Descriptive epidemiology of persistent diarrhoea among young children in rural northern India

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In order to determine the descriptive epidemiology of persistent diarrhoea in rural northern India, a cohort of 963 children aged 0–71 months was followed prospectively for 12 months through weekly household visits. The incidence of persistent diarrhoea was 6.3 per 100 child-years among those aged 0–71 months, and was highest (31 per 100 child-years) among those aged 0–11 months. There were no significant sex-related differences in the incidence of the disease, and the overall seasonal distribution of acute and persistent diarrhoea was similar. The persistence of diarrhoeal symptoms was significantly correlated with a higher initial mean stool frequency ($P < 0.01$) and passage of gross blood with stools ($P < 0.001$). Persistent diarrhoea was an important problem among children during the first 2 years of life.

Established enteric pathogens were isolated during the initial illness in 46.4% of persistent and 55.4% of acute episodes. Pathogens isolated during persistent episodes included enterotoxigenic Escherichia coli *(ETEC 9.3%),* Salmonella *spp. (4.7%), as well as campylobacter (4.7%),* Shigella *spp. (2.3%),* Entamoeba histolytica *(2.3%), and rotavirus (2.3%). Similar proportions of these pathogens were isolated also during episodes of acute diarrhoea. Multiple pathogens were isolated in 7% of the persistent and 5% of the acute episodes.* E. coli *that manifested aggregative adherence (EAEC-A) was more common (34.9% versus 12.3%) in persistent than acute episodes ($P < 0.01$), and initial faecal excretion of EAEC-A was significantly associated with the persistence of a diarrhoeal episode.*

Most diarrhoeal illnesses among children in developing countries are self-limiting, but a proportion follow a prolonged or persistent course. Persistent diarrhoea is important for at least the following reasons: the risk of mortality associated with diarrhoea increases significantly with the duration of the illness (*1*, *2*); and prolonged episodes probably have a greater impact on nutritional status than acute diarrhoea (*2–5*). Although estimates indicate that 3–20% of episodes of acute diarrhoea become persistent (*3*, *6–7*), the true incidence of persistent diarrhoea is not well established.

Recently, persistent diarrhoea was identified as a priority area of research by WHO and, as an initial step, it was recommended that in developing countries community-based longitudinal studies should be carried out to determine more accurately the magnitude and epidemiological characteristics of persistent diarrhoea. Here, we report the descriptive epidemiology of persistent diarrhoea among a cohort of rural northern Indian children aged under 6 years who were followed prospectively.

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Materials and methods

Setting

The study was carried out in Anangpur–Palla, a village located in a rural community 30 km south of Delhi. The climate is marked by three distinctive seasons: hot dry pre-monsoon (April to June), monsoon (July to October), and a cool dry winter (November to March). An initial demographic survey of the entire village in September 1984 identified 4800 inhabitants, of whom 91% were Hindus, in 715 households. The median number of individuals per household was seven, and 35% of the heads of households were factory workers, 19% local artisans, and 14% farmers. The literacy rate was low—35% of the heads of households were illiterate and only 11% had been educated beyond high school level, while most mothers (80%) were illiterate. The median per capita family income in 1983 was 1200 rupees (range, 400–5000 rupees. Wells were used by 45% of the population, while 55% used handpumps to obtain water.

Assembly of the cohort

The verbal, informed consent of the children's parents or guardians was obtained after the details of the study procedure had been explained to them. A total of 794 out of 821 children who resided in the village and who were aged <60 months on 1 May 1985 were enrolled in the study (27 children refused to participate or were not available during the survey). Enrolled children were followed until they died, out-migrated, or until the end of the study on 30 April 1986. During the study period, all newborns ($n = 50$) in initially enrolled households and all permanent immigrants into the village aged <60 months ($n = 119$) were also enrolled. In the period of the study 15 children died (five from diarrhoea), while 45 out-migrated.

Surveillance for morbidity

From 1 May 1985 to 30 April 1986 every enrolled child was visited once a week by a team of interviewers who were specially trained but kept unaware of the study's specific aims. Children not available during a scheduled visit were visited the next day. Subjects were not available for interview on 2728 (6%) of the 42776 total visits over the 12-month study period.

During the visits the number and consistency of stools recorded each day, the presence or absence of blood or mucus in the stools, and any episodes of vomiting or fever were noted. Also, at each visit, children who were suffering from diarrhoea were examined for signs of dehydration. Stool specimens were collected by parents only from children aged <36 months, either on the first day of an episode of diarrhoea or on the following day, while the child was having diarrhoea. Such specimens were obtained for 222 of 294 episodes that involved children aged <36 months.

The feeding status of children was recorded at the last visit each month and at every visit during episodes of diarrhoea. A doctor made unannounced visits to each house every 2 weeks to validate the data collected by the interviewers.

Medical services

Medical care and immunization services were available to the village population from the local primary health centre (PHC). Mothers were instructed to use home-made oral rehydration salt solutions and to continue feeding their children with breast milk and other foods during episodes of diarrhoea. Children who suffered from dysentery or lower respiratory tract infections were referred to the PHC as soon as they were identified, as also were those with diarrhoea that persisted for at least 15 days. At the PHC, all three groups were prescribed co-trimoxazole, and, if they did not respond to this treatment, were referred to the paediatric service at the nearest hospital.

Definitions

An episode of diarrhoea was defined as the passage of four or more loose or watery stools per day. An episode was considered to have ended on the last day with diarrhoea that was followed by three consecutive non-diarrhoeal days. For breast-fed infants aged <3 months, it was required that mothers consider also loose motions as diarrhoeal stools. Dysentery was diagnosed when stools mixed with gross blood were observed or reported. Episodes that lasted more than 14 days were defined as persistent.

Microbiological procedures

Faecal specimens from children with diarrhoea were collected in triplicate; the first was placed in Cary-Blair transport medium, the second in a vial containing 10% phosphate-buffered saline (pH 7.4), and the third was tested immediately by the concentration method for *Giardia lamblia* and *Entamoeba histolytica* in a saline iodine preparation. Specimens collected in the transport medium were plated within 2 hours onto MacConkey agar, selenite F broth, or deoxycholate-citrate agar. Microorganisms were identified tentatively after overnight incubation at 37 °C.[a] Subcultures from the selenite F broth were made on Salmonella–Shigella (SS) agar, and *Salmonella* spp. and *Shigella* spp. were identified biochemically (*7*). *Campylobacter jejuni* was cultured by first plating the stools onto modified Skirrow's agar and incubating the plates at elevated temperature and reduced oxygen partial pressure, and was identified by its growth and Gram-stain characteristics (*8*).

Three lactose-positive colonies of *Escherichia coli* with similar morphology were harvested from the MacConkey agar and stored on nutrient agar slants (*8*, *9*). The colonies were blotted onto six nitrocellulose filter papers, each of which was hybridized with DNA probes to detect diarrhoeogenic *E. coli*, including those that were enterotoxigenic (LT, STP, and STH) (*10*), enteroinvasive (EIEC) (*11*), enterohaemorrhagic (EHEC) (*12*), and enteropathogenic (EPEC) (*13*). An adherence assay was carried out on all the *E. coli* strains using monolayers of Hep-2 cells grown on circular coverslips (13-mm diameter) in 24-well tissue-culture plates (Costar, Cambridge, MA, USA) in the presence of alpha-D-mannoside (*13–15*). The Hep-2 cell assay was run with coded

[a] *WHO manual for laboratory investigations of acute enteric infections*. Unpublished document WHO/CCD/83.3.

bacteria in the Center for Vaccine Development, MD, USA; neither the probe results nor the clinical data were known by the individual who interpreted the adherence patterns. Samples of *E. coli* were designated as enteroadherent only if they adhered to Hep-2 tissue culture cells and were negative by LT, STP, EIEC and EHEC probes. Enteroadherence was categorized into the three phenotypes discussed below (*15*).

In the aggregative type (EAEC-A), aggregates of bacteria assumed a characteristic "stacked-brick" pattern that was evident both on the surface of the Hep-2 cells as well as on the glass coverslips. The localized type (EAEC-L) was characterized by the formation of tight clusters of bacteria or "micro-colonies", where virtually all the adherent bacteria formed tightly clumped clusters on the Hep-2 cell surface; aggregates of bacteria only rarely occurred that were not associated with Hep-2 cells. Finally, in the diffuse type (EAEC-D) the tissue culture cells were uniformly covered with adherent bacteria, mostly as single organisms; no aggregates of bacteria occurred on the surface of the Hep-2 cells or on the cover slips.

Faecal specimens collected in phosphate-buffered saline and stored at −20 °C were tested for rotavirus and adenovirus. Rotavirus was detected using both screening and blocking enzyme-linked immunosorbent assay (ELISA) methods.[b] Enteric adenovirus was detected using two ELISA methods, the first of which was genus-specific and the second was specific only for adenovirus type 40 or type 41 (*16*).

Analysis

The incidences of episodes of diarrhoea in various age categories were calculated by dividing the number of episodes, i.e., those that were not present at the onset of follow-up, by the number of person-days of follow-up for children of a particular age range. Here, person-days of follow-up refer only to the weekly periods when information about children was obtained. As noted above, only 6% of the weekly visits failed to yield morbidity information. The incidences obtained were analysed using appropriate statistical techniques (*17*). For comparisons of dichotomous variables, associations were expressed as an odds ratio that was statistically validated using the χ^2 test. To evaluate the association between EAEC-A and the persistence of diarrhoea, independently of confounders, we estimated the corrected odds ratio using the Mantel–Haenzel technique. The simultaneous effects of various confounders were further determined using a logistic regression model, with persistence as the dependent variable and candidate associational factors as independent variables. The Mann–Whitney test was used to make group comparisons of dimensional variables with skewed distributions. All statistical tests were interpreted using a two-tailed approach to estimate the *P*-value.

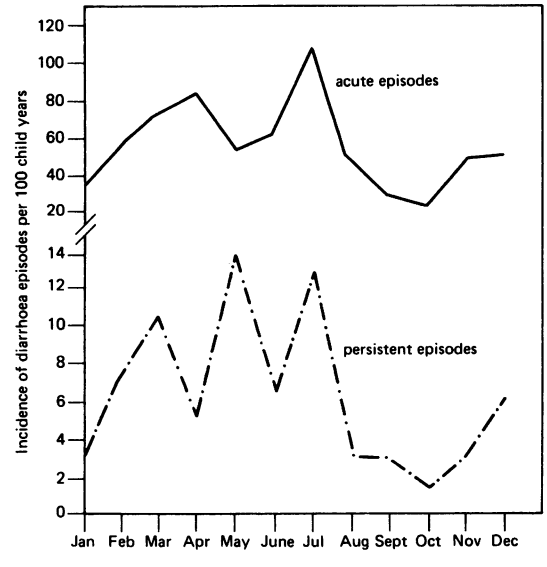
Results

A total of 471 episodes of diarrhoea occurred among 349 of the children during the 1-year follow-up period, which was equivalent to 768 child-years. The incidences of diarrhoea, by weeks of duration, are shown in Table 1. For persistent diarrhoea (duration >14 days) the incidence was 6.3 episodes per 100 child-years, while that for acute diarrhoea was 55.1 episodes per 100 child-years (Table 1).

The highest incidence of persistent diarrhoea occurred among infants (0–11 months) and was significantly greater than that for children aged 12–23, 24–35, or 36–71 months. For infants the incidence of persistent diarrhoea was greatest among 3–5-month-olds. Over two thirds (73%) of all episodes of persistent diarrhoea occurred in children aged less than 2 years. The difference in the incidence of persistent diarrhoea in males (5.7) and females (6.9) was statistically not significant. The monthly incidence of both acute and persistent diarrhoea is shown in Fig. 1.

The 48 persistent episodes of diarrhoea that were observed in the study involved 46 children. The

Fig. 1. **Monthly incidence of acute and persistent episodes of diarrhoea among the study children.**



[b] See footnote *a*, p. 282.

Table 1: **Incidence of persistent and acute diarrhoea (by duration in weeks) in a cohort of rural Indian children aged ⩽71 months, by age group, 1 May 1985 to 30 April 1986**

Age group (months)	Child-years at risk	Acute episodes[a]			Persistent episodes[a]		
		⩽1 week	1–2 weeks	Total	2–3 weeks	>3 weeks	Total
0–2	1.8	1 (55.8)	1 (55.8)	2 (111.6)	0 (0.0)	0 (0.0)	0 (0.0)
3–5	10.7	8 (74.9)	6 (56.2)	14 (131.0)	2 (16.0)	2 (16.0)	4 (37.4)
6–11	55.2	27 (48.9)	30 (54.3)	57 (103.2)	11 (19.9)	6 (10.9)	17 (30.8)
0–11	67.7	36 (53.2)	37 (54.7)	73 (107.9)[b]	13 (19.2)	8 (11.9)	21 (31.0)[b]
12–23	151.9	36 (23.7)	70 (46.1)	106 (69.8)	8 (5.3)	6 (3.9)	14 (9.2)
24–35	138.3	35 (25.3)	37 (26.8)	72 (52.1)	7 (5.1)	1 (0.7)	8 (5.8)
36–47	154.2	34 (22.1)	50 (32.4)	84 (54.5)	2 (1.3)	1 (0.7)	3 (1.9)
48–59	145.2	15 (10.3)	41 (28.3)	56 (38.6)	0 (0.0)	0 (0.0)	0 (0.0)
60–71	110.8	10 (9.0)	22 (19.9)	32 (28.9)	2 (1.8)	0 (0.0)	2 (1.8)
Total	768.1	166 (21.6)	257 (33.5)	423 (55.1)	32 (4.2)	16 (2.1)	48 (6.3)

[a] In each pair, the first figure shows the number of episodes in eligible children, while the figure in parentheses shows the incidence expressed as the number of episodes per 100 child-years.
[b] 0–11 months' acute versus 12–23 months' acute diarrhoea, $z = 2.89$, $P < 0.001$; 0–11 months' persistent versus 12–23 months' persistent diarrhoea, $z = 3.74$, $P < 0.0001$; 0–11 months' acute versus 12–71 months' acute diarrhoea, $z = 6.13$, $P < 0.0001$; 0–11 months' persistent versus 12–71 months' persistent diarrhoea, $z = 8.54$, $P < 0.0001$.

median proportion of study days spent observing episodes of diarrhoea was 11.3% for the 46 children who suffered one or more persistent episodes and 2.8 for the 303 children who experienced episodes that did not last longer than 2 weeks ($P < 0.00001$). The incidence of diarrhoea among the 46 children (227.4 episodes per 100 child-years) who suffered a persistent episode was significantly higher than that among the 303 (132.3 episodes per 100 child-years) who exhibited one or more acute episodes of diarrhoea ($z = 7.17$, $P < 0.0001$).

Table 2 shows the distribution of some of the characteristics of the episodes that occurred during the initial week of illness for children with acute or persistent diarrhoea. Persistent episodes were more frequently associated with bloody stools ($P < 0.001$) and a higher stool frequency ($P < 0.006$); also a slightly, but not significantly, higher case fatality rate was observed among children with persistent diarrhoea.

Table 3 shows the distribution of established enteric pathogens that were identified in the stools of children with acute or persistent diarrhoea. Such pathogens were isolated in 46.4% of persistent and in 55.4% of acute episodes. Multiple pathogens were isolated in 7% of the persistent and in 5% of the acute episodes. Although the excretion rates of ETEC-LT, *Salmonella* spp., *Shigella* spp. and *E. histolytica* were higher in persistent than in the acute episodes, these differences were not significant.

In addition to the established pathogens, Table 3 also shows the distribution of Hep-2-cell-adherent *E. coli* isolates of different phenotypes. Of these, EAEC-A was isolated in a substantially higher proportion of persistent (34.9%) than acute (12.3%) episodes (odds ratio 3.8, $P < 0.001$). To test whether this association arose spuriously because of confounding, we calculated the odds ratio, using the Mantel-Haenzel technique, for the association between isolation of aggregative adherence (AA) and

Table 2: **Distribution of clinical characteristics during the first week of illness and the occurrence of death in episodes of acute and persistent diarrhoea in a cohort of rural Indian children aged ⩽71 months**

Characteristic	All episodes ($n = 471$)	Acute episodes ($n = 423$)	Persistent episodes ($n = 48$)	Odds ratio
Watery stools	38.2%	38.1%	39.6%	1.1
Bloody stools	8.7%	7.3%	20.8%	3.3[a]
Frequency of watery or bloody stools[b]	8.1 (4–25)	8.1 (4–25)	8.9[c] (4–14)	—
Fever	8.5%	8.5%	8.3%	1.0
Vomiting	8.7%	9.0%	6.3%	0.7
Deaths[d]	1.5%	1.4%	2.1%	1.5

[a] $P < 0.001$ for persistent versus acute episodes.
[b] Shown is the maximum frequency in any 24-hour period during the first week of illness; values in parentheses give the range.
[c] $P = 0.006$ for persistent versus acute episodes.
[d] Includes only deaths caused by diarrhoea.

Table 3: **Distribution of enteric pathogens isolated in stools obtained during the first week of acute and persistent diarrhoeal episodes in a cohort of rural Indian children aged <36 months, 1 May 1985 to 30 April 1986**

Agents	All episodes (n = 222)	Acute episodes (n = 179)	Persistent episodes (n = 43)	Odds ratio
Established pathogens:[a]				
Rotavirus	2.3	2.2	2.3	1.0
Adenovirus (40, 41)	0.5	0.6	0	—
Salmonella spp.	3.2	2.8	4.7	1.7
Shigella spp.	1.8	1.7	2.3	1.4
Aeromonas spp.	2.3	2.8	0	—
Vibrio cholerae	0	0	0	
Giardia lamblia	2.3[b] (5.4)[c]	2.2[b] (5.6)	2.3[b] (4.6)	1.0[b] (1.2)
Entamoeba histolytica	0.5[b] (4.5)	0[b] (3.9)	2.3[b] (7.0)	—[b] (1.0)
Campylobacter	5.6	5.6	4.7	0.8
EPEC[d]	6.3	7.3	2.3	0.3
ETEC	13.5	15.1	9.3	0.6
LT	7.7	7.3	9.3	1.3
ST	3.6	4.5	0	—
LT/ST	2.3	2.8	0	—
EIEC	0	0	0	—
EHEC	0	0	0	—
Multiple pathogens	5.4	5.0	7.0	1.5
No established pathogen	46.4	45.3	51.2	0.7
Potential pathogens:[e]				
LA	6.8	7.8	2.3	0.3
DA	5.0	6.1	0	—
AA	16.7	12.3	34.9	3.8[f]
DA/AA[g]	0.9	1.1	0	—
LA/AA[g]	0.5	0.6	0	—
LA/DA[g]	0.5	0.6	0	—

[a] Isolated as single pathogens; excludes episodes with multiple isolations, which are shown separately.
[b] Vegetative stage detected with or without cysts.
[c] Figures in parentheses apply only to cysts with no vegetative stage.
[d] As detected by enteroadherence factor (EAF) probe; these results overlap with those of the Hep-2 cell assay.
[e] LA = localized adherence; DA = diffuse adherence; AA = aggregative adherence.
[f] Acute versus persistent, $P < 0.001$.
[g] Mixed isolations pertain to conditions where three strains tested from the same patient showed different patterns of adherence.

the persistence of diarrhoea corrected for the following: the child's age (0–11 months, 12–23 months, or 24–36 months), sex, breast-feeding status (yes/no on date of onset), income ($\leq$ median or $>$ median), maternal education level (none versus some), father's education level (none versus some), season of onset (pre-monsoon, monsoon, or winter), antibiotic intake (yes/no), antecedent nutritional status (weight-for-age $\leq 70\%$ or $>70\%$ of median value). The odds ratio and significance level were essentially invariant for all these associations. In order to assess the simultaneous confounding effect of all the associations, these variables, together with information on the isolation of AA as an independent variable were fitted to a logistic regression model. The stratified analysis for nutrition and logistic regression was restricted to 144 episodes (116 acute; 28 persistent) for which the weights of children within the previous 3 months were available. The corrected odds ratio, independent of any confounding effects, was 3.5 (95% confidence interval: 1.1–10.9, $P < 0.03$).

Discussion

The results suggest that persistent diarrhoea was an important problem for children during the first 2 years of life in the study population and that children who suffered a persistent episode of diarrhoea experienced a significantly higher diarrhoeal burden over the 1-year study period. *E. coli* that showed aggregative adherence to Hep-2 cells was associated with a significantly higher proportion of persistent than acute episodes.

Potential limitations of the study

Several potential limitations need to be considered in interpreting these findings. The frequency of household visits was once a week rather than 2–3 times a week, and this may have led to an under-reporting of short-duration episodes, although it is less likely to have affected the identification of persistent episodes. Also, under-identification of very short episodes

could have biased the results, particularly with regard to clinical features.

Our assessment of the duration of diarrhoea required an arbitrary definition of a diarrhoeal day as well as the start and end of an episode. There are currently no universally accepted definitions for these variables and our results, therefore, cannot be extrapolated to settings where different definitions are employed. Moreover, since the incidence of persistent diarrhoea may be influenced, *inter alia*, by nutritional profiles, feeding practices, and the epidemiological features of diarrhoeal pathogens our results may not apply to populations that differ in these characteristics. Lastly, the outcome of persistent episodes of diarrhoea may have been influenced by the treatment administered at the hospital to which the children were referred.

Incidence of persistent diarrhoea

The overall incidence of diarrhoea in the study population was low. For example, in an investigation of a neighbouring community, Ghai et al., who defined diarrhoea as the passage of 3 or more liquid stools, as opposed to the 4 or more in our study, reported an overall incidence of 1.6 episodes per child per annum among under-5-year-olds (*18*). Studies from several other countries have reported annual diarrhoeal attack rates of 3 to 7 per child (*7, 19*). The incidence of persistent diarrhoea in our cohort (6.4 episodes per 100 child-years) is similar to that reported in Egypt (*19*), and Guatemala (*20*), while higher incidences have been reported in Bangladesh (*7*) and Ethiopia (*21*). Overall, 10% of all episodes of diarrhoea that we found lasted longer than 2 weeks; in other community-based studies, 3–23% of diarrhoeal episodes resolved after more than 2 weeks (*3, 7, 19*). The peak incidence of persistent diarrhoea occurred during the first year of life, particularly among children aged 3–5 months and this is consistent with the results of a study by McAuliffe et al. who reported that persistent diarrhoea peaked in children aged 3–6 months in Brazil (*3*). The higher risk of prolonged diarrhoeal illnesses in early infancy may be related to host characteristics, to the introduction of all or specific foods to the diet, or to a reduction in the prevalence of breast-feeding after the initial few months of life.

Persistent episodes as a marker of diarrhoeal morbidity

In the study 6% of children suffered from one or more episodes of persistent diarrhoea. The 48 persistent episodes that were observed involved 46 children, indicating that recurrence of persistent diarrhoea was not a common feature during the 1-year follow-up period. However, our data are consistent with previous observations that prolonged episodes affect primarily children who experience an overall higher prevalence of diarrhoea (*3, 22*).

Both our data and those from the study in Brazil reported by McAuliffe et al. indicate that persistent episodes of diarrhoea are not restricted to a particular season of the year. Such a pattern is consistent with the observed association of several different pathogens with persistent episodes of diarrhoea reported by other workers.

Episodic characteristics of persistent diarrhoea

There was a positive correlation between both dysenteric illness and initial stool frequency with subsequent persistent diarrhoea. However, the predictive value of this association was too low (24.4% for dysentric illness) to be of diagnostic importance. Halliday et al. reported a significant association between stool frequency, elevated blood urea levels, bacterial etiology, and the risk of persistence (*23*), but other workers have observed no such correlation (*24, 25*).

Etiological candidates of persistent diarrhoea

Of the several pathogens that were excreted during acute or persistent episodes, only EAEC-A was significantly associated with persistent rather than with acute episodes. This is supported by recent histopathological findings caused by EAEC-A in an animal model, which were characterized by shortening of the villi and the lack of an epithelial cover that left a denuded connective tissue core with a haemorrhagic surface (*26*).

The role of different phenotypes of adherent *E. coli* in causing acute or persistent diarrhoea is still not properly understood. In one study in Chile the excretion rates of EAEC-L and EAEC-A were significantly greater in children with diarrhoea than in nondiarrhoeal controls (*28*), but the diarrhoeal episodes were not classified by duration. In the same study, both patients with diarrhoea and nondiarrhoeal controls excreted EAEC-D at a similar frequency. *E. coli* strains that exhibited localized adherence caused diarrhoea in adult volunteers (*29*) but similar studies have not been performed with EAEC-A. The possible role of EAEC-A in episodes of persistent diarrhoea is therefore preliminary and needs to be investigated in other settings.

Our search for etiological agents did not include *Cryptosporidium* spp., which have been recognized to be common protozoal parasites associated with diarrhoea in the tropics (*27*). Also, *Aeromonas* spp. may have been under-identified because selective culture media were not used.

In a prospective study in Brazil, excretion of multiple pathogens was more commonly associated with persistent than with acute episodes of diarrhoea (*3*). Although the excretion of multiple pathogens in the present study was higher among patients with persistent rather than acute diarrhoea, these differences were not statistically significant. However, since serial stools were not collected, only simultaneous, but not sequential, infection with more than one pathogen could have been identified.

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Résumé

Epidémiologie descriptive de la diarrhée persistante chez les jeunes enfants d'une région rurale du nord de l'Inde

Au 1er mai 1986, on a analysé l'incidence et les caractéristiques saisonnières, cliniques et microbiologiques de la diarrhée persistante dans une cohorte de 963 enfants âgés de 0 à 71 mois, à Anangpur-Palla, une communauté rurale située près de Delhi. Du 1er mai 1985 au 30 avril 1986, ces enfants avaient été soumis à une surveillance hebdomadaire, à la recherche d'une morbidité par diarrhée ou de tout autre phénomène apparenté. On a recueilli des échantillons de selles chez les enfants âgés de moins de 36 mois, qu'ils présentent ou non une diarrhée. On a retrouvé les germes suivants: rotavirus, adénovirus, *Escherichia coli*, *Salmonella* spp., *Shigella* ssp., *Campylobacter jejuni*, *Aeromonas* ssp., *Giardia lamblia* et *Entamoeba histolytica*. On a utilisé des sondes d'ADN pour caractériser les diverses souches d'*E. coli*: entérotoxinogènes, entéropathogènes, entérohémorragiques ou entéro-invasives. Les souches d'*E. coli* réagissant négativement avec les sondes mais adhérant aux cellules Hep-2 ont été appelées entéroadhésives (ECEA), catégorie elle-même subdivisée en fonction des caractéristiques de l'adhérence en souches d'*E. coli* entéroadhésives localisées (ECEA-L), diffuses (ECEA-D) ou agrégantes (ECEA-A).

Au cours de la période de suivi qui a représenté 768 années-enfants, on a enregistré un total de 471 épisodes diarrhéiques. L'incidence globale de la diarrhée (61 pour 100 années-enfants) chez les enfants âgés de 0 à 71 mois a été faible. Pour la diarrhée persistante (durée >14 jours) l'incidence a été de 6,3 pour 100 années-enfants pour les enfants âgés de 0 à 71 mois et de 31 pour 100 années-enfants entre 0 et 12 mois. On a observé une forte diminution de l'incidence des épisodes de diarrhée persistante au cours de la deuxième année et des années suivantes, alors qu'elle était progressive pour les épisodes de diarrhée aiguë. Plus des deux tiers (73%) de tous les épisodes de diarrhée persistante se sont produits chez des enfants de moins de 24 mois. Les 48 épisodes de diarrhée persistante observés l'ont été chez 46 enfants pour lesquels la proportion de jours d'observation avec diarrhée a été plus grande que chez les 303 enfants n'ayant jamais souffert de diarrhée persistante (médiane de 11,3% contre 2,8%; $P < 0,00001$). L'incidence de la diarrhée persistante n'a été associée à aucune différence liée au sexe et les caractéristiques saisonnières générales des épisodes persistants ont été les mêmes que celles des épisodes aigus. Cependant, on a observé une corrélation significative entre la persistance des symptômes diarrhéiques et une fréquence moyenne initiale des selles élevée ($P < 0,006$) ou la présence de sang macroscopique dans les selles ($P < 0,001$).

On a isolé des germes entériques connus dans 46,4% des épisodes de diarrhée persistante et dans 55,4% des épisodes aigus. Les plus couramment associés à la diarrhée persistante ont été *Escherichia coli* entérotoxinogène (9,3%), *Salmonella* spp. (4,7%), *Campylobacter* (4,7%), *Shigella* spp., *G. lamblia*, *E. histolytica* et les rotavirus (2,3% chacun). On a isolé des germes multiples dans 7% des épisodes persistants et 5% des épisodes aigus.

Parmi les phénotypes ECEA, le type ECEA-A a été isolé dans 34,9% des épisodes persistants alors qu'il n'a été isolé que dans 12,3% des épisodes aigus ($P < 0,001$); l'excrétion d'ECEA-L et d'ECEA-D pendant les épisodes aigus ou persistants n'a pas présenté de différence significative.

References

1. **Bhan, M.K. et al.** Major factors in diarrhea-related mortality among rural children. *Indian journal of medical research*, **83**: 9–12 (1986).
2. **Larcher, V.F. et al.** Protracted diarrhea in infancy. Analysis of 82 cases with particular reference to diagnosis and management. *Archives of disease in childhood*, **52**: 597–605 (1977).

3. **McAuliffe, J.F. et al.** Prolonged and recurring diarrhea in the north-east of Brazil: examination of cases from a community-based study. *Journal of pediatric gastroenterology and nutrition*, **5**: 902–906 (1986).
4. **Black, R.E. et al.** Development of nutritious, hygienic weaning food to reduce diarrhea in Peru. *Annual report, 1982–1983*.
5. **Black, R.E. et al.** Effect of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh. *Pediatrics*, **73**: 799–805 (1984).
6. **Lie, K.J. et al.** Diarrhoea among infants in a crowded area of Djakarta, Indonesia: a longitudinal study from birth to two years. *Bulletin of the World Health Organization*, **34**: 197–210 (1966).
7. **Black, R.E. et al.** Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II. Incidence of diarrhea and association with known pathogens. *American journal of epidemiology*, **115**: 315–324 (1982).
8. **Ahmed, M.Z. et al.** The detection of enteropathogens in acute diarrhea in a family cohort population in rural Egypt. *American journal of tropical medicine and hygiene*, **35**: 1013–1022 (1986).
9. **Merson, M.H. et al.** The use of a colony pool for diagnosis of enterotoxigenic *Escherichia coli* diarrhea. *Journal of clinical microbiology*, **9**: 493–497 (1973).
10. **Lenata, C.F. et al.** Sensitivity and specificity of DNA probes with the stool blot technique for detection of *Escherichia coli* enterotoxin. *Journal of infectious diseases*, **152**: 1087–1090 (1985).
11. **Wood, P.K. et al.** Comparison of a DNA probe with the Sereny's test in the identification of invasive strains of shigella and *Escherichia coli*. *Journal of clinical microbiology*, **24**: 498–500 (1986).
12. **Levine, M.M. et al.** DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *Journal of infectious diseases*, **156**: 175–182 (1987).
13. **Levine, M.M. et al.** The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. *Journal of infectious diseases*, **152**: 550–559 (1985).
14. **Methewson, J.J. et al.** A newly recognised cause of traveller's diarrhea: enteroadherent *Escherichia coli*. *Journal of infectious diseases*, **151**: 471–475 (1985).
15. **Nataro, J.P. et al.** Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. *Journal of infectious diseases*, **152**: 560–565 (1985).
16. **Bhan, M.K. et al.** Role of enteric adenoviruses and rotaviruses in mild and severe acute enteritis. *The pediatric infectious diseases journal*, **7**: 320–323 (1988).
17. **Kleinbaum, D.G. et al.** *Epidemiologic research: principles and quantitative methods*. Belmot, CA, Lifetime Learning Publications, 1982.
18. **Ghai, O.P. et al.** Epidemiology of diarrhea in infants and pre-school children in a rural community near Delhi. *Indian pediatrics*, **6**: 263–271 (1969).
19. **Alamy, M.A.E.** The incidence of diarrheal disease in a defined population of rural Egypt. *American journal of tropical medicine and hygiene*, **35**: 1006–1012 (1986).
20. **Gordon, J.E. et al.** Nutrition and infection field study in Guatemalan villages, 1959–1964. VI. Acute diarrheal disease and nutritional disorders in general disease incidence. *Archives of environmental health*, **16**: 424–437 (1968).
21. **Freij, W.S. et al.** Exploring child health and its ecology. The Kirkos Study in Addis Ababa: an evolution of procedures in the measurement of acute morbidity and the search for causal structure. *Acta paediatrica Scandinavica*, **267** (suppl): 1–120 (1977).
22. **Black, R.E. et al.** Malnutrition is a determining factor in diarrheal duration, but not incidence, among young children in a longitudinal study in rural Bangladesh. *American journal of clinical nutrition*, **37**: 87–94 (1984).
23. **Halliday, K. et al.** Persistent post-enteritis diarrhea in childhood. *Medical journal of Australia*, **1**: 18–20 (1982).
24. **Gribbin, M. et al.** Delayed recovery following acute gastroenteritis. *Acta paediatrica Belgica*, **29**: 167–176 (1976).
25. **Gribbin, M. et al.** A twelve month prospective survey of admissions to the gastroenteritis unit of a children's hospital. *Acta paediatrica Belgica* **29**: 69–77 (1976).
26. **Vial, P.A. et al.** Characterization of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrheal disease. *Journal of infectious diseases*, **158**: 70–79 (1988).
27. **Navin, T.R. & Juranek, D.D.** Cryptosporidiosis: clinical, epidemiologic and parasitological review. *Reviews of infectious diseases*, **6**: 313–327 (1984).
28. **Nataro, J.P. et al.** Patterns of adherence of diarrheogenic *Escherichia coli* to Hep-2 cells. *Pediatric infectious diseases*, **6**: 829–831 (1987).
29. **Mathewson, J.J. et al.** Pathogenicity of enteroadherent *Escherichia coli* in adult volunteers. *Journal of infectious diseases*, **154**: 524–527 (1986).