

## LONGITUDINAL STUDIES OF INFECTIOUS DISEASES AND PHYSICAL GROWTH OF CHILDREN IN RURAL BANGLADESH

### II. INCIDENCE OF DIARRHEA AND ASSOCIATION WITH KNOWN PATHOGENS

ROBERT E. BLACK,<sup>1,2</sup> KENNETH H. BROWN,<sup>1,3</sup> STAN BECKER,<sup>1</sup> A. R. M. ABDUL ALIM<sup>1</sup> AND  
IMDADUL HUQ<sup>1</sup>

**Black, R. E. (U. of Maryland School of Medicine, Baltimore, MD 21201), K. H. Brown, S. Becker, A. R. M. A. Alim and I. Huq. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II. Incidence of diarrhea and association with known pathogens. *Am J Epidemiol* 1982;115:315-24.**

Longitudinal studies were done in two villages in rural Bangladesh to learn more about the interactions between infectious diseases and the nutritional status of children. Diarrheal diseases, identified by surveillance of 197 children aged 2-60 months, were studied for bacterial, viral and parasitic enteropathogens in 1978-1979. The annual incidence of diarrhea was highest in children aged 2-11 months, and declined progressively with age from seven to four episodes per child per year. An enteropathogen was identified from rectal cultures taken during diarrhea in 51% of episodes and from 6% of monthly cultures taken when diarrhea was not present. Enterotoxigenic *Escherichia coli* were the pathogens found most frequently, followed by shigellae and rotaviruses. Diarrheal episodes associated with shigellae had the longest duration, while episodes associated with *Vibrio cholerae* or with rotavirus were more frequently associated with dehydration. *E. coli* diarrhea had a peak incidence during the hot months, and shigellosis was more frequent during the cool, dry months.

**cholera; diarrhea; diarrhea, infantile; dysentery, bacillary; *Escherichia coli*; malnutrition; rotavirus; *Shigella***

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Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; LT, heat-labile toxin; ST, heat-stable toxin.

<sup>1</sup> International Centre for Diarrhoeal Diseases Research, Bangladesh, Dacca, Bangladesh.

<sup>2</sup> Center for Vaccine Development, Division of Infectious Diseases, U. of Maryland School of Medicine, 29 S. Greene St., Baltimore, MD 21201 (address for reprint requests).

<sup>3</sup> Division of Geographic Medicine, Department of Medicine, and Gastroenterology and Nutrition Unit, Department of Pediatrics, Johns Hopkins U. School of Medicine, and Division of Human Nutrition, Department of International Health, Johns Hopkins U. School of Hygiene and Public Health, Baltimore, MD.

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Diarrheal diseases are among the most important causes of death of children in developing countries (1-3). Furthermore, these highly prevalent illnesses have been found to be major determinants of growth retardation and malnutrition (4-6).

Previous studies (7-9) of the epidemiology of diarrheal diseases in children in developing countries have been able to detect an enteropathogen in less than 20 per cent of stool specimens from children with diarrhea. This lack of information about the causes of diarrhea had precluded the complete exposition of the epidemiology of the diarrheal syndromes. Furthermore, it led some to conclude inaccurately that a sizeable proportion of the diarrhea in children in developing countries was the result of malnutrition, rather than a series of infectious illnesses.

Using new techniques to detect bacterial and viral agents, it is now possible to identify an enteropathogen for over half of the diarrheal episodes in young children in developing countries (10, 11). The application of these techniques in a longitudinal study permits a more complete description of the epidemiology of the infections associated with diarrhea. Furthermore, the availability of these laboratory data in a study of the effects of diarrhea on growth also for the first time permits an assessment of the nutritional consequences of diarrhea associated with specific enteropathogens.

Longitudinal studies were undertaken in two villages in rural Bangladesh to learn more about the interactions between infectious illnesses and the nutritional status of children (12). Illnesses were detected by a village-based system of surveillance, and specimens from the diarrheal episodes identified were examined for bacterial, viral and parasitic enteropathogens. This report concerns the relation of these pathogens to disease and the epidemiology of diarrhea associated with specific agents.

#### MATERIALS AND METHODS

Two villages, Enayet Nagar and Sepai Kandi, in the Matlab field research area of the International Centre for Diarrhoeal Diseases Research, Bangladesh (formerly the Cholera Research Laboratory), were chosen for study. The villages, study population and surveillance methodology are described in an accompanying paper (12). From these villages, 197 children aged 2-48 months were included during the year of study (March 30, 1978 to March 28, 1979). These children were observed for 61,536 days.

Surveillance workers visited and examined each child every other day. At each visit, the worker asked the mother about the presence of symptoms on that or the preceding day and recorded information on the number and consistency of the child's stools, presence of blood or mucus in the stool, vomiting, dehydration, fever and anorexia, as well as the presence of other symptoms and signs of illness. A Bangladeshi physician visited each child every week, reviewed the information recorded by the field worker, examined the child, and, based on predetermined definitions of illness, coded the occurrence of specific illnesses on each day of the preceding week. An episode of diarrhea was defined as at least one day with four or more liquid stools. That episode was considered resolved on any day with fewer than three stools; however, if a child had three or more liquid stools on any day within three days after an episode of diarrhea, that day was included in the same episode. An episode of diarrhea was considered dysentery if blood or mucus was noted in the child's stools.

On the first day that a child was noted to have diarrhea, two rectal swabs were obtained by the worker. Two swabs were also obtained from each child in the study on a fixed day of each month, whether or not diarrhea was present. One swab was placed in Cary-Blair transport media and the other in a vial containing phosphate

buffered saline (pH 7.4); these swabs were taken to the Matlab field laboratory.

**Laboratory methods.** Swabs from the transport medium were plated within several hours of collection on *Salmonella-Shigella*, trypticase-tellurite-gelatin, and MacConkey's agars. This swab was also enriched overnight in bile peptone and then plated again on trypticase-tellurite-gelatin agar. After 18 hours of incubation, the plates were examined for salmonellae, shigellae and vibrios by standard methods (13). Five lactose-positive colonies with the typical morphology of *Escherichia coli* and a pool of 10 other lactose-positive colonies were removed from the MacConkey's agar and stored on nutrient agar slants (14). These five individual colonies and pool of 10 colonies were tested for heat-labile toxin (LT) by the Chinese hamster ovary cell assay and for heat-stable toxin (ST) by the infant mouse assay (14).

Fecal material from the other swab, which had been diluted in phosphate buffered saline, was tested for rotavirus by an enzyme-linked immunosorbent assay (15, 16). Samples were confirmed to be positive if the reaction was blocked by preincubation of the specimen with specific anti-rotavirus antibody. Positive specimens were further confirmed with an enzyme-linked immunosorbent assay, which tested specimens in parallel wells coated with immune and nonimmune serum (17).

For diarrheal episodes in which no enteropathogen could be detected using these methods, a fresh stool specimen was collected from the child and examined for intestinal parasites by saline and iodine preparations; 109 (24 per cent) of such episodes were studied. In this study only stools containing vegetative stage *Giardia lamblia* or *Entamoeba histolytica* were considered positive. Additionally, for diarrheal episodes without a demonstrated enteropathogen, *E. coli* from rectal swabs collected during these episodes were tested for invasiveness by the

Sereny assay (18). Five colonies of *E. coli* were pooled, and 0.05 ml of a suspension containing approximately  $10^9$  cells per ml was inoculated into the conjunctival sacs of guinea pigs with the uninoculated eye serving as a control. These guinea pigs were observed for seven days for evidence of keratoconjunctivitis. Sereny testing was done for 286 (63 per cent) of the episodes from which no pathogen was identified in the initial testing.

**Statistical methods.** Data from laboratory examination of rectal swabs and stools were linked by computer with diarrheal morbidity information from the alternate-day illness surveillance. Enteropathogens were considered to be associated with a diarrheal episode if they were identified from a specimen obtained during or one day before or after the episode. Enteropathogens identified from other specimens were not considered to be associated with an episode, although some positive specimens probably represented carriage of the organism during early convalescence. The age-, sex-, and pathogen-specific incidences of diarrhea were calculated, using the number of days at risk as determined by village surveillance of study children (12). Statistical comparisons were done with Student's *t*, chi-square and Fisher's exact tests, as appropriate.

## RESULTS

During the year of study, 941 episodes of diarrhea were identified; 920 were studied for enteric pathogens. A bacterial, viral or parasitic agent was identified in 465 (51 per cent) of the 920 diarrheal episodes (table 1). Enterotoxigenic *E. coli* (ETEC), the most frequently noted agents, were found in 27 per cent of episodes as a single infection and in 4 per cent of episodes in combination with another pathogen. Nearly half of the ETEC produced only ST, while a quarter each produced only LT or both ST and LT. *Shigellae* were the second most common

TABLE 1

*Enteropathogens identified by routine monthly specimens and by diarrheal specimens in a cohort of 197 rural Bangladeshi children aged 2-60 months studied in 1978-1979*

Enteropathogen	Routine		Diarrhea	
	No.	%	No.	%
<i>Escherichia coli</i> ST	22	1.1	120	13.0
<i>Shigella</i>	20	1.0	118	12.8
<i>Escherichia coli</i> ST/LT	28	1.4	65	7.1
<i>Escherichia coli</i> LT	38	1.8	63	6.8
Rotavirus	12	0.6	35	3.8
<i>Escherichia coli</i> and <i>Shigella</i>	0	0	26	2.8
Non-O group 1 <i>Vibrio</i>	4	0.2	10	1.1
<i>Escherichia coli</i> and rotavirus	1	0.05	6	0.7
<i>Giardia lamblia</i>	0	0	5	0.5
<i>Vibrio cholerae</i> O group 1	1	0.05	3	0.3
<i>Shigella</i> and rotavirus	0	0	2	0.2
<i>Entamoeba histolytica</i>	0	0	2	0.2
Other	0	0	10	1.1
None	1934	94.0	455	49.5
Total	2060	100.0	920*	100.0

\* 21 additional episodes were not tested for enteropathogens.

LT = heat-labile toxin; ST = heat-stable toxin.

pathogens identified: *S. flexneri* accounted for 67 per cent of these infections and the other three serogroups of *Shigella* were each responsible for nine to 12 per cent. Rotaviruses were the third most frequently identified pathogens. *Vibrio cholerae*, both O group 1 and non-O group 1, were found infrequently. Most mixed infections included two of the three most frequent pathogens, ETEC, *Shigella* and rotavirus. Other infections included one episode associated with *Salmonella* and nine episodes with combinations of pathogens, such as *V. cholerae* O group 1 with ETEC (three), or *Shigella*, ETEC and *G. lamblia* (one), and *V. cholerae* non-O group 1 with ETEC (one), rotavirus (one), *Shigella* (one), or *Salmonella* (one) and *Shigella* with *G. lamblia* (one).

Of 188 episodes considered dysentery, shigellae were isolated in 117 cases (62 per cent). *E. histolytica* were associated with two episodes of dysentery and no pathogen could be found for most of the rest. No invasive *E. coli* were found in specimens from 48 episodes of *Shigella*-negative dysentery.

Six per cent of cultures not associated with diarrhea yielded an enteropathogen; nearly three-quarters of these were ETEC. The rest were predominantly shigellae and rotaviruses. Single infections with ETEC, *Shigella* and rotavirus were significantly ( $p < 0.001$ ) associated with diarrheal illnesses when compared with the identification of these agents from regular cultures from the same children, taken when diarrhea was not present. Non-O group 1 vibrios were also significantly ( $p < 0.01$ ) associated with diarrheal episodes.

Eight-five per cent of ST ETEC-positive cultures were associated with diarrhea compared with only 62 per cent of LT ETEC-positive cultures ( $p < 0.001$ ) or 70 per cent of ST/LT ETEC cultures ( $p < 0.01$ ). Eight-six per cent of *Shigella*-positive cultures were associated with diarrheal illnesses, as were 74 per cent of rotavirus-positive specimens.

The incidence of all diarrheal episodes was highest in children aged 2-11 months and declined progressively with age from seven to four episodes per child

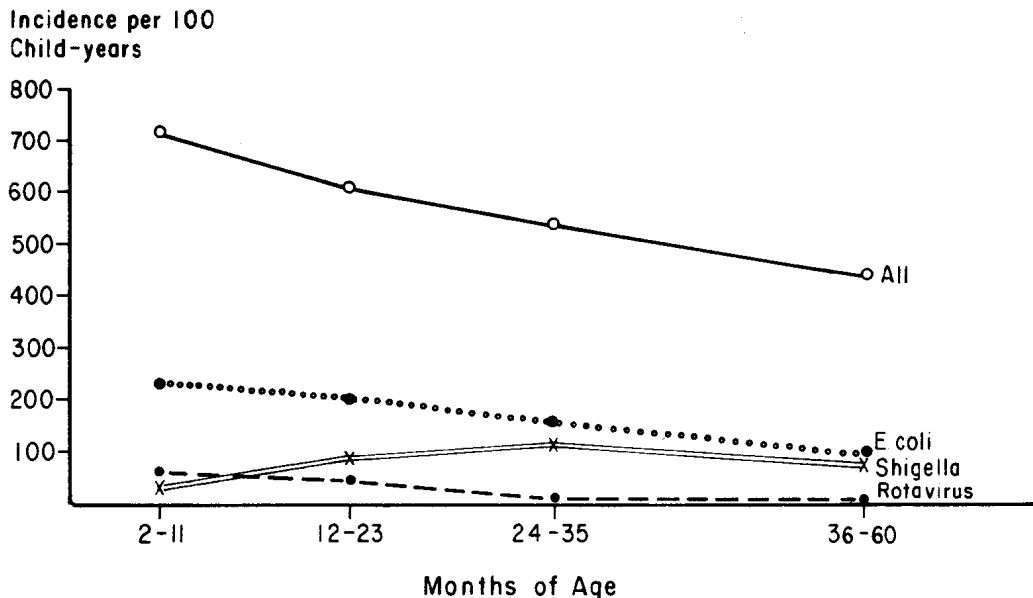


FIGURE 1. Annual incidence of diarrhea by enteropathogen and age in a cohort of 197 rural Bangladeshi children aged 2-60 months studied in 1978-1979.

per year (figure 1). The annual incidence of ETEC diarrhea decreased from more than two episodes per child to one episode in the oldest age group. The incidence of rotavirus diarrhea was approximately one-half an episode per year per child during the first two years of life and decreased to a negligible level thereafter. In contrast, *Shigella*-associated diarrhea had a low incidence in the youngest age group and a peak incidence in children 24-35 months old. There were no significant differences in the incidences of diarrhea according to the sex of the child.

The incidence of all diarrheal episodes showed little seasonal variation; however, the incidence of diarrhea associated with some specific enteropathogens did show seasonal changes (figure 2). ETEC diarrhea occurred most frequently in the hot months from April to September, while shigellosis had a higher incidence in the cool, dry months. The incidence of rotavirus diarrhea was relatively constant, except for a single peak in December.

Dehydration, noted at home or at the

time the child visited the treatment facility, was more common with rotavirus diarrhea than with diarrhea associated with each of the three types of ETEC (all  $p < 0.001$ ), shigellae ( $p < 0.001$ ) or diarrhea without a demonstrated enteropathogen ( $p < 0.001$ ) (table 2). This significant association was also found when only children with rotavirus diarrhea who were less than 24 months old (the age group of nearly all the children with rotavirus diarrhea) were compared. Severe dehydration occurred during three episodes: one associated with *V. cholerae* O group 1, one with ST ETEC, and one with both *V. cholerae* and ETEC.

Fever was reported on 12 per cent of all days of diarrhea (considering only days on which diarrhea was the only illness noted) and was significantly more frequent ( $p < 0.01$ ) on days with ETEC, *Shigella* or rotavirus diarrheas than on days without recorded illness (four per cent). Fever was reported on 33 per cent of days with rotavirus diarrhea, compared with seven per cent with ETEC diarrhea ( $p < 0.001$ ), 11 per cent with diarrhea

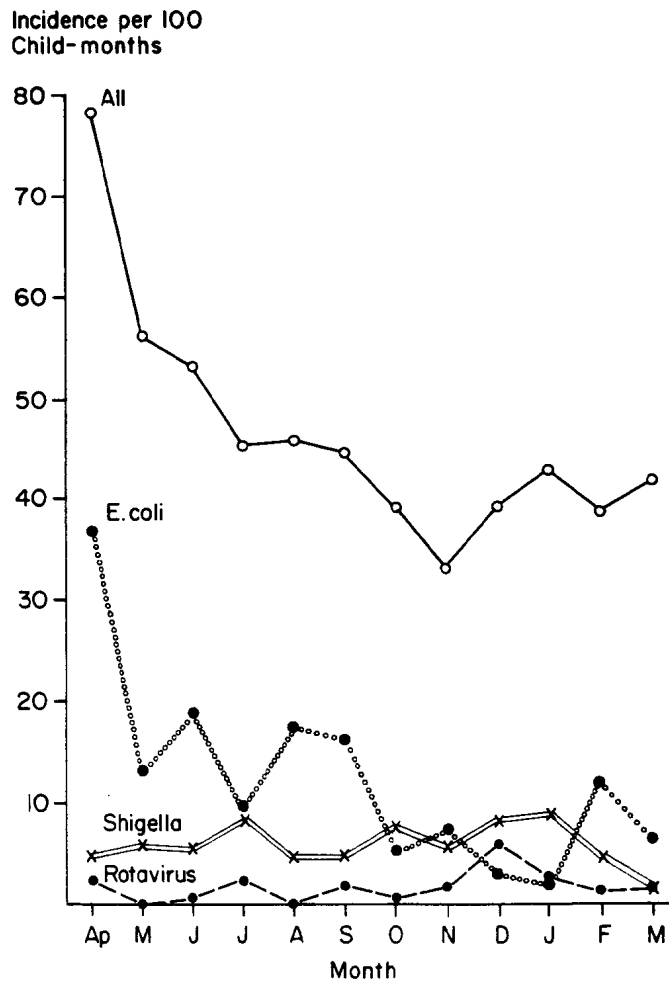


FIGURE 2. Monthly incidence of diarrhea by enteropathogen and calendar month in a cohort of 197 rural Bangladeshi children aged 2–60 months studied in March, 1978–March, 1979.

without a recognized pathogen ( $p < 0.001$ ), and 18 per cent with *Shigella* diarrhea ( $p < 0.01$ ). Fever was significantly more frequent with *Shigella* diarrhea than with diarrhea associated with ETEC or diarrhea with no identified pathogen (both  $p < 0.001$ ).

The median duration of the various types of diarrhea was 4–5 days, with the exception of shigellosis, which had a median duration of seven days (table 3). Likewise, the mean duration of *Shigella* diarrhea was significantly longer (all  $p < 0.02$ ) than the duration of any of the other types of diarrhea. The median duration of

rotavirus diarrhea was 9.5 days ( $n = 10$ ) in children less than 12 months old, and four days ( $n = 21$ ) in children 12–33 months old. The duration of other types of diarrhea did not vary with the age of the child. With all types of diarrhea, a small proportion of illnesses persisted longer than 20 days; occasionally, nondehydrating diarrhea was continuous for several months.

Overall, children had diarrhea on 12.8 per cent of all days of observation (table 4). The prevalence of diarrhea was highest during the second six months of life and then decreased progressively. ETEC

TABLE 2  
Percentage of children experiencing dehydration during diarrheal episodes\* in a cohort of 197 rural Bangladeshi children aged 2-60 months studied in 1978-1979

Pathogen identified	No. of episodes	No. with dehydration	% with dehydration
<i>Vibrio cholerae</i> O group 1	3	1	33
Rotavirus	35	9	26
Non-O group 1 <i>Vibrio</i>	10	1	10
<i>Escherichia coli</i>			
ST	120	6	5
LT	63	1	2
ST/LT	65	0	0
None	455	4	1
<i>Shigella</i>	118	0	0

\* Excludes episodes with isolation of multiple pathogens.

TABLE 3  
Duration of diarrheal episodes\* associated with enterotoxigenic *Escherichia coli*, *Shigella* or rotavirus and of episodes without an identified enteropathogen in a cohort of 197 rural Bangladeshi children aged 2-60 months studied in 1978-1979

Pathogen identified	Total episodes	Duration (days)				Median	Mean $\pm$ SEM
		% distribution					
		1-3	4-9	10-19	$\geq 20$		
<i>Escherichia coli</i>							
ST	120	40	35	18	7	5	7.4 $\pm$ 0.8
LT	63	44	26	23	8	5	8.5 $\pm$ 1.4
ST/LT	65	49	33	9	8	4	6.2 $\pm$ 0.9
<i>Shigella</i>	118	23	39	21	16	7	10.7 $\pm$ 1.0
Rotavirus	35	43	37	17	3	4	6.2 $\pm$ 1.1
None	455	31	44	17	8	5	7.9 $\pm$ 0.4

\* Excludes episodes with isolation of multiple pathogens.

diarrhea had the highest prevalence in all but the oldest age group; in that group, *Shigella* diarrhea had a slightly higher prevalence. Rotavirus diarrhea had the second highest prevalence in study children less than a year old.

#### DISCUSSION

In this study in rural Bangladesh we were able to identify an enteropathogen associated with over half of the diarrheal episodes of a cohort of children less than 5 years old. As in previous studies of children in developing countries, less than 20 per cent of episodes were associated with the long-accepted bacterial pathogens: *Shigella*, *Vibrio cholerae* and *Salmonella* (7-9). By contrast, 35 per cent of episodes

were associated with two recently recognized pathogens: enterotoxin-producing *E. coli* and rotavirus.

Enterotoxigenic *E. coli* (ETEC) have been found in the stools of a substantial proportion of children who have been treated for diarrhea at medical facilities in several developing countries (11, 19, 20). Furthermore, in the few studies that also sought ETEC from appropriately selected control children who did not have diarrhea, ETEC were more often isolated from ill children than from controls (19, 20). In this study, ETEC were the most frequent pathogens identified and were strongly associated with diarrheal illnesses. ETEC producing only ST were the predominant strains, although strains pro-

TABLE 4

Prevalence (per 1000 days of observation) of diarrhea by enteropathogen identified and age in a cohort of 197 rural Bangladeshi children aged 2-60 months studied in 1978-1979

Enteropathogen identified	Age group (months)					All (61536)
	2-5 (1652)*	6-11 (6644)	12-23 (19875)	24-35 (15515)	36-60 (17850)	
Enterotoxigenic <i>Escherichia coli</i>	30	44	34	36	15	30
<i>Shigella</i>	0	8	26	24	18	20
<i>Shigella/E. coli</i> rotavirus†	24	17	9	7	3	8
Other	16	7	7	8	6	7
Rotavirus	13	12	5	<1	<1	3
None	84	84	52	53	60	59
Total	167	173	133	128	103	128

\* Days of observation.

† Includes mixed infections with two of these three pathogens.

ducing only LT or both ST and LT each accounted for about one-quarter of all ETEC. In a previous community-based study of diarrheal incidence and etiology in another village in the same research area in Bangladesh, we found that ETEC were the enteropathogens found most frequently and that the peak incidence of ETEC-associated diarrhea was in children 9-11 months of age (10). Likewise, in this study we noted that the highest incidence of ETEC diarrhea was in the first year of life and that it decreased progressively with age. Considering our findings that the incidence of ETEC diarrhea ranges from 1-2 episodes per child per year in the first five years of life and that additional asymptomatic infections exist, it is obvious that repeated infections with ETEC occur throughout childhood. Additional studies in these villages indicate that these frequent infections of children may result largely from eating contaminated foods during weaning (29). Field studies in this ETEC-endemic area suggest that immunity does develop against ETEC (21), and studies in volunteers demonstrate that serotype homologous, but not heterologous, immunity occurs after episodes of ETEC diarrhea (22).

Our recovery of shigellae from 16 per cent of specimens from diarrheal episodes

and the peak incidence of *Shigella*-associated diarrhea in the third year of life are similar to findings of previous studies of children in communities with poor sanitation (7-10, 23). As in these other communities, *S. flexneri* was the predominant serotype. Diarrhea associated with *Shigella* had a longer duration than other types of diarrhea; 16 per cent of episodes lasted three weeks or longer. This chronicity has been noted previously (9); however, it is still unclear if this is due to continuous *Shigella* infection or to persistent intestinal mucosal abnormalities after the acute infection. The role of ampicillin treatment in shortening or perhaps prolonging the diarrhea cannot be assessed.

No pathogens could be found during 69 episodes of dysentery. Some of these may have also been due to *Shigella*. Although several rectal cultures were often done during diarrheal episodes, only a single culture was available for some episodes. Since a single culture, even with direct plating, will identify shigellae in only 60 to 70 per cent of ill persons known to be infected (24), some cases of *Shigella* infection were undoubtedly missed. We did not examine stool cultures for *Campylobacter jejuni*, a cause of diarrhea and dysentery that has subsequently been isolated from

five per cent of children with dysentery and 12 per cent of children with diarrhea, who were studied during the second year of this study (25). Other known causes of dysenteric illnesses, such as *E. histolytica* or invasive *E. coli*, were sought in this study and were not found frequently.

In studies of diarrhea in children less than two years old in treatment facilities in many developing and developed countries, rotaviruses have been the enteropathogens found most frequently (11, 26, 27). This preeminence of rotavirus as the cause of diarrhea among hospitalized children is undoubtedly related to the greater degree of dehydration with rotavirus diarrhea than with most other types of diarrhea, a characteristic that has been noted in comparisons of hospitalized children (26, 27) and in community-based prospective studies (10, 28). The greater frequency of dehydration with rotavirus diarrhea is evident in this study, where it accounted for less than four per cent of all diarrheal episodes, but 39 per cent of episodes associated with dehydration. In this study, as in our previous community-based study in the same area, the incidence of rotavirus diarrhea was high only in the first two years of life (10). These studies suggest that symptomatic infection with rotavirus occurs one to two times in Bangladeshi children in the first two years of life. Additional studies of antirotavirus antibody titers of the children enrolled in this study further suggest that numerous asymptomatic infections may occur during the first five years of life (30). Although rotavirus infections had a slightly higher incidence in the cool months, transmission was noted the year round.

The demonstration of an enteropathogen associated with the majority of diarrheal episodes permits some observations on the relative importance of illnesses associated with specific bacterial and viral agents. Rotavirus-associated diarrhea may be more likely to result in death

due to dehydration, if treatment is not available. On the other hand, ETEC- and *Shigella*-associated diarrhea result in more morbidity, because of their high incidence and prevalence throughout childhood. Furthermore, ETEC-diarrhea may have the greatest adverse effect on growth due to its high incidence during the first two years of life.

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