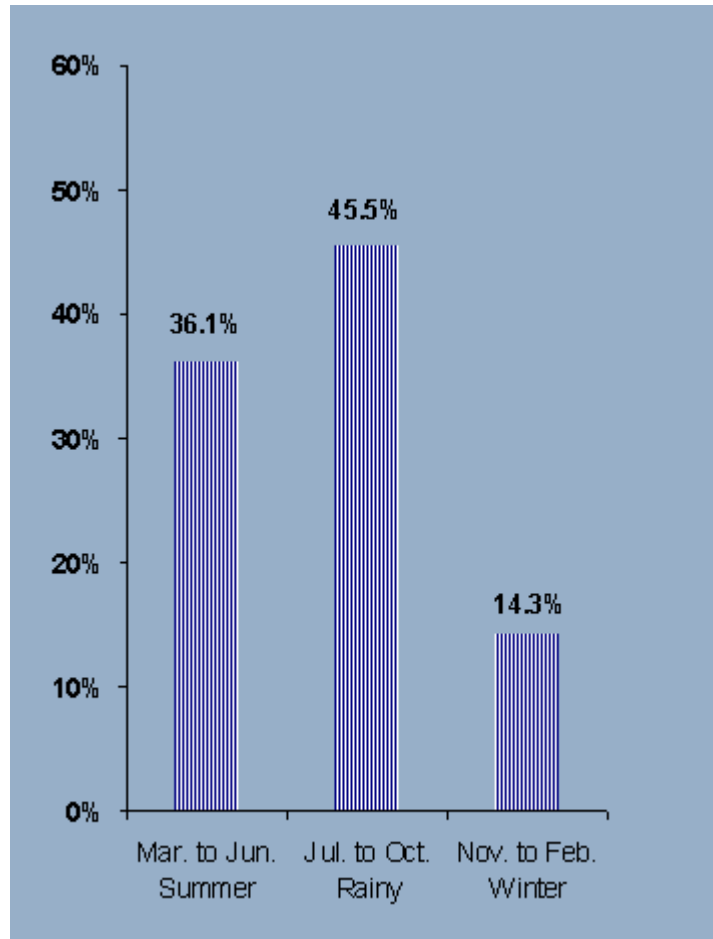


Fig. 1: Pattern of isolation of faecal *Escherichia coli* from hand-rinsing samples



DISCUSSION

Infective diarrhoea results from faeco-oral transmission. Hence, *E. coli*, as an indicator of faecal contamination, could be used for searching for the sources of potential contamination of infants and their foods by diarrhoeal pathogens.

The number of bacteria was not determined, because in our setting it was not technically feasible. Moreover, delay in transportation of samples in peptone water would have resulted in multiplication of all organisms, leading to false interpretation. It is true, however, that the higher the level of contamination in the samples, the greater the danger of infection from the sources (11). From a practical point of view, we considered the presence of *E. coli* in any sample as indicative of recent faecal pollution, hence a potential source of diarrhoeal pathogens.

Pinfold (12) has discussed the advantages of *E. coli* as an indicator of faeco-oral contamination and disease.

Mothers' hands have a strikingly high rate of faecal contamination (40%), much higher than their children (17%), whether exclusively breastfed or receiving complementary feeding (Fig. 1) both

suffer from similarly defective personal hygiene and lack of environmental sanitation. The difference between mothers and their infants is very significant (chi-square = 23.96; df = 1; $p < 10^{-6}$). Leftover food, and water are even more polluted: 17 of the 29 samples (60%). Yet, because of the small sample size, the 95% confidence limits are broad, from 39% (n=11) to 76% (n=22). Not surprisingly, feeding utensils are also heavily polluted (30%). The *E. coli* isolation rates from cups and spoons (32%), and from bottles and teats (27%) are almost equal. The heavy pollution of leftover food, drinks and utensils might, at least in part, explain that in our study community the risk of diarrhoea is three times higher in partially breastfed babies than in exclusive breastfed ones, as we have shown earlier (15).

A significant increase in contamination was observed during the rainy season ($p < 0.0001$). Rowland *et al.* (16) also found more contamination during the rainy season in Africa.

Our findings highlight once more the importance of prolonged exclusive breast feeding. Giving infants leftover foods should be discouraged and infant feeding with freshly prepared food encouraged. Mathur *et al.* came to the same conclusion (3). Mothers' and infants' hands are an important source of faecal contamination. This again emphasises the importance of personal hygiene, particularly frequent hand washing of mothers and their infants. The same view was reflected in previous studies (13,14). Still, one should not forget that the root causes are crushing poverty and, as one of its many consequences, maternal illiteracy.

In conclusion, our study quantifies the heavy exposure of infants to faecal contamination by their mothers and by other sources in rural Bengal. In this regard, isolating *E. coli* is easier than isolating and identifying diarrhoeal pathogens. The latter is time-consuming and all too frequently suffers from the inadequate laboratory facilities in developing countries. Hence, *E. coli* isolation can help develop and monitor intervention programmes and health education activities.

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Snow Mountain-Like Virus Identified in Young Children with Winter Vomiting Disease in South Africa

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ABSTRACT

Human caliciviruses have been reported to be associated with both epidemics of acute diarrhoeal illness and with sporadic cases of gastroenteritis in children. In this study, we report the identification of genogroup II small round-structured viruses or human caliciviruses associated with an outbreak of winter vomiting disease in South Africa. The virus was initially identified by electron microscopic examination of the stools and then further characterized by recombinant immunoassay with expressed capsid proteins to human caliciviruses from genogroups I and II. Both antigenically by the EIA and by sequence analysis of a region of the RNA-dependent RNA polymerase gene, the virus was shown to belong to genogroup II11 of the human *Caliciviridae*.

Key words: Calicivirus; Gastroenteritis; Disease outbreaks

INTRODUCTION

A group of small round viruses, which have been associated with epidemics of gastroenteritis world-wide and which show some surface structure under the electron microscope (EM), have been collectively termed small round-structured viruses (SRSV) (1). The identification and characterization of this group of morphologically similar viruses has been hampered by a lack of appropriate biologic tools, such as an *in vitro* cultivation system or an animal model. However, the recent cloning and sequencing of the viral genome of two members of this group, Southampton virus (2) and Norwalk virus (3,4), has enabled their molecular characterization. The structural organization and polarity of the viral genome has confirmed that these viruses are human members of the *Caliciviridae* (2,4).

Several studies, using reverse-transcriptase polymerase chain reaction (RT-PCR) and sequence analysis of the amplicon, have been reported to investigate the relationships between human viruses within this family (5-8). Comparative analyses of a region of the RNA-dependent RNA polymerase gene have been used for predicting overall genetic relatedness between the viruses (9-13). These studies have correlated well with studies which have included sequence analysis of the capsid gene (9-11). Two major genetic groups or "genogroups" of small round-structured viruses have been described using these techniques (7,9-11,13). The representative viruses of these two genogroups are Norwalk virus (genogroup I1) and Snow Mountain virus (genogroup III) (13).

Despite the advances made in molecular epidemiological techniques for studying the small round-structured caliciviruses, the "antigenic relationships between them remain unclear. Immunoassays using recombinant viral capsid have demonstrated that, at least two and probably more, antigenic groups exist (14-17). The two broad "serogroups," which have been defined so far, correspond to the two genogroups described by sequence analysis of regions of the RNA-dependent RNA polymerase gene which has, thus, been used as a surrogate marker for studying the two genogroups. Immune-electron microscopy (IEM) studies, using convalescent sera from adult volunteers, have indicated that multiple virus "types" exist, although the relationships within the broader serogroups are still to be determined (19,20).

In South Africa, two successive outbreaks of SRSV-associated gastroenteritis in adults have recently been reported (21). SRSVs were initially identified in each outbreak by electron microscopy (EM) and subsequently typed by IEM (as Hawaii virus-like), and by the recombinant ELISA technique (as Norwalk virus-like). Thus, members of both genogroups of human caliciviruses are present in South Africa. Further reports indicate the association of human caliciviruses with paediatric diarrhoea (22,23). In this study, we report the identification of a human calicivirus associated with acute winter vomiting disease in South Africa. The virus was shown to belong to genogroup II (Snow Mountain-like virus) by a recombinant immunoassay and by sequence analysis of a region of the RNA-dependent RNA polymerase gene.

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MATERIALS AND METHODS

Derivation of the viral strain

During the winter month of June 1994, a family practice comprising four general practitioners in the town of Middelburg in the Eastern Cape Province of South Africa saw many cases of what the practitioners described by clinical criteria as "winter vomiting disease." The main symptoms included sudden onset of vomiting and abdominal cramps., There was no diarrhoea in the majority of cases, although a few patients had upper respiratory tract infections. The patients were mostly young children from the Middelburg area, and it appears that they were not exposed to a common source of infection. Random cases were still being reported six months later.

Several stool specimens from this area showed the presence of SRSVs by electron microscopy (EM). One patient showing typical symptoms during this period, a 22-month old female child, presented with sudden onset vomiting without diarrhoea or respiratory tract symptoms. The child had no siblings and did not attend a day care facility. A stool sample from this child, who resided in Clubville, was collected on 9 June 1994 and sent to the National Institute for Virology, Sandringham for EM. In keeping with the common terminology used for this group of viruses and the procedure suggested by Wanmg (13), the strain has been designated HuCV/Clubville/94/SA.

Electron microscopy

For EM, 1 mL of stool was initially suspended in 9 mL distilled water. The suspension was clarified by centrifugation at 1000 rpm (100 Gg) for 10 minutes. The clarified solution was then subjected to

ultracentrifugation at 37,000 rpm (150,000 Gg) for two hours. The resulting pellet was resuspended in one drop of supernatant and placed on a carbon-coated, 100 mesh, copper grid. Excess fluid was blotted off, and one drop of 2% phosphotungstic acid (pH 6.9) was added for negative staining. After subjecting the grid to ultraviolet light, it was examined in a Joel 1200 transmission electron microscope.

The stool sample was also subjected to routine virological examination, including culture on Vervet monkey (*Cercopithecus aethiops*) kidney cells (MA104) at 37 ° C and inoculation into 24/48 hour suckling mice. A portion of the stool was stored for 3 months at -70 ° C and then retrieved for molecular and immunologic studies.

Reagents used for the immunoassays

Recombinant Norwalk virus (rNV) capsids expressed in a baculovirus system were available and diluted to a concentration of 1 µg/mL (7). The rNV was used for inoculating guinea pigs and rabbits, as described previously, to produce hyperimmune antisera for use in a Norwalk virus antigen detection ELISA (7,16).

A recombinant protein capsid (rMX), also expressed in a baculovirus system, was derived from a Snow Mountain-like virus recovered from the stool of a Mexican child (18). This recombinant has been used for generating hyper-immune sera to the MX agent for use in an antigen detection ELISA, similar to that for Norwalk virus (18).

Antigen detection immunoassays

An ELISA for the detection of Norwalk virus antigen in stool specimens was performed as described elsewhere in detail (16). The conjugate used was horseradish peroxidase-conjugated goat anti-human immunoglobulin (DAKO Ltd, High Wycombe, United Kingdom), which was added at a 1:5000 dilution and the substrate was 3,3',5,5'-tetramethylbenzidine (TMB, Sigma Chemical Company, Poole, United Kingdom). The optical density values were determined at 405 nm on a spectrophotometer, (Titertek Multiscan, Flow Laboratories McLean VA).

An ELISA used for the detection of Snow Mountain-like virus (MX agent) has been described in detail (18). Procedures for this ELISA were followed using the same conjugate and substrate described above.

Reverse-transcriptase PCR

The cetyltrimethylammonium bromide (CTAB) method described elsewhere was used for extracting viral RNA for RT-PCR (5). In brief, 10% stool suspensions were extracted with an equal volume of 1, 1,2-trichloro-1,2,2-trifluoroethane and concentrated with 8% polyethylene glycol 6000 and 0.4M NaCl. The pellets were resuspended, digested with proteinase K, and the viral RNA was extracted with phenol-chloroform in the presence of CTAB. The viral RNA was precipitated with ethanol and resuspended in de-ionised water.

Two pairs of primers described previously were used for the RT-PCR reaction (8). However, it was found that the optimal combination pair was by using one primer from each accepted pair. RT-PCR was, therefore, performed using the primer pair 36 and 51. The RT-PCR method has been described previously (5,8). In brief, the purified viral RNA was reverse transcribed for one hour at 42 ° C with avian myeloblastosis virus reverse transcriptase in the presence of RNasin and the sense primer. PCR was performed by adding a master mixture containing the anti-sense primer and *Taq* polymerase to the RT mixture. Amplification was performed over 39 cycles at 94 ° C for one minute, 50 ° C for 1.5 minutes and 72 ° C for one minute. The PCR products were analysed by agarose gel electrophoresis.

PCR product sequencing

Following amplification, the PCR products were purified and sequenced in both directions using the primers 36 and 51 with the PCR Product Sequenase system (United States Biochemicals, Cleveland). The system was used as specified by the manufacturer with slight modifications. The sequencing reactions were run through 6% urea sequencing gels at 80 W.

RESULTS

Electron microscopy

Examination of the stool specimen by EM showed clumps of viral particles. The sizes of the clumps varied and contained between 12 and over 200 particles. Measurement of the virus particle from micrographs showed the mean diameter to be 33 nm. The surface of the virus was structured, and the edges of the particles had a ragged outline (Fig. 1). On the basis of size and morphology, the virus was identified as a small round-structured virus.

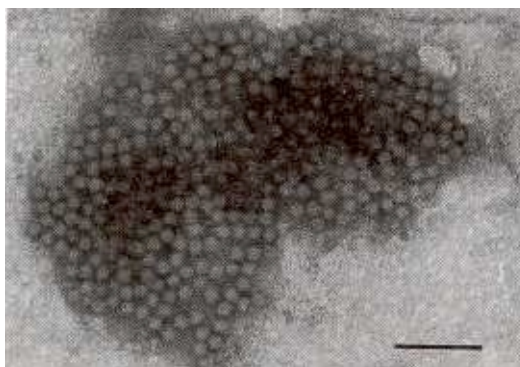


Fig. 1: Electron micrograph of the 33 nm small round-structured viruses observed in the diarrhoeal stool of a young child with winter vomiting disease in South Africa

No virus was isolated from tissue culture on MA 104 cells or by inoculation into suckling mice.

Detection of human calicivirus antigen in stool

In the ELISA for detecting Norwalk virus antigen, the stool was negative. However, the stool was positive for Snow Mountain-like virus antigen using a newly described ELISA (18). The optimal densities values were >2.000 , and the negative and positive control stools fell within normal boundaries. Antigenically, therefore, Cv/94/SA would be grouped in the Snow Mountain serogroup.

Amplification by RT-PCR

Three primer pairs were used in an attempt for amplifying a region of the RNA polymerase gene. The initial primer pair (36/35) was selected because of its high base pair match with other caliciviruses (13). However, no bands depicting the amplified product were observed. The second pair (3/51) is internal to 36/35., although it was also unsuccessful for the amplification of the RNA polymerase gene from Cv/94/SA. However, using a primer from each pair (i.e. primers 36/51), we amplified a product of approximately 360 base pairs, which is the expected size.

Sequence analysis of the PCR product

DNA sequences of the PCR products of the RNA polymerase gene were obtained in both orientations using the same primers which had been used for amplification. Repeated sequencing of the DNA showed excellent reproducibility. The nucleotide sequence of the RNA-dependent RNA polymerase gene strain Cv/94/SA was compared with the corresponding region (from base pair 4505 to 4958) of Norwalk virus

and Snow Mountain virus. The nucleotide similarity in this region of the polymerase gene of Cv/94/SA is 59.6% to Norwalk virus and 78.7% to Snow Mountain virus.

The predicted amino acid sequence of this region is shown in Figure 3 and compared it with the prototype viruses for genogroup I and II. Although there were 76 base pair substitutions compared to Snow Mountain virus within this region, only 10 changes to the predicted amino acid occurred (Fig. 4). The amino acid homology of Cv/94/SA to Norwalk virus and Snow Mountain virus was 59.3% and 91.5% respectively, indicating the grouping of Cv/94/SA within the Snow Mountain virus genogroup.

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1451aa
NV8fIIa/68/US IE---L-YAE-AK-KN-F---TA----N-QIMTESFS--S-LT-S-E
Cv/94/SA      xxDGPIIFEHSRYRYHYDADYSRW DSTQQR AVLAAALEIMVRFSAEPQL
SMA/76/US     NE-----FS-----K--P--H--

NV8fIIa/68/US -EV--Q----EM---YV-RVK-----F-----V---V---V--N--II-----
Cv/94/SA      AQIVAEDLLAPSVVDVGD FKITINEGLPSGVPCTSQWNSLAHWLLTLCAL
SMA/76/US     -----M-----

NV8fIIa/68/US --A---S--VV-SM-YF----
Cv/94/SA      SEVTGLGPDIIQANS MYSFYG
SMA/76/US     ----N-A-----LF-----

```

Fig. 2: Alignment of the predicted amino acid sequence of the region of the RNA polymerase gene. The sequences shown are aligned from amino acid 1454 to 1571 of the predicted ORF1 of the Norwalk virus and Snow Mountain virus genomes. The highly conserved RNA polymerase GLPSG motif is shown in bold print.

DISCUSSION

In this study we report on the identification of a small round-structured virus, characterized as a Snow Mountain agent-like virus, recovered from the stool of children with classic winter vomiting disease in South Africa. The human caliciviruses have been reported to be associated with epidemic gastroenteritis (21) and with paediatric gastroenteritis (22,23) in South Africa. Nevertheless, this report is interesting in that (i) the virus is associated with winter vomiting disease and not with the diarrhoeal episodes used to identify the cases of paediatric gastroenteritis in the previous studies (22,23), and (ii) the standard primer pairs did not amplify Cv/94/SA, whereas they were used successfully in other studies (5,8).

The outbreak of "winter vomiting disease" that which occurred in Middelburg coincided with the coldest winter in 30 years, which may have some significance in the epidemiology of this outbreak. Furthermore, the majority of cases were associated clinically, not with diarrhoea, but with the sudden onset of vomiting and, in some cases, upper respiratory tract infections. This observation highlights the need to include not only diarrhoeal stools when screening for the involvement of human caliciviruses in clinical disease. The availability of the recombinant EIAs for this screening will greatly aid in the further elucidation of the role of these viruses in clinical disease.

Sequence analysis of the RNA-dependent RNA polymerase gene has been found to be a powerful technique for the early characterization of human strains within the *Caliciviridae* and has been used fairly extensively for studying strains from the United Kingdom (7,9,24), the United States (10,11,13), and Japan (13). Limited comparisons have been made with the human caliciviruses identified in South Africa (22). In this study we have obtained the sequence of a region of the RNA polymerase gene of a South African human calicivirus, Clubville/94/SA, and compared it with the prototype viral strains of the two human genogroup within this family.

The deduced amino acid sequence of the RNA polymerase gene of Clubville/94/SA is over 90% homologous to Snow Mountain virus compared to barely 60% to Norwalk virus, and indicates that Clubville/94/SA belongs to genogroup II. Further, these results were supported by the immunoassay studies using recombinant capsid antigen expressed in a baculovirus system (17). Clubville/94/SA reacted to high titre with the hyper-immune sera generated by the genogroup 11 vital antigen (Snow Mountain-like virus), but failed to react with that raised against the expressed Norwalk virus antigen. The highly conserved RNA polymerase GLPSG motif was confirmed to be in the correct position with respect to the gene and was conserved in Clubville/94/SA. Unfortunately the anti-sense primer runs into the second conserved motif YGDD which is present in all RNA polymerase genes. Nevertheless, the first two amino acids of this motif were present as shown in Figure 4, and it may be reasonable to assume that the 6 base pairs on the 3' end of the primer must be conserved for the amplification reaction to have been successful. When we compared the deduced amino acid sequence of the polymerase gene of Clubville/94/SA with other human strains belonging to genogroup II11, it was interesting to note that a higher degree of homology was found with OTH-25/89/Japan recovered from a 2-year old child in Japan (13), 4S/90/UK recovered from a 10-month old in the UK (7), and to other South African strains (22). The deduced amino acid sequence of these two strains was reported to be 100% homologous (7); strains Clubville/94/SA differed by 1 amino acid from these two strains, compared to the 10 amino acid substitutions noted with Snow Mountain virus.

It is noteworthy that, based on the sequence of the RNA polymerase gene of these strains, all three have been categorized as belonging to genogroup II11 with Snow Mountain virus. Antigenic studies, using the recombinant Norwalk and Snow Mountain virus-like protein (3,17), have shown that these viruses, derived from different countries, are antigenically related to each other and to Snow Mountain virus (13). The data presented here supports the use of sequence analysis of the RNA polymerase gene to type members of the human caliciviruses. The single exception to this is Southampton virus, which was antigenically typed as similar to Snow Mountain virus, but has an RNA polymerase gene similar to Norwalk virus (2,13).

Wang and colleagues have proposed an interim scheme for the nomenclature of the human caliciviruses found in stools and associated with gastroenteritis (13). We have adopted this approach in this study, and support the concept proposed to avoid unnecessary confusion as this field is explored further.

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From the Internet

The first topic reprinted here is from the World Health Organization (<http://www.who.org>). It is the report of the WHO vaccine research development steering committee on diarrhoeal diseases. The committee was chaired by Professor Philippe Sansonetti, Pasteur Institute, Paris. It is a crisp, focused document that should be of value to all interested in the use of vaccines to prevent infectious diarrhoeas. Clearly, the task ahead is still formidable but much is being done and progress is being made. In this vast field, priorities must be set and that is what the report does with great clarity.

The second topic is from the July-September 1997 (Volume 3, Number 3) issue of **Emerging Infectious Diseases**. One of the excellent publications of the USA Centres for Disease Control and Prevention in Atlanta, it is in the public domain: its contents may be reprinted without special permission. The JDDR wishes,

however, to explicitly acknowledge the source and the authors of the paper "[Multidrug-Resistant Enteroaggregative Escherichia coli Associated with Persistent Diarrhea in Kenyan Children](#)" by W.K. Sang, J.O. Oundo, J.K. Mwituria, P.G. Waiyaki, Kenya Medical Research Institute, Nairobi Kenya; M. Yoh, T. Iida, and T. Honda, Osaka University, Osaka, Japan.

Emerging Infectious Diseases was accessed through a circuitous route, starting with an interesting site: <http://www.medscape.com/>. Medscape.com is free, and packed with interesting material. It also has many hyperlinks to, amongst many others, Medline and a number of medical journals, including **Emerging and Infectious Diseases**. For those with an easy access to the World Wide Web it is worth exploring.

R. Eckels

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day period indicated satisfactory tolerance of the KY diet with adequate weight gain. The weight gain and stool output was however higher in lactose intolerant children, with the worst results seen with K-Y and buffalo milk combination. While lactose intolerant children with PD do have higher rates of therapeutic failure, our data indicate that a traditional diet and yogurt combination can be used satisfactory for nutritional rehabilitation in over 80% of such children."

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"The emergence of *Vibrio cholerae* 0139 Bengal in 1992, its rapid spread in an epidemic form, in which it replaced existing strains of *V. cholerae* 01 during 1992 and 1993, and the subsequent reemergence of *V. cholerae* 01 of the El Tor biotype in Bangladesh since 1994 have raised questions regarding the origin of the reemerged El Tor vibrios. We studied 50 El Tor vibrio strains isolated in Bangladesh and four other countries in Asia and Africa before the emergence of *V. cholerae* 0139 and 32 strains isolated in Bangladesh during and after the epidemic caused by *V. cholerae* 0139 to determine

whether the reemerged El Tor vibrios were genetically different from the El Tor vibrios which existed before the emergence of *V. cholerae* 0139. Analysis of restriction fragment length polymorphisms in genes for conserved rRNA, cholera toxin (*ctxA*), and zonula occludens toxin (*zot*) or in DNA sequences flanking these genes showed that the El Tor strains isolated before the emergence of *V. cholerae* 0139 belonged to four different ribotypes and four different *ctx* genotypes. Of 32 El Tor strains isolated after the emergence of 0139 vibrios, 30 strains (93.7%) including all the clinical isolates belonged to a single new ribotype and a distinctly different *ctx* genotype. These results provide evidence that the reemerged El Tor strains represent a new clone of El Tor vibrios distinctly different from the earlier clones of El Tor vibrios which were replaced by the 0139 vibrios. Further analysis showed that all the strains carried the structural and regulatory genes for toxin-coregulated pilus (*tcpA*, *tcpI*, and *toxR*). All strains of the new clone produced cholera toxin (CT) in vitro, as assayed by the G_{M1}-dependent enzyme-linked immunosorbent assay, and the level of CT production was comparable to that of previous epidemic isolates or El Tor vibrios. Further studies are required to assess the epidemic potential of the newly emerged clone of *V. cholerae* 01 and to understand the mechanism of emergence of new clones of toxigenic *V. cholerae*."

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"Background: Glucose-based oral rehydration solutions (ORS) available in the United States do not appear to reduce the severity or duration of diarrhea. The use of cereal-derived ORS and cereal-based feedings appears to diminish the severity of illness in studies conducted in the developing world. To our knowledge, no controlled trials of cereal-derived ORS or cereal-based feedings have been performed in the United States. **Methods:** We performed a randomized, double-blind trial of two ORS feeding regimens in outpatients with diarrhea. Patients aged 2-13 months with acute watery diarrhea were enrolled. Subjects received standard glucose-based ORS in alternation with soy-based, lactose-free infant formula (Group 1) or rice syrup solid containing ORS in alternation with rice-based, lactose-free infant formula (Group 2). Subjects were visited at home daily to determine the severity of illness and characteristics of the stool. **Results:** After the first 2 days, significantly more Group 1 subjects continued to have diarrhea than did Group 2 subjects (median duration of diarrhea 3 vs. 2 days) as demonstrated by Kaplan-Meier survival curves ($p=0.04$). **Conclusion:** We conclude that infants fed a regimen consisting of rice syrup solid containing ORS and rice formula resolved their diarrhea sooner than did infants fed a regimen of standard glucose-based ORS with soy formula. The relative contributions of ORS and formula to this more rapid recovery can be elucidated by further studies."

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"A study was carried out in 3 villages near Calcutta, India, having a population of 5464, between August 1992 and December 1994. A cohort of rural children below 4 years of age was prospectively observed to determine the clinico-epidemiological aspects of mucoid diarrhoea and examine propensity to invasiveness. Overall, the incidence of diarrhoea was 1.7 episodes/child/year, and that of mucoid and bloody dysentery was 0.8 and 0.2 episodes/child/year, respectively. Children aged 6-11 months had a higher incidence of mucoid diarrhoea (1.3 episodes/child/year) and the peak season occurred in June and July. Multivariate analysis using logistic regression showed that mucoid diarrhoea and bloody dysentery were closely similar in both clinical and laboratory findings, including raised faecal leucocyte count (>10/high power microscope field [hpf]). However, abdominal pain occurred more frequently in bloody dysentery than in mucoid diarrhoea. Faecal leucocyte count (>10/hpf) can therefore be used as an indicator for invasiveness of mucoid diarrhoea at the community level."

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"Lactation counsellors were trained to advise mothers of partially breast-fed infants who were admitted to hospital because of diarrhoea, so that they could start exclusive breast-feeding during their hospital stay. Infants (n = 250) up to 12 weeks of age were randomized to intervention and control groups. Mothers in the intervention group were individually advised by the counsellors, while mothers in the control group received only routine group health education. During follow-up at home by the counsellors a week later, only the mothers in the intervention group were counselled. All the mothers were evaluated for infant feeding practices at home two weeks after discharge. Among the 125 mother-infant pairs in each group, 60% of mothers in the intervention group were breast-feeding exclusively at discharge compared with only 6% in the control group (P<0.001); two weeks later, these rates rose to 75% and 8% in the intervention and control groups, respectively (P<0.001). However, 49% of mothers in the control group reverted back to bottle-feeding compared with 12% in the intervention group (P<0.001). Thus, individual counselling had a positive impact on mothers to start exclusive breast-feeding during

hospitalization. and to continue the practice at home. Maternal and child health facilities should include lactation counselling as an integral part of their programmes to improve infant feeding practices."

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"An updated review of nonvaccine interventions for the prevention of childhood diarrhoea in developing countries is presented. The importance of various key preventive strategies (breast-feeding, water supply and sanitation improvements) is confirmed and certain aspects of others (promotion of personal and domestic hygiene, weaning education/food hygiene) are refined. Evidence is also presented to suggest that, subject to cost-effectiveness examination, two other strategies - vitamin A supplementation and the prevention of low birth weight - should be promoted to the first category of interventions, as classified by Feachem, i.e. those which are considered to have high effectiveness and strong feasibility."

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"Epidemiological studies have demonstrated that hands may be an important vehicle for transmission of shigellosis. The present study was carried out to find out the survival potential of *Shigella dysenteriae* 1 on fingers of volunteers. Finger surface was inoculated with 10^5 cfu of *S. dysenteriae* 1 and then the bacteria were detected using conventional culture, PCR and fluorescent antibody (FA) techniques after different time intervals. It was found that *S. dysenteriae* 1 survived for up to one hour in culturable form but up to four hours in non-culturable form on human fingers. The non-culturable *S. dysenteriae* was detected by PCR and FA techniques. This study elaborates on the role that fingers have in the transmission of shigellae."

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Eng. Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Yaba, Lagos

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"*Objective.* To study the etiologic role of toroviruses as a cause of gastroenteritis in humans. *Methods.* The design was a case-control study. We compared the rate of torovirus detection in fecal specimens from a selection of children with acute or persistent diarrhea and controls without diarrhea from a study of childhood diarrhea in an urban Brazilian slum. Stool samples were coded and tested in a blinded fashion for the presence of torovirus antigen by enzyme-linked immunosorbent assay, other enteropathogens, toxins and fecal leukocytes. *Results.* Thirty-three children with acute diarrhea, 41 children with persistent diarrhea and 17 controls were enlisted in the study. Torovirus antigen was detected in 9 (27%) samples from children with acute diarrhea, 11 (27%) samples from children with persistent diarrhea and none of the samples from controls ($P < 0.05$). In addition the presence of enteroaggregative *E. coli* was associated with persistent diarrhea and the presence of *Cryptosporidium* oocysts was common although not significant ($P = 0.08$); torovirus and *Cryptosporidium* occurred in different subsets of samples, whereas torovirus and enteroaggregative *Escherichia coli* were commonly found in combination. *Conclusions.* These data indicate that toroviruses, alone or in combination with enteroaggregative *E. coli*, may play a pathogenic role in acute and possibly persistent diarrhea. Further studies are warranted to determine the etiologic role of toroviruses in gastroenteritis."

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"*Aeromonas* isolates from patients with diarrhea in Bangladesh ($n = 69$), from healthy controls ($n = 11$), and from surface water ($n = 40$) were analyzed with respect to their hybridization groups (HGs) by the aid of fatty acid methyl ester (FAME) characterization and DNA fingerprinting by AFLP, biochemical phenotypes (PhenePlate [PhP] types), and the production of hemolysin and cytotoxin. The aim of the investigation was to find out whether certain strains carrying virulence factors predominated among patient isolates. According to FAME and/or AFLP analysis, most human isolates were allocated to DNA HGs 4 (*Aeromonas caviae*) and 1 (*A. hydrophila*). Most environmental strains were

allocated to HG8 (*A. veronii* biogroup *sobria*) and HG4 (*A. caviae*), and only one was of HG1. According to PhP typing, the diversity among patient isolates was lower than that among other strains, and two dominating PhP types (types BD-1 and BD-2) were identified in 29 and 30% of the patient isolates, respectively. PhP type BD-1 was also common among the environmental isolates, whereas PhP type BD-2 was only identified in two of the other isolates. Twenty-five of 26 isolates belonging to HG1 were of the same PhP type (BD-2), whereas isolates of other common HGs were more diverse according to their PhP types. Hemolytic and cytotoxin-producing strains occurred more frequently among the environmental isolates than among patient isolates. However, the hemolytic and cytotoxic activities among human isolates was strongly correlated to the HG1/BD-2 type, which, in addition, showed high cytotoxin titers (median values. 1/512 compared to 1/128 for cytotoxin-positive isolates belonging to other types). Thus, the HG1/BD-2 type may represent a pathogenic *A. hydrophila* type that is able to produce diarrhea in humans."

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"To determine risk factors for diarrhea, the authors followed an open cohort of 1,314 children from Guinea-Bissau by weekly diarrhea recall interviews between April 1987 and March 1990. Data on feeding practices and measles infection were available for all children and, for 531 children, comprehensive data on explanatory variables were recorded. Of 57 variables, seven were independently associated with an increased incidence of diarrhea. These were a recent (in the past 14 days) diarrheal episode, male sex, being weaned from breast milk, not being looked after by the mother, head of the household being <30 years old, eating cold leftovers, and drinking water from an unprotected public water supply. In breastfed children, only three variables were associated with diarrhea, including prior diarrhea, male sex, and not being looked after by the mother. Among weaned children, six variables delineated increased rates of diarrhea, including unprotected public water supply, eating of cold leftovers, and lack of maternal education. Major determinants of persistent diarrhea included weaning, lack of maternal education, and having pigs in the home. It is concluded that, in addition to the promotion of breastfeeding, important interventions against diarrhea include improvements in water supply, hygiene, and food handling. However, because of effect modification by breastfeeding, the largest effects of these interventions will probably be among weaned children.

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"This study was conducted in a hospital setting to determine whether enterotoxigenic strains of *Bacteroides fragilis* (ETBF) were associated with childhood diarrhoea. ETBF was isolated from 6 (2.6%) of 226 patients and 3 (1.7%) of 172 controls and was found mostly in children between 1-5 yr of age. The syndrome associated with ETBF was secretory in nature with watery diarrhoea and of mild severity. ETBF may be associated with diarrhoeal illness in children but is not a major problem in this part of the country."

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292. Pal T, Al-Swehi NA, Herpay M, Chugh TD. Identification of enteroinvasive *Escherichia coli* and *Shigella* strains in pediatric patients by an IpaC-specific enzyme-linked immunosorbent assay. J Clin Microbiol 1997 Jul;35(7):1757-60. 25 ref, Eng. Department of Microbiology, University Medical School, H-7643 Pecs, Hungary

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"Diarrheal episodes with enterotoxigenic *Escherichia coli* (ETEC) were prospectively monitored during the first 2 years of life in a cohort of 235 infants from Leon, Nicaragua. ETEC was an etiological finding in 38% (310 of 808) of diarrheal episodes and in 19% (277 of 1,472) of samples taken as asymptomatic controls at defined age intervals ($P < 0.0001$). The majority of diarrheal episodes (80%) occurred before 12 months of age. The major ETEC type was characterized by colonization factor CFA I and elaboration of both heat-labile enterotoxin and heat-stable enterotoxin (ST). The proportion of *E. coli* strains with CFA I was significantly higher in cases with diarrhea ($P = 0.002$). The second most prevalent type showed putative colonization factor PCFO166 and production of ST. The prevalence of PCFO166 was approximately 20%, higher than reported before. Children with a first CFA I episode contracted a second ETEC CFA I infection 24% of the time, compared with 46% for ETEC strains of any subtype. Most of the ETEC episodes were of moderate severity, and only 5% (15 of 310) were characterized as severe. In conclusion, our results give valuable information for the planning of intervention studies using ETEC vaccines."

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"Three morphologic patterns of interaction between bacteria and enterocytes have been observed in colonic biopsy specimens from AIDS patients with chronic diarrhea in the United States. The DNA encoding virulence factors and the HEp-2 cell adherence patterns of *Escherichia coli* strains isolated from the stools of eight symptomatic AIDS patients were compared with those of five control strains with known adherence patterns. One clinical isolate from a patient with attaching-and-effacing enteropathy displayed the localized adherence attaching-and-effacing pattern typical of enteropathogenic *E. coli* on HEp-2 cells, five isolates displayed the "stacked-brick" aggregative adherence pattern typical of enteroaggregative *E. coli* strains, and one isolate showed the pattern characteristic of diffusely adherent *E. coli*. One patient's isolate displayed features of all three patterns. No clinical isolate hybridized with standard probes for enteropathogenic, enteroaggregative, diffusely adherent, enterotoxigenic, and enteroinvasive *E. coli* strains. Thus, isolates from symptomatic AIDS patients in the United States can display the same interactive patterns with HEp-2 cells as the agents of pediatric or traveler's diarrhea, but lack their typical virulence factors."

295. Qadri F, Jonson G, Begum YA, Wennerås C, Albert MJ, Salam MA, Svennerholm A-M. Immune response to the mannose-sensitive hemagglutinin in patients with cholera due to *Vibrio cholerae* O1 and O139. Clin Diagn Lab Immunol 1997 Jul;4(4):429-34. 36 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"The mannose-sensitive hemagglutinin (MSHA) is a type 4 pilus present in *Vibrio cholerae* O1 strains of the El Tor biotype, as well as in strains of serogroup O139. It has been shown to be a colonization antigen in animal models. The aim of this study was to investigate systemic and local antibody responses to MSHA in adult patients with cholera due to *V. cholerae* O1 and O139. Twenty-four of 28 (86%) patients with O1 cholera and 11 of 17 (65%) patients with O139 cholera showed significant increases in MSHA-specific immunoglobulin A (IgA) and IgM antibody-secreting cells (ASCs) 7 days after the onset of disease. However, the magnitude of the ASC response in O1 cholera patients was significantly higher than that in the O139 cholera patients in both IgA-producing ($P=0.015$) and IgM-producing ($P=0.029$) cells. Both groups of patients responded with antibody responses to MSHA in plasma, seroconverting with both IgA (63 to 70% of patients) and IgG (43 to 59% of patients) antibodies. Compared to the MSHA-specific antibody levels determined in healthy controls ($n=10$), more than 90% of O1 and O139 cholera patients showed responses to MSHA of both the IgA and the IgG isotypes. About 70% of the patients in both groups also had antibody responses to MSHA in their feces. In summary, we demonstrated that MSHA is immunogenic, giving rise to both systemic and local antibodies in patients with cholera due to both O1 and O139 serogroups."

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"The frequency of intestinal parasitic infections was studied retrospectively in 1,029 cancer patients presenting with symptoms of diarrhoea. Intestinal parasites were diagnosed by stool examination, using both the direct and concentration techniques and also the modified acid fast stain. Parasitic infection was found in 16.5% of the cases. The majority of the patients with intestinal parasitosis had cancer of the haemopoietic system

and were on anticancer chemotherapy. The most prevalent parasites were *Entamoeba histolytica/Entamoeba dispar* (8.5%) and *Giardia lamblia* (3.1%). Much more rare were *Strongyloides stercoralis* (0.6%), *Cryptosporidium parvum* (0.3%) and *Isospora belli* (0.1%). All the patients with intestinal parasites were negative for HIV antibodies."

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"Objective. A community-based, randomized, double-blind intervention trial was conducted to measure the impact of zinc supplementation on young Guatemalan children's morbidity from diarrhea and respiratory infections. **Methods.** Children aged 6 to 9 months were randomly assigned to receive 4 mL of a beverage containing 10 mg of zinc (as zinc sulfate) daily (7 d/wk) for 7 months (n=45) or a placebo (n=44). Morbidity data were collected daily. Diagnoses of diarrhea, fever, and anorexia were based on mothers' definitions. Respiratory infections were defined as the presence of at least two of the following symptoms: runny nose, cough, wheezing, difficulty breathing, or fever. **Results.** High rates of diarrhea and respiratory infections were reported. Children from the placebo group had a 20% episodic prevalence of diarrhea, with 8 episodes/100 d, and a 7% episodic prevalence of respiratory infections, with 3 episodes/100d. The median incidence of diarrhea among children who received zinc supplementation was reduced by 22% (Wilcoxon rank test), with larger reductions among boys and among children with weight-for-length at baseline lower than the median of the sample (39% reductions in both subgroups). Zinc supplementation also produced a 67% reduction in the percentage of children who had one or more episodes of persistent diarrhea (X^2 test). No significant effects were found on the episodic prevalence of diarrhea, the number of days per episode, or the episodic prevalence or incidence of respiratory infections. **Conclusions.** The large impact of zinc supplementation on diarrhea incidence suggests that young, rural Guatemalan children may be zinc deficient and that zinc supplementation may be an effective intervention to improve their health and growth."

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"Adhesion and subsequent colonisation are important events in the infection by *Vibrio cholerae* 0139 Bengal. To determine in details the pathological changes in the gut mucosa, an epidemic strain of 0139 Bengal was inoculated in a rabbit ileal loop model.

Electron microscopic studies were done at different time intervals after inoculation of the strain to see the histological changes at the ultrastructural level. From 10 hours onwards, cellular invasive processes with presence of bacteria in the lamina propria and other associated inflammatory changes were revealed."

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"The acceptability of prepackaged rice-based (Oresol-R) and flavoured (Oresol-F) glucose-based oral rehydration salts (ORS) solutions was compared with that of standard glucose-based ORS (Oresol-G) in a randomized field trial. Additionally, it is determined if presenting rice-based ORS as a solution that would help stop diarrhoea (Oresol-K) enhanced its acceptability. A total of 437 non-dehydrated children aged less than five years presenting to health centres with acute diarrhoea were randomly assigned to the three ORS groups. Acceptability was determined by the amounts of ORS consumed at home by children still with diarrhoea on 24- or 48-hour follow-up. The amounts of ORS consumed by children given Oresol-R (54 [95% CL 38-70] mL/kg/24 h) and Oresol-F (47 [24-70]) were similar to the amount of Oresol-G (44 [32-56]). ORS consumption was not affected by the child's age, nutritional status, feeding before the episode, duration of diarrhoea at health centre visit, maternal education and previous ORS use. Informing the caretaker that rice-based ORS would help stop diarrhoea did not lead to increased consumption of the solution (Oresol-R 54 [38-70] mL/kg/24 h; Oresol-K 50 [32-68]). Solution preparation was likewise similar among the treatment groups. Reactions to the different ORS types were generally favourable but did not differ between the groups."

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"A community-based, double-blind, randomized trial was conducted in a population of low socioeconomic status in urban India to determine whether daily zinc supplementation reduces the incidence and prevalence of acute diarrhea, especially in those with zinc deficiency. Children 6-35 mo of age were randomly assigned to zinc (n = 286) and control (n = 293) groups and received a supplement daily for 6 mo. Zinc gluconate (10 mg elemental Zn) was given, with both zinc and control groups also receiving multivitamins. The primary outcome measures determined by home visits every fifth day and physician examinations were the number of acute diarrheal episodes (incidence) and total diarrheal days (prevalence). Zinc supplementation had no effect in children 6-11 mo old. In children aged >11 mo there was significantly less diarrhea in the zinc group. In boys >11 mo old, supplementation resulted in a 26% (95% CI: 13%, 38%) lower diarrheal incidence and a 35% (95% CI: 20%, 50%) lower prevalence. In zinc-supplemented girls >11 mo of age, the incidence was 17% (95% CI: 2%, 30%) lower and the prevalence was 19% (95% CI: 4%, 47%) lower. Overall, zinc supplementation resulted in a 17% (95% CI: 1%, 30%) lower diarrhea incidence in children with plasma zinc concentrations <9.18 µmol/L at enrollment and a 33% (95% CI: 6%, 52%) lower incidence in children with concentrations <50 µmol/L. In conclusion, zinc supplementation had a significant effect on acute diarrheal morbidity in children >11 mo old and in children with low plasma zinc concentrations."

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"Background & Aims: Net sodium absorption from oral rehydration solution is increased by both glucose-sodium cotransport and solvent drag. The aim of this study was to measure the relative importance of glucose-sodium cotransport and solvent drag in the stimulation of net sodium absorption by oral rehydration solution. **Methods:** Total intestinal perfusion was used in normal subjects with and without intrajejunal cholera toxin using three test solutions containing 100 mmol/L sodium and either 100 mmol/L mannitol (control), 100 mmol/L glucose, or no additional solute (hypotonic solution). The increase in sodium absorption greater than control with hypotonic solution represented sodium absorption stimulated by solvent drag; the further increase in sodium absorption induced by glucose, greater than that noted with the hypotonic solution, represented sodium absorption stimulated by cotransport. **Results:** Without cholera toxin, solvent drag and cotransport promoted sodium absorption at rates of 62 and 33 mmol/h, respectively. With cholera toxin, solvent drag and cotransport promoted sodium absorption at rates of 44 and 71 mmol/h, respectively. **Conclusions:** Net sodium absorption caused by cotransport increased more than twofold after exposure of the intestine to cholera toxin ($P < 0.003$). This could be mediated by increased cotransport, a change in the stoichiometry of cotransport, or an increase in chloride permeability."

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"In Bolivia, few data are available to guide empiric therapy for bloody diarrhea. A study was conducted between December 1994 and April 1995 to identify organisms causing bloody diarrhea in Bolivian children. Rectal swabs from children <5 years old with bloody diarrhea were examined for *Salmonella*, *Shigella*, and *Campylobacter* organisms; fecal specimens were examined for *Entamoeba histolytica*. A bacterial pathogen was identified in specimens from 55 patients (41%). *Shigella* organisms were found in 39 specimens (29%); 37 isolates (95%) were resistant to ampicillin, 35 (90%) to trimethoprim-sulfamethoxazole, and 24 (62%) to chloramphenicol, but all were susceptible to nalidixic acid. Only 1 of 133 stool specimens contained *E. histolytica* trophozoites. Multidrug-resistant *Shigella* species are a frequent cause of bloody diarrhea in Bolivian children; *E. histolytica* is uncommon. Clinical predictors described in this study may help identify patients most likely to have *Shigella* infection. Laboratory surveillance is essential to monitor antimicrobial resistance and guide empiric treatment."

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"Background & Aims: We have shown that addition of gum arabic (GA) to a 90 mmol/L sodium-111 mmol/L glucose oral rehydration solution (ORS) enhances its effectiveness for water and electrolyte absorption in normal rats. The present study extends these observations on GA in ORS to two rat models of diarrheal disease. **Methods:** Juvenile rats were either treated for 1 week with magnesium citrate-phenolphthalein to produce chronic osmotic-secretory diarrhea or lumenally exposed to 10 mmol/L theophylline to induce jejunal secretion. In both models jejunal perfusion was used to assess absorption. **Results:** Addition of 2.5 or 5.0 g/L GA to ORS increased roughly twofold absorption of sodium, potassium, and water in the model of chronic osmotic-secretory diarrhea. Rats perfused with GA-supplemented ORS showed an expansion of the basolateral intercellular spaces between villus absorptive epithelial cells and the lamina propria, reflecting enhanced water and sodium absorption. Similarly, addition of 2.5, 5.0, or 10.0 g/L GA to the ORS neutralized theophylline-induced abolition of net sodium and potassium absorption and reversed water and glucose malabsorption. **Conclusion:** These experimental studies in models of diarrhea suggest that GA may be a useful additive to ORS for the potentiation of water and electrolyte absorption."

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"Background - The nitric oxide (NO) precursor L-arginine has been shown to produce variable effects on intestinal absorptive function, including ion transport. **Aims** - To determine whether there is an optimal concentration of L-arginine, promoting proabsorptive effects from oral rehydration solutions (ORS) with 90 or 60 mM sodium. **Subjects and methods** - In vivo perfusion of rat jejunum with determination of net water absorption, unidirectional fluid exchanges, sodium and calcium transport, and glucose absorption. **Results** - L-Arginine (1 mM) added to the 90 mM sodium ORS increased intestinal absorption of both sodium and water. Higher concentrations of L-arginine (2 to 10 mM) lacked this stimulatory effect. At 20 mM, L-arginine decreased sodium absorption below baseline. With a 60 mM sodium ORS, 2 mM L-arginine had a maximal fluid and electrolyte proabsorptive effect. At 20 mM L-arginine, net water absorption was indistinguishable from that obtained in the absence of L-arginine, and lower than with 2 mM L-arginine. Sodium absorption remained raised above baseline in perfusions with 10 and 20 mM L-arginine. Morphologically, villi from perfusions with increased absorption showed a large expansion of intercellular and lamina propria intercellular spaces. **Conclusions** - Low concentrations of L-arginine seem to stimulate water and electrolyte absorption by the small intestine. This effect is consistent with NO induced vasodilation, whereas higher L-arginine concentrations may be vasoconstrictive and thereby reverse fluid and electrolyte transport."

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