Enteric Adenovirus Infection among Infants with Diarrhea in Rural Bangladesh

KERSTIN JARECKI-KHAN, SAUL R. TZIPORI,† AND LEANNE E. UNICOMB*

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

A total of 4,409 stool specimens from infants less than 5 years of age seeking treatment for diarrhea in Matlab, Bangladesh, were tested for the presence of adenoviruses by using an enzyme immunoassay (EIA). EIA-positive stool samples were serotyped with monoclonal antibodies specific for adenovirus type 40 (Ad40) and Ad41 and group antigen, inoculated into Graham G293 cells, and retested by EIA. Of adenovirus-positive cultures, 125 (2.8%) specimens were confirmed as enteric adenoviruses (EAds), of which 51 (40.8%) were typed as Ad40 and 74 (59.2%) were typed as Ad41, and 12 of 4,409 (0.3%) were identified as nonenteric adenoviruses. A slight peak of incidence of EAd infection was observed in the cool, dry months, and an outbreak of Ad40 infections occurred in March 1988, when the detection rate of EAd reached 12.3%. Information on age, gender, and symptoms was available for 80 infants infected with adenovirus only. Age distribution was similar for types 40 and 41 and nonenteric adenovirus; the median ages were 11, 12, and 12 months, respectively. The ratio of males to females for the 80 infants varied according to serotype; Ad40 had the highest male/female ratio, 2.17. The symptoms experienced by the 80 children were similar for each adenovirus type. The most common clinical features of EAd infection were watery diarrhea (87.5%), more than eight loose bowel movements per day in the 24-h period prior to presentation (68.8%), with vomiting (80.0%), abdominal pain (76.3%), and low-grade fever (95.0%); these symptoms are significantly similar to symptoms of infants infected with group A rotavirus. EAd infection generally gave rise to mild to moderate dehydration, which is significantly similar to dehydration produced by infection with rotavirus.

Epidemiological studies in developing and developed countries have succeeded in establishing an etiological role of enteric adenovirus (EAd) in infantile gastroenteritis in some instances, but their importance remains uncertain. On the basis of enzyme immunoassay (EIA) techniques, EAds were found to be present in 0.9 to 33.3% of stool specimens collected from infants with diarrhea (1, 12, 14, 17, 21, 24, 26), with lower rates generally reported from developing countries (0.9 to 13.9%). In studies which were long enough to observe seasonal variation, a few clusters of EAd infection incidences were noted in summer in Sweden (21) and in a South African rural environment (20), but no seasonality was found in studies conducted in Thailand (12) and the United States (14).

Infections have been reported to occur most commonly in children less than 2 years of age (2, 12, 17, 21), and clinical features have been characterized as watery, nonbloody diarrhea lasting 2 to 22 days with up to eight loose bowel movements per day (4, 14, 21, 24, 26). Fever and vomiting have been described as mild (4, 7, 14, 21, 26), and gastroenteritis is sometimes accompanied by respiratory symptoms (14, 21, 26).

Our study aimed to determine the prevalence and epidemiological importance of EAd causing infantile gastroenteritis in Bangladesh with a description of clinical features that characterize an EAd infection in a rural population.

MATERIALS AND METHODS

Study population. Stool specimens were obtained from 4,409 patients less than 5 years of age who sought treatment

for acute diarrhea and produced a stool sample between June 1987 and May 1989 as well as from August 1989 to May 1990 at one of the three diarrhea treatment centers in the rural area of Matlab as part of the passive surveillance of a cholera vaccine trial which commenced in 1985 (5).

Stools were collected on visits to the Matlab hospital or to one of two neighboring diarrhea treatment centers, and the nature of diarrhea was categorized as previously described (5). Samples were submitted for bacterial culture, and aliquots were stored at −20°C for detection of enteric viruses.

Clinical information such as type of diarrhea, number of loose movements in the 24-h period prior to presentation, duration of diarrhea prior to presentation, vomiting, rectal temperature, status of radial pulse, skin turgor, dryness of mucous membranes, and whether eyes and/or fontanelles were sunken was collected from patients at the time of presentation between June 1987 and May 1989.

Dehydration was assessed on the basis of the latter five clinical signs. When any of these signs were normal a score of 0 was given. Dry mucous membranes were scored as 1, weak pulse was scored as 1, absent pulse was scored as 2, skin turgor displaying slow return was scored as 1, turgor displaying very slow return was scored as 2, sunken eyes was scored as 1, deeply sunken eyes was scored as 2, and sunken fontanelle was scored as 1. Only patients with fontanelles were included. Dehydration was considered severe if a patient's score was 4 or above, moderate if the score was 2 or 3, and mild if the score was 0 or 1.

Bacterial culture. Stools were cultured for vibrios, salmonellae, and shigellae by standard methods described in the World Health Organization manual (25).

Detection of group A rotavirus. Stool suspensions were prepared as 10% (wt/vol) phosphate-buffered saline (PBS) (pH 7.2) extracts and tested for group A rotavirus with a

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* Corresponding author.
† Present address: Department of Pathology, School of Veterinary Medicine, Tufts University, North Grafton, MA 01536.

commercial kit (Dakopatts, Glostrup, Denmark) incorporating polyclonal antibodies to rotavirus.

Determination of virus titer. The concentration of infectious virus in preparations of standard adenovirus was determined by a fluorescent focus assay. To serial dilutions of test virus (50 μl per well) in a 96-well sterile microtiter plate (Nunc, Roskilde, Denmark), 50 μl of cell suspension (Graham G293 cells or intestinal Henle H407 cells) at a concentration of 10^5/ml in Dulbecco's modified Eagle medium with 2.5% fetal bovine serum per well was added, and plates were incubated for 3 days. After aspiration of the growth medium, the infected monolayers were fixed with 70% (vol/vol) acetone at room temperature for 2 min and air dried.

To determine the number of infected cells, the fixed monolayers were stained with an antiadenovirus rabbit hyperimmune serum (RCH) which was provided by the Microbiology Department, Royal Children's Hospital, Melbourne, Australia, diluted 1/100 in PBS (50 μl per well) for 30 min at 37°C, washed three times with PBS, and then similarly reacted with 25 μl of anti-rabbit immunoglobulin G F(ab')$_2$-fluorescein isothiocyanate conjugate (Silenus, Melbourne, Australia) diluted 1/100 in PBS for 30 min at 37°C. The plates were washed as described above, and the number of fluorescing cells was counted at low magnification (×100). Titers were expressed as fluorescent-cell-forming units (FFU) per milliliter.

Virus propagation. Prototype strains of adenovirus type 40 (Ad40; VR-931), Ad41 (VR-930), and Ad5 (VR-5) were purchased from the American Type Culture Collection; Ad2 was provided by Roger Glass (Centers for Disease Control, Atlanta, Ga.). Virus stocks of the EAd strains Ad40 and Ad41 were cultivated in G293 cells (9) for the first four passages and then in the intestinal Henle H407 cell line (11); the nonenteric strains Ad2 and Ad5 were cultivated throughout in HeLa cells. Virus stocks with titers of 10^5 FFU/ml were then used to raise hyperimmune serum in laboratory animals and also employed as positive controls in the antigen detection EIA (see below).

Stool samples positive in adenovirus EIA and EAd EIA (see below) were also cultivated in G293 cells. Stool extracts (10%, wt/vol) were prepared in Dulbecco's modified Eagle medium with 100 U of penicillin, 100 μg of streptomycin, 50 μg of gentamicin, and 2 μg of amphotericin B (Fungizone) per ml, held at room temperature for 2 h, and then centrifuged at 12,000 × g for 30 min. For each stool sample, 0.1 ml of supernatant was added to a well of a 24-well sterile culture plate, 1 ml of G293 cell suspension in Dulbecco's modified Eagle medium with a 2.5% fetal bovine serum was added, and plates were incubated for up to 1 week at 37°C in 5% CO_2. After one freeze-thaw cycle, supernatants were retested in the EAd EIA (see below).

Preparation of hyperimmune antisera. Tissue culture supernatants (TC-SNTs) each containing 10^5 FFU of Ad40, Ad41, Ad2, and Ad5 per ml were clarified by centrifugation at 10,000 × g for 20 min and used to immunize animals. Guinea pigs were inoculated subcutaneously with 1 ml of a mixture of the serotypes with equal volumes of Freund's complete adjuvant. The second booster was given 3 weeks later with Freund's incomplete adjuvant, and the third administration was with clarified TC-SNT alone after another 3 weeks. Blood was collected by exsanguination 10 days after the final injection.

EIA for detection of adenovirus group antigen. Antiadenovirus rabbit serum (RCH) used to coat microtiter plates (Nunc Immunoplate) was diluted 1/4,000 in 0.06 M carbon-ate-bicarbonate buffer (pH 9.6), 100 μl was added to each well, and wells were incubated overnight at 4°C. One hundred microliters of stool samples prepared as 10% (wt/vol) PBS suspensions were added to a single well and incubated for 2 h at 37°C. Guinea pig antiserum to adenovirus (100 μl per well), diluted 1/1,000 in PBS containing 0.05% (vol/vol) Tween 20 and 2% (wt/vol) skim milk powder (PBST-SMP) was added, and plates were incubated for 2 h at room temperature. Anti-guinea pig horseradish peroxidase conjugate (Silenus) (100 μl per well) diluted 1/500 in PBST-SMP was reacted for 1 h at 37°C. Substrate containing 3,3'5,5'-tetramethylbenzidine was added and incubated at room temperature for 10 min. The optical density at 450 nm (OD_{450}) was measured after the addition of 2 N H_2SO_4 by using a Titertek EIA reader. Each working step started with four cycles of washing with PBST. The specificity of the EIA was monitored by inclusion of wells containing TC-SNT with 10^3 FFU of Ad5 per ml, TC-SNT from uninfected G293 cells, and PBST-SMP. The positive-negative cutoff was arbitrarily set at 0.15 OD units.

EIA for detection of Ad40 and Ad41 (EAd EIA). Monoclonal antibodies MA 5-8 (specific for Ad40), MA 5-15 (specific for Ad41), and MA 1-3 (group antigen specific) and horse serum against adenovirus were provided by J. C. de Jong of Rijkinstituut Voor Volksgezondheid en Milieuhygiene, Bilthoven, the Netherlands.

Serotyping was performed on all adenovirus EIA-positive samples. For each sample, four wells of a microtiter plate were coated with 100 μl of horse serum against adenovirus diluted 1/500 in 0.06 M carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C. Stool suspension (100 μl per well) further diluted one-fourth in PBST-SMP was added to four wells and incubated for 2 h at 37°C. One hundred microliters of each of the three monoclonal antibodies diluted 1/1,000 in PBST-SMP was added to each of three wells, and the fourth received PBST-SMP. Plates were incubated for 2 h at 37°C and reacted with 100 μl of anti-mouse immunoglobulin horseradish peroxidase conjugate at a dilution of 1/500 for 1 h at 37°C. 3,3',5,5'-Tetramethylbenzidine substrate was added and OD measurements were taken as described above. Three washings were performed prior to each working step. TC-SNT of Ad2, Ad40, and Ad41 were included in each test as positive controls, and PBST-SMP was included as a negative control. A specimen was considered to contain Ad40 or Ad41 if the OD_{450} reading for respective wells was at least twice as great as the reading for the negative wells and greater than 0.2 OD units. Samples were considered to contain nonenteric adenoviruses (NAds) if a reaction with MA 1-3 only was observed.

RESULTS

Detection of EAds. Of the 4,409 specimens tested by adenovirus EIA and EAd EIA, 150 (3.4%) were initially found to contain EAds, of which 55 contained Ad40 strains and 95 contained Ad41 strains, and 19 were found to contain NAds. All positive stool samples were cultivated in G293 cells (as described above) and retested in the EAd EIA for confirmation. A case of EAd or NAd infection in this study was therefore defined as one in which tissue culture confirmation was obtained. Following cultivation in G293 cells, 125 (2.8%) patients were found to shed EAds in stool; 51 of 125 (40.8%) specimens contained Ad40, and 74 of 125 (59.2%) were typed as Ad41 (Table 1). In addition, adenoviruses other than group F (NAds) were detected in 12 samples (0.3%).

TABLE 1. Prevalence of EAd and NAd in stool specimens[a]

Virus	No. of specimens					
	Total EIA positive[b]	Culture positive[c]	With *Shigella* spp.[d]	With *V. cholerae*[d]	With *Salmonella* spp.[d]	With rotavirus[d]
Ad40	55	51	5	1		2
Ad41	95	74	10	3	1	9
NAd	19	12	2			1

[a] Specimens from children less than 5 years of age who presented with diarrhea to the Matlab diarrhea treatment centers between June 1987 and August 1990.
[b] Prior to culture.
[c] Specimens positive by EAd EIA and then, after one cycle of culture in G293 cells, positive again by EAd EIA.
[d] Of culture-positive specimens.

Infections with other enteropathogens. Among the population of 4,409 infants, 1,042 (23.6%) were infected with group A rotavirus, 382 (8.7%) were infected with *Vibrio cholerae*, 822 (18.6%) were infected with *Shigella* species, and 12 (0.3%) were infected with *Salmonella* species. As shown in Table 1, among EAd-infected infants, *Shigella* spp., *Vibrio* spp., *Salmonella* spp., and rotavirus were present in 31 of 125 (24.8%) specimens. The rate of coinfection of patients shedding NAd was 3 of 12 (25.0%).

Seasonality of EAd infections. EAds were isolated throughout the year, and a peak of EAd infection was observed in February to March 1988, when the isolation rate reached 12.3% (in March) (Fig. 1). Another peak of EAd infection was found in February to March 1989 and appeared again in 1990. Furthermore, a minor peak of EAd infections was observed in October 1988, coinciding with a major flood in Bangladesh (Fig. 1).

Age and gender of patients from whom Ad was isolated. Characteristics of patients with diarrhea from whom only EAd or NAd (confirmed by cell culture) was isolated were examined. A total of 80 of 125 patients infected with adenovirus were found to excrete EAd or NAd only and had full clinical information available. Age and gender distribution among the 80 infants is given in Table 2, which shows that 78.8% of infections of all adenoviruses detected occurred in infants less than 2 years of age. The highest incidence of

infection with EAd was among the 7- to 12-month age group, and the rate of isolation of Ad40 was found to be markedly higher (18.7%) than the rate of isolation for the younger age group (7.5%) and 13- to 24-month-old infants (12.5%). For Ad41 infections, the frequencies in the 7- to 12- and 13- to 24-month age groups were similar, and the median ages of infection with Ad40, Ad41, and NAd were 11, 12, and 12 months, respectively. NAds were found at similar frequencies in the 7- to 12-month age group and in infants more than 2 years of age. Significantly more infants in the 25- to 60-month age group were infected with EAd than with rotavirus (Table 2).

Serotype-related differences were observed in the gender distribution. EAd infection was more common in males; the male/female ratios were 2.17 (26/12) and 1.62 (21/13) for Ad40 and Ad41, respectively. An equal distribution between the sexes was observed with NAd infection (male/female ratio, 8/8 [1.0]), and differences in the male/female ratio between Ad40 and Ad41 and between Ad40 and NAd were not significant ($\chi^2 = 0.35$, $P = 0.56$; and $\chi^2 = 1.61$, $P = 0.20$, respectively).

Clinical features of EAd infection. Table 3 shows information on the 80 infants with infection with either serotype of EAd. This infection was characterized by watery diarrhea, which was present in 86.1% of EAd infections, lasted for 1 to 4 days prior to presentation (in 88.9% of the cases), and was
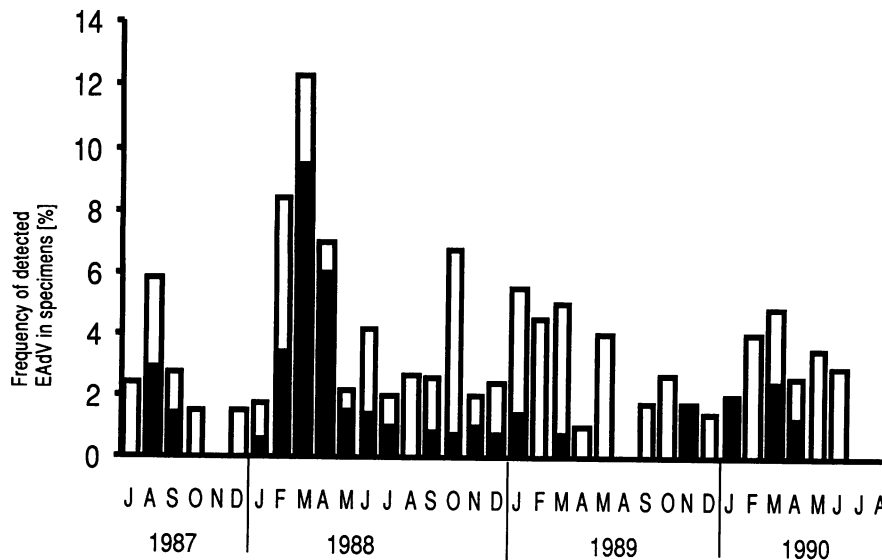

FIG. 1. Seasonal distribution of culture-confirmed EAd infection in Matlab from 1987 to 1990. □, Ad41; ■, Ad40.

TABLE 2. Age and gender distribution[a]

Age (mo)	No. (%) of infants infected with[b]:			
	Ad40	Ad41	NAd	RV
0–6	6 (15.8)	5 (14.7)	1 (12.5)	62 (12.2)
7–12	15 (39.5)	12 (35.3)	4 (50.0)	250 (49.2)
13–24	10 (26.3)	10 (29.4)	0 (0.0)	171 (37.7)
25–60	7 (18.4)	7 (20.6)	3 (37.5)	25 (4.9)[c]
Total	38	34	8	508

[a] Distribution among EAd- and NAd-infected infants ($n = 80$) and group A rotavirus (RV)-infected infants for whom no other pathogen was found ($n = 508$) and for whom clinical data were available. Infection was confirmed by tissue culture.

[b] Male/female ratios for Ad40, Ad41, NAd, and rotavirus infections were 2.17, 1.62, 1.00, and 1.53, respectively.

[c] $P < 0.0001$ by Fisher's exact test (one tailed) between EAd-infected infants and rotavirus-infected infants in this age group.

generally associated with eight or more loose movements per day (68.1% of patients). Blood was rarely found (in stools of only 8.4% of EAd-infected infants), but vomiting (79.2% of EAd infections), low-grade fever (94.4% of EAd infections), and abdominal pain (76.4% of EAd infections) commonly accompanied diarrhea. Most infants had a hospital stay of 1

to 3 days. The symptoms of infants infected with NAd were similar to those of infants infected with EAd.

Symptoms experienced by infants from whom EAd was isolated in the absence of other enteric pathogens were compared with those of group A rotavirus (only)-infected infants from the same geographic area (Table 3). The symptoms which were different between EAd- and RV-infected infants were type of diarrhea and vomiting. Watery diarrhea was experienced by more infants with rotavirus (484 of 508) than by those with EAd (62 of 72) ($\chi^2 = 9.6$, $P = 0.002$), and mucoid diarrhea was experienced more commonly by EAd-infected infants (4 of 72) than rotavirus-infected infants (6 of 508) (Fisher's exact test, one-tailed; $P = 0.002$). Vomiting was experienced more commonly among rotavirus-infected infants (462 of 508) than EAd-infected infants (57 of 72) (Mantel-Haenszel $\chi^2 = 9.3$, $P = 0.002$). All other symptoms were statistically similar.

Severity of dehydration of infants infected with EAd was examined by using a scoring system, as described above, in which a severe score significantly correlated with receipt of intravenous therapy. EAd diarrhea was most commonly accompanied by mild and moderate dehydration. Severity was compared with that of dehydration experienced by patients infected with rotavirus only (Table 4). EAd infection produced dehydration in patients similar to that found with

TABLE 3. Symptoms associated with culture-confirmed EAd and NAd infections[a]

Symptom	No. (%) of children with:		
	EAd ($n = 72$)	NAd ($n = 8$)	RV ($n = 508$)
Type of diarrhea:			
Watery, no blood	62 (86.1)	8 (100)	484 (95.3)[b]
Nonwatery, no blood	4 (5.5)	0 (0)	6 (1.2)[b]
With blood	6 (8.4)	0 (0)	18 (3.5)
Number of loose stools in previous 24 h:			
1–4	8 (11.1)	2 (25.0)	48 (9.5)
5–7	15 (20.8)	0 (0.0)	112 (22.0)
8+	49 (68.1)	6 (75.0)	348 (68.5)
Duration of diarrhea (days) prior to presentation:			
1–4	64 (88.9)	5 (62.5)	454 (89.4)
5–7	7 (9.7)	1 (12.5)	33 (6.5)
8–14	1 (1.4)	1 (12.5)	14 (28.0)
14+	0 (0.0)	1 (12.5)	7 (13.0)
Vomiting:			
Yes	57 (79.2)	7 (87.5)	462 (90.9)[b]
No	15 (20.8)	1 (12.5)	46 (9.1)
Temperature:			
96.0–101.9°F (35.6–38.8°C)	68 (94.4)	8 (100)	430 (84.6)
≥102.3°F (≥39.1°C)	4 (5.6)	0 (0.0)	78 (15.4)
Abdominal pain:			
Present	55 (76.4)	6 (75.0)	334 (65.7)
Absent	17 (23.6)	2 (25.0)	174 (34.3)
No. of days in hospital:			
0	2 (2.8)	0 (0.0)	9 (1.8)
1–3	66 (91.7)	8 (100)	440 (86.6)
4+	4 (5.5)	0 (0.0)	59 (11.6)
Degree of malnutrition[c]:			
<74%	42 (58.3)	6 (75.0)	345 (67.9)
75–80%	22 (30.6)	2 (25.0)	97 (19.1)
>81%	8 (11.1)	0 (0.0)	66 (13.0)

[a] EAd and NAd were detected in stools from 80 children less than 5 years of age with diarrhea from whom no other diarrheal pathogen was detected and from 508 children less than 5 years of age with group A rotavirus (RV) only detected in stools. Samples were collected at the Matlab diarrhea treatment centers between June 1987 and May 1989.

[b] Difference between EAd-infected and rotavirus-infected infants is significant ($P = 0.002$ using Mantel-Haenzel χ^2 and Fisher's exact [one-tailed] tests where appropriate).

[c] Percentage of National Center for Health Statistics standards.

TABLE 4. Comparison of severity of dehydration in adenovirus and rotavirus infections[a]

Severity of dehydration[b]	No. (%) of patients infected with:	
	EAd only (n = 72)	Group A rotavirus only (n = 508)
Mild	42 (58.3)	275 (54.1)
Moderate	22 (30.6)	173 (34.1)
Severe	8 (11.1)	60 (11.8)

[a] Comparison between patients infected with tissue culture-confirmed EAd only (Ad40 and Ad41) and patients infected with group A rotavirus only. Patients were treated from June 1987 to May 1989 and were less than 5 years of age.

[b] Severity of dehydration was based on the following signs: weak or absent pulse, decreased skin turgor, dry mucous membrane, sunken eyes, and sunken fontanelle. When any of these signs were normal, a score of 0 was given. Other scores were as follows: dry mucous membranes, 1; weak pulse, 1; absent pulse, 2; skin turgor with slow return, 1; skin turgor with very slow return, 2; sunken eyes, 1; deeply sunken eyes, 2; and sunken fontanelle, 1. Only patients with fontanelles were included. Dehydration was considered severe if a patient's score was 4 or more, moderate if the score was 2 or 3, and mild if the score was 0 or 1.

rotavirus infection; a statistical difference was not found (comparing the two viruses and the three levels of dehydration; $\chi^2 = 0.41$, $P = 0.81$).

Nutritional status. Weight-for-age data (expressed as percentages of the NCHS standard) were available for the 80 infants infected with adenovirus for whom no other pathogen was detected. Using the classification system of Gomez et al. (8), we found no significant difference in weight for age data between Ad40-, Ad41-, and NAd-infected infants with the chi-square test, where 36 of 38, 31 of 34, and 8 of 8 weighed less than 80% of the standard weight for their age, respectively (Table 3). There was no significant difference in the nutritional status trend between EAd-infected infants and RV-infected infants.

DISCUSSION

Stool samples from infants with diarrhea were tested for EAd, and 2.8% of the infants were found to excrete enteric serotypes as confirmed by cell culture passage of EIA-positive stools; no specimens from infants without diarrhea were tested in this study. Other studies using similar reagents (i.e., specific monoclonal antibodies) for detection of enteric serotypes have found similar rates of excretion among diarrhea cases in developing countries (12, 15), whereas studies in developed and some developing countries have found much higher rates of detection (6, 14). A study utilizing DNA hybridization with Ad40- and Ad41-specific probes gave the high detection rate of 13.2% (20). In this study we found high rates of detection of EAd during particular months.

For the purpose of this study we considered a stool sample to contain EAd if, upon culture, the supernatant also yielded EAd by EIA. This may have led to an underestimation, since 25 stools were considered EAd-positive after initial testing but failed to yield virus after culture. Also, highly specific monoclonal antibodies can fail to detect virus strains which have lost their determinants by antigenic drift (18), which can lead to underestimation. It may also be possible that higher rates of detection are due to outbreaks; the studies described by Cruz et al. (6) and Tiemessen et al. (20), in which high detection rates were found, were conducted over 1-year periods.

Ad41 (isolated from 59.2% of patients) was found to be more common than Ad40 (isolated from 40.8%), similar to the findings of Brown (3) in Canada; other investigators who discriminated between EAd serotypes observed varying frequencies of the 2 types (20, 21, 24). Ad40 has previously been associated with an outbreak at an orphanage in Japan (4), and as shown in Fig. 1, we observed an outbreak of Ad40 in February to March 1988.

In addition to the peak of Ad40 infections in March 1988, small peaks of EAd infections were observed during the two winter or dry seasons covered. Such a seasonal trend has not been observed in previous studies in which the period covered has allowed observation of seasonality to be made (1, 20). The study by Bhan et al. (1), which was conducted in India, where the climate is similar, found EAds to be more common in the wet, warm months of July to September.

NAds were detected in only 0.3% of specimens tested, which is much lower than rates described by a number of investigators (1, 12, 14, 15, 21, 24), who detected rates ranging between 1.0 and 8.8%. To capture adenovirus particles, the initial detection test used rabbit hyperimmune antiserum which was raised against an EAd strain purified from a stool sample. It is possible that the antiserum may have contained predominantly EAd-specific antibodies and therefore, some NAds could have been missed.

EAd was found to be associated with other enteric pathogens in almost one-fourth of the cases. Enterotoxigenic *Escherichia coli* was not tested for and in this age group could have accounted for a reasonable number of coinfections. When the association with other pathogens has been studied, the frequencies have tended to be higher (6, 12). Exclusion of the cases in which pathogens other than adenovirus were detected and for which full data were available enabled the study of age and gender distribution and clinical features of EAd diarrhea. Of EAd-infected infants, 82.2% were observed to acquire infection by 2 years of age, a finding that correlates with a seroprevalence study conducted by our group (13) in which approximately 85% of Bangladeshi children had acquired anti-EAd neutralizing antibodies by 2 years of age. Also, Uhnoo and others (21) reported that 70% of EAd infections occurred in infants less than 2 years of age. We found little difference among the adenovirus types. Our finding of Ad40 infection being more common among males has also been observed elsewhere (21).

Clinical features described here are highly compatible with observations of other investigators (14, 21). Other studies have found EAds in a greater number of stool samples with blood and/or mucus and in a lower number of infants with fever (1, 14). It has been generally thought that EAd diarrhea is milder than that caused by rotavirus (22), but dehydration in EAd infections has been documented before (22), and our results show that the degree and frequency of dehydration are the same as for rotavirus diarrhea. Of the clinical features that were examined, only the consistency of stool and frequency of vomiting varied significantly in comparison with symptoms of rotavirus-infected infants in the same age group.

EAds have been detected in stools of Bangladeshi infants at a low rate and may be associated with occasional outbreaks. Morbidity associated with EAd alone is significantly similar to morbidity associated with rotavirus.

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