

A Two-Year Study of Bacterial, Viral, and Parasitic Agents Associated with Diarrhea in Rural Bangladesh

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Enteric pathogens associated with diarrhea were studied for two years at a diarrhea treatment center in rural Bangladesh. Enterotoxigenic *Escherichia coli* (ETEC) was the most frequently identified pathogen for patients of all ages. Rotavirus and ETEC were isolated from ~50% and ~25%, respectively, of patients less than two years of age. A bacterial or viral pathogen was identified for 70% of these young children and for 56% of all patients with diarrhea. Most ETEC isolates were obtained in the hot dry months of March and April and the hot wet months of August and September. Rotavirus identification peaked in the cool dry months of December and January, but infected patients were found year-round. The low case-fatality rates for patients with watery diarrhea and substantial dehydration further document the usefulness of treating patients with diarrhea with either a glucose- or sucrose-base electrolyte solution such as those used in this treatment center.

Diarrhea, especially in children, is a major cause of morbidity and mortality in developing countries [1]. Although classic enteric bacterial pathogens are not isolated from most patients with diarrhea, recent studies indicate that enterotoxigenic *Escherichia coli* (ETEC) and rotaviruses may frequently cause diarrhea [2-4]. Although these agents have been isolated from patients in developing countries, the studies have been brief, and the results do not permit an accurate assessment of the relative importance of the various causative agents. We therefore studied patients during a two-year period at a treatment center in rural Bangladesh to determine the frequency, severity, and seasonality of diarrhea associated with ETEC, rotavirus, and other enteric agents.

Materials and Methods

Population studied. The Matlab field research area of the International Centre for Diarrhoeal

Diseases Research, Bangladesh (formerly the Cholera Research Laboratory) is located in a riverine, rural area. A central treatment facility, staffed by physicians and paraprofessionals, provides free therapy for patients with diarrhea who come directly or are brought by speedboat or jeep ambulances stationed in the field area. For a two-year period data for all patients who lived in the research area and were treated for diarrhea were gathered for a study of enteric pathogens.

Between February 1977 and January 1978 (year 1), 8,139 patients were included in the study, and from February 1978 to January 1979 (year 2), 6,352 patients were enrolled. In years 1 and 2, respectively, 43% and 41% of all patients were children less than two years of age; 22% each year were children two through nine years of age, and 35% and 37% were ≥ 10 years of age.

When the patient visited the treatment center, a physician or nurse did a standard clinical evaluation of the degree of dehydration on the basis of signs such as skin elasticity, sunken eyes or fontanelle, pulse, respirations, volume of urine, and level of consciousness. Dehydration was classified as none, mild (corresponding to $< 5\%$ loss of body weight), moderate (5%–10%), or severe ($> 10\%$).

Patients judged to have no or mild dehydration were treated with oral electrolyte solution (composition in milliequivalents/liter: Na^+ , 90; K^+ , 20; Cl^- , 80; HCO_3^- , 30) containing 20 g of glucose/liter

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(year 1) or 40 g of sucrose/liter (year 2) [5]. Patients with moderate or severe dehydration were given a solution (composition in milliequivalents/liter: Na⁺, 134; K⁺, 13; Cl⁻, 86; HCO₃⁻, 48) iv to replace their estimated fluid deficit and were also given the oral solution for maintenance therapy. Because laboratory findings were not available at the time of admission, the assessment of dehydration and the choice of treatment were not influenced by the identification of an enteropathogen. Only persons whose histories indicated a dysenteric illness were given ampicillin or trimethoprim-sulfamethoxazole on admission.

Laboratory studies. On admission rectal swabs were taken from all patients and plated directly on salmonella-shigella, trypticase-tellurite-gelatin, and MacConkey's agars. Part of each specimen was enriched overnight in bile peptone and then plated on trypticase-tellurite-gelatin agar. The plates were examined for salmonellae, shigellae, and vibrios [6] by standard methods. Vibriolike colonies identified on trypticase-tellurite-gelatin plates were further characterized in terms of biochemical, serotypic, and salt tolerance properties and classified as *Vibrio cholerae* group O:1, non-group O:1 vibrios, *Vibrio parahaemolyticus*, or group F vibriolike organisms [6, 7].

A sample of patients, stratified by age group, was further studied for ETEC infection. During year 1, studies were performed with 4,498 patients (52% of the patients less than two years of age, 67% of the patients two through 19 years of age, and 79% of the patients ≥20 years of age). During year 2 the sample was modified because of the results from year 1, and 2,042 patients (19% of the patients less than two years of age, 66% of the patients two through 19 years of age, and 40% of the patients ≥20 years of age) were studied. From each culture selected for toxin studies, 10 lactose-positive colonies with typical *E. coli* morphology were removed from MacConkey's agar plates and pooled on nutrient agar slants. These pools were tested with the Chinese hamster ovary cell assay for heat-labile toxin (LT) and with the infant mouse assay for heat-stable toxin (ST) [8].

During year 1, fresh stool specimens from 40% of the patients were treated with saline and iodine preparations and examined for intestinal parasites. In this study only stools containing vegetative-stage *Giardia lamblia* or *Entamoeba histolytica* were considered positive.

Beginning in December 1977, a second rectal

swab was taken from each patient and refrigerated in phosphate-buffered saline for less than one month (and generally less than one week) before being tested by enzyme-linked immunosorbent assay for rotavirus antigen [9, 10]. Positive results were confirmed by testing about 30 positive specimens per month with an enzyme-linked immunosorbent assay involving wells coated with immune and nonimmune sera [11]. Of 404 specimens retested, 380 (94%) were confirmed as positive.

Analysis. The frequency with which patients were infected with the pathogens was calculated directly; however, since only subgroups of patients were tested for ETEC and for parasites, those results had to be extrapolated for the entire group of patients. Because rotavirus was tested for only the last two months of year 1, we included these data in the analysis of seasonality, degree of dehydration, and hospital death rates but did not try to determine the overall frequency of infection with rotavirus in year 1.

Results

For both year 1 and year 2, ETEC was the pathogen most frequently isolated from all patients and adults; however, it was the second most often isolated (after rotavirus) from children less than two years of age and the second most often isolated (after *V. cholerae*) from children two through nine years of age. Most of the ETEC produced only ST, fewer produced both ST and LT, and still fewer produced only LT (table 1).

Rotavirus was found in the stools of 46% of the children less than two years of age and in the stools of 12% and 9%, respectively, of older children and adults. *V. cholerae* group O:1 was rarely identified for children less than two years of age but was an important pathogen in older children and adults, while non-group O:1 vibrios were found in the stools of 4%–11% of the patients of each age group for both years of the study. Group F vibriolike organisms were associated with diarrhea for 3% of all patients in year 1 but rarely in year 2. *V. parahaemolyticus* and *Salmonella* were rarely isolated during the study, but *Shigella* was isolated from 5%–6% of all of the patients treated at the center for diarrhea. Vegetative *G. lamblia* was identified from 4% of the older children and adults, and vegetative *E. histolytica* was identified from 13% of the older children and 8% of the adults. For 2,039 patients tested for

Table 1. Percentage of patients with diarrhea associated with enteric pathogens by age in the periods February 1977–January 1978 (year 1) and February 1978–January 1979 (year 2).

Pathogen identified	Age group, study year (no. of patients)							
	<2 years		2–9 years		≥10 years		All ages	
	1 (3,500)	2 (2,614)	1 (1,815)	2 (1,391)	1 (2,824)	2 (2,347)	1 (8,139)	2 (6,352)
Enterotoxigenic <i>Escherichia coli</i> *								
ST	12	17	12	15	16	22	14	18
ST/LT	6	7	6	7	14	19	9	11
LT	5	4	3	1	3	2	4	2
Total	23	28	22	24	33	42	26	32
Rotavirus	...	46	...	12	...	9	...	24
<i>Vibrio cholerae</i>	2	2	29	34	14	14	12	14
Non-group O:1 vibrios	8	5	6	4	8	11	7	7
<i>Shigella</i>	4	5	8	10	4	5	5	6
<i>Entamoeba histolytica</i>	1	...	13	...	8	...	4	...
<i>Giardia lamblia</i>	<1	...	4	...	4	...	2	...
Group F vibriolike organisms	4	<1	2	<1	2	<1	3	<1
<i>Salmonella</i>	<1	<1	<1	<1	<1	<1	<1	<1
<i>Vibrio parahaemolyticus</i>	0	<1	0	0	<1	<1	<1	<1

NOTE. This study was conducted at a diarrhea treatment center (Matlab) in rural Bangladesh.

* ST = strain producing heat-stable enterotoxin; LT = strain producing heat-labile enterotoxin.

bacterial pathogens, including ETEC, and for rotavirus (but not for parasites) in year 2, an enteropathogen was identified for 70% of the children less than two years of age, 47% of the children two through nine years of age, and 56% of the adults. Infection with more than one pathogen was found for about 20% of all patients.

The seasonal patterns of occurrence of the three most common enteric pathogens are illustrated in figure 1. Infections with *V. cholerae* were decidedly seasonal, with a peak occurrence during the hot monsoon period. In contrast, infections with ETEC had two seasonal peaks, one in the hot dry months of March and April and the other in August through September. Although the highest numbers of patients with diarrhea associated with rotavirus were seen in the cool dry months of December 1977 and January 1978, there was no comparable peak in year 2 of the study. The number of infections with non-group O:1 vibrios and group F vibriolike organisms peaked in April and May, whereas the incidence of shigella infections peaked between June and August. No seasonal pattern could be determined for infections with *G. lamblia* or *E. histolytica*.

The degree of dehydration at the time that patients visited the treatment center during year 1 was tabulated for those infected with *V. cholerae*, ETEC, or rotavirus after data for patients with known mixed infections were excluded. Among

children less than two years of age, moderate or severe dehydration requiring inpatient therapy occurred in 24 (40%) of 60 patients with cholera; this proportion was significantly higher than the 68 (20%) of 340 and 76 (16%) of 473 patients with ETEC and rotavirus diarrhea, respectively (both $P < 0.001$, χ^2). Among adults moderate or severe dehydration was found in 307 (77%) of 398 patients with cholera and 269 (43%) of 624 with ETEC diarrhea ($P < 0.001$). There were no significant differences in the levels of dehydration accompanying diarrhea associated with ETEC of different toxin types in children or adults.

In spite of substantial dehydration in patients of

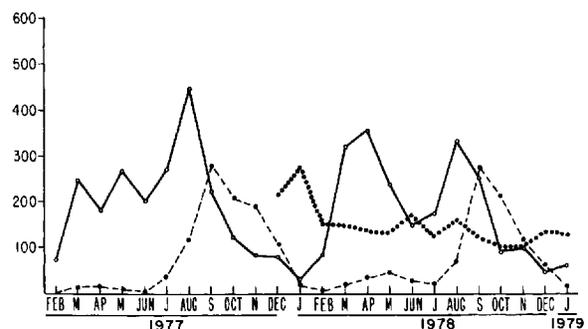


Figure 1. Visits to a diarrhea treatment center (Matlab) in rural Bangladesh for diarrhea associated with enterotoxigenic *Escherichia coli* (○—○), *Vibrio cholerae* (●---●), or rotavirus (●●●●) for the period February 1977–January 1979.

Table 2. Case-fatality rate, by pathogen associated with diarrhea, February 1977–January 1979.

Pathogen identified	No. dead/no. of cases (% case-fatality rate)
<i>Salmonella</i>	1/38 (2.6)
<i>Shigella</i>	9/782 (1.2)
Group F vibriolike organisms	2/245 (0.8)
Non-group O:1 vibrios	2/1,032 (0.3)
<i>Vibrio cholerae</i>	4/1,864 (0.2)
Rotavirus	5/2,112 (0.2)
Enterotoxigenic <i>Escherichia coli</i>	0/2,279
All diarrhea	37/14,499 (0.3)

NOTE. This study was conducted at a diarrhea treatment center (Matlab) in rural Bangladesh.

all ages, the hospital case-fatality rate for this two-year period was very low (table 2). Furthermore, there were no significant differences in fatality rate between year 1, when glucose-electrolyte oral therapy solution was used, and year 2, when a sucrose-base solution was used. The fatality rate for patients with diarrhea associated with *Shigella* was higher than those for patients infected with *V. cholerae*, both group O:1 (Fisher's exact test, $P < 0.01$) and non-group O:1 ($P < 0.02$), rotavirus ($P < 0.01$), or ETEC ($P < 0.001$).

Discussion

ETEC organisms were the pathogens most frequently isolated from patients of all ages and were the second most frequently isolated (after rotavirus) from young children coming to the treatment center in rural Bangladesh. Our study indicates that rotavirus is the most common pathogen for children less than two years of age visiting a treatment center for diarrhea; this finding is in agreement with other studies in both developed and developing countries [3, 4, 12, 13]. Older children and adults may also have had symptomatic infections caused by rotavirus, but because of frequent concomitant infections with other pathogens it is difficult to determine whether rotavirus caused diarrhea for the infected adults in this study.

In this highly endemic area cholera remains an important cause of life-threatening diarrhea. Although few children less than two years of age had cholera, *V. cholerae* was the most frequently isolated pathogen from children two through nine years of age and was the second most frequently isolated (after ETEC) in adults. The observation

that relatively few young children had cholera whereas many had diarrhea caused by other pathogens suggests that *V. cholerae* may have a pattern of transmission different from that of other agents or, less likely, that infants have substantial immunity to *V. cholerae* but not to other organisms.

A bacterial or viral pathogen could be identified for 70% of the children less than two years of age and for a majority of all patients. These proportions are substantially higher than those observed before the recognition of ETEC and rotavirus [14, 15]. Furthermore, *G. lamblia* and *E. histolytica*, which were identified from 6% of the patients in year 1, probably caused some of the episodes in year 2. The failure to find a pathogen for a minority of patients may be due in part to the relative insensitivity of some of the techniques used, such as testing a pool of 10 colonies of *E. coli* for toxin production rather than testing multiple individual isolates [8]. Also, the assays we used could not detect some agents of diarrhea, including bacterial pathogens such as invasive or enteropathogenic *E. coli*, *Campylobacter fetus*, and *Yersinia enterocolitica* and viruses such as parvovirus-like agents and other recently reported particles (adenoviruses, caliciviruses, coronavirus-like agents) that may be associated with diarrhea [16–19].

Patients with cholera were generally more severely dehydrated than patients with diarrhea associated with other pathogens, including LT- and ST/LT-producing *E. coli*, which produce an enterotoxin similar to that of *V. cholerae*. This finding suggests that these organisms differ in the amount of toxin released or in other properties of virulence such as the ability to adhere to the mucosal surface and to colonize the small bowel.

The low case-fatality rates for patients who had watery diarrhea and substantial dehydration at the time that they visited the treatment center further document the effectiveness of oral rehydration therapy in conjunction with sufficient iv fluid to correct shock. Recent comparative studies have demonstrated that a sucrose-base electrolyte formula is nearly as good as a glucose-base formula for oral fluid replacement [5, 20]. Our experience with the two solutions, each used for one year of this study in a center annually treating 6,000–8,000 patients with diarrhea, supports this conclusion in that the case-fatality rates for the two years of study were comparable.

References

1. Walsh, J. A., Warren, K. S. Selective primary health care. An interim strategy for disease control in developing countries. *N. Engl. J. Med.* 301:967-974, 1979.
2. Donta, S. T., Wallace, R. B., Whipp, S. C., Olarte, J. Enterotoxigenic *Escherichia coli* and diarrheal disease in Mexican children. *J. Infect. Dis.* 135:482-485, 1977.
3. Ryder, R. W., Sack, D. A., Kapikian, A. Z., McLaughlin, J. C., Chakraborty, J., Rahman, A. S. M. M., Merson, M. H., Wells, J. G. Enterotoxigenic *Escherichia coli* and reovirus-like agent in rural Bangladesh. *Lancet* 1:659-663, 1976.
4. Anonymous. Rotavirus gastroenteritis [editorial]. *Br. Med. J.* 2:784-785, 1977.
5. Black, R. E., Merson, M. H., Taylor, P. R., Yolken, R. H., Yunus, M., Alim, A. R. M. A., Sack, D. A. Comparison of glucose with sucrose in oral rehydration therapy of rotavirus diarrhea in infants and young children. *Pediatrics*, 1980 (in press).
6. Collwell, R. R. Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and related *Vibrio* species. *J. Bacteriol.* 104:410-433, 1970.
7. Furniss, A. L., Lee, J. V., Donovan, T. J. Group F, a new vibrio [letter]. *Lancet* 2:565-566, 1977.
8. Merson, M. H., Sack, R. B., Kibriya, A. K. M. G., Mahmood, A., Ahmed, Q. S., Huq, I. The use of colony pools for diagnosis of enterotoxigenic *Escherichia coli* diarrhea. *J. Clin. Microbiol.* 9:493-497, 1979.
9. Yolken, R. H., Kim, H. W., Clem, T., Wyatt, R. G., Kalica, A. R., Chanock, R. M., Kapikian, A. Z. Enzyme-linked immunoassay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. *Lancet* 2:263-266, 1977.
10. Yolken, R. H., Wyatt, R. G., Kapikian, A. Z. ELISA for rotavirus [letter]. *Lancet* 2:819, 1977.
11. Yolken, R. H., Stopa, P. J. Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotavirus. *J. Clin. Microbiol.* 10:703-707, 1979.
12. Kapikian, A. Z., Kim, H. W., Wyatt, R. G., Cline, W. L., Arrobio, J. O., Brandt, C. D., Rodriguez, W. J., Sack, D. A., Chanock, R. M., Parrott, R. H. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. *N. Engl. J. Med.* 294:966-972, 1976.
13. Hieber, J. P., Shelton, S., Nelson, J. D., Leon, J., Mohs, E. Comparison of human rotavirus disease in tropical and temperate settings. *Am. J. Dis. Child.* 132:853-858, 1978.
14. Feldman, R. A., Bhat, P., Kamath, K. R. Infection and disease in a group of South Indian families. IV. Bacteriologic methods and a report of the frequency of enteric bacterial infection in preschool children. *Am. J. Epidemiol.* 92:367-375, 1970.
15. Gordon, J. E., Behar, M., Scrimshaw, N. S. Acute diarrheal disease in less developed countries. I. An epidemiological basis for control. *Bull. W.H.O.* 31:1-7, 1964.
16. Black, R. E., Jackson, R. J., Tsai, T. F., Medvesky, M. G., Shayegani, M., Feeley, J. C., MacLeod, K. I. E., Wakeley, A. M. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *N. Engl. J. Med.* 298:76-79, 1978.
17. Butzler, J. P., Dekeyser, P., Detrain, M., Dehaen, F. Related *Vibrio* in stools. *J. Pediatr.* 82:493-495, 1973.
18. Schreiber, D. S., Trier, J. S., Blacklow, N. R. Recent advances in viral gastroenteritis. *Gastroenterology* 73:174-183, 1977.
19. Gary, G. W., Hierholzer, J. C., Black, R. E. Characteristics of noncultivable adenoviruses associated with diarrhea in infants: a new subgroup of human adenoviruses. *J. Clin. Microbiol.* 10:96-103, 1979.
20. Sack, D. A., Chowdhury, A. M. A. K., Eusof, A., Ali, M. A., Merson, M. H., Islam, S., Black, R. E., Brown, K. H. Oral rehydration in rotavirus diarrhoea: a double blind comparison of sucrose with glucose electrolyte solution. *Lancet* 2:280-283, 1978.