

## Persistent Diarrhea Signals a Critical Period of Increased Diarrhea Burdens and Nutritional Shortfalls: A Prospective Cohort Study among Children in Northeastern Brazil

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Persistent diarrhea (PD; duration  $\geq 14$  days) is a growing part of the global burden of diarrheal diseases. A 45-month prospective cohort study (with illness, nutritional, and microbiologic surveillance) was conducted in a shantytown in northeastern Brazil, to elucidate the epidemiology, nutritional impact, and causes of PD in early childhood (0–3 years of age). A nested case-control design was used to examine children's diarrhea burden and nutritional status before and after a first PD illness. PD illnesses accounted for 8% of episodes and 34% of days of diarrhea. First PD illnesses were preceded by a doubling of acute diarrhea burdens, were followed by further 2.6–3.5-fold increased diarrhea burdens for 18 months, and were associated with acute weight shortfalls. Exclusively breast-fed children had 8-fold lower diarrhea rates than did weaned children. PD-associated etiologic agents included *Cryptosporidium*, *Giardia*, enteric adenoviruses, and enterotoxigenic *Escherichia coli*. PD signals growth shortfalls and increased diarrhea burdens; children with PD merit extended support, and the illness warrants further study to elucidate its prevention, treatment, and impact.

The 1997 report by Murray and Lopez on disability-adjusted life years (DALYs) for specific diseases notes that the 3 leading causes of global DALYs are, in descending order, lower respiratory infections, diarrheal diseases, and perinatal disorders [1, 2]. These investigators also showed that 5 of the 10 leading

killers were communicable diseases and perinatal and nutritional disorders mostly affecting children [1, 2]. Endemic diarrhea in developing areas continues to be one of the world's leading causes of DALYs, especially among children <5 years old, in whom attack rates are 2–12 illnesses per child per year [3, 4].

Although the majority of diarrheal episodes resolve within 1–2 weeks, a proportion of illnesses persists longer [3, 5]. We have found that the occurrence of a persistent-diarrhea (PD) illness (duration  $\geq 14$  days) identifies those children with the heaviest diarrhea burdens (i.e., those who will spend  $\geq 15\%$  of their days with diarrhea) [3]. When a PD illness occurs, the development or worsening of malnutrition becomes more likely, a vicious cycle of deteriorating nutritional status and continuing diarrhea may become established, and there is a substantial risk of death [6]. “Graveyard surveillance” (i.e., weekly interviews with the local gravedigger) in the rural community of Guaiuba in northeastern Brazil revealed that 70% of childhood deaths were due to diarrhea and that half of these deaths were due to PD illnesses [7].

Many studies have found a substantial effect of diarrheal

Received 3 June 1999; revised 28 January 2000; electronically published 15 May 2000.

Presented in part: 8th annual meeting of the International Centers for Tropical Disease Research, National Institutes of Health, Bethesda, MD, 26–28 April 1999.

Informed consent was obtained from the children's parents and guardians, and the study was approved by the human investigation committees at the Federal University of Ceará, the Johns Hopkins University School of Medicine, and the University of Virginia.

Grant support: National Institute of Allergy and Infectious Diseases grant U01AI26512. The work of J.P.N. is supported by Public Health Service grant AI33096.

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The Journal of Infectious Diseases 2000;181:1643–51

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0022-1899/2000/18105-0017\$02.00

illnesses (especially PD or recurrent diarrhea) and enteric infections (with or without diarrhea) on nutritional status, as well as an association between prior malnutrition and subsequent frequency and duration of diarrhea, thus documenting the reciprocal cycle of diarrhea and malnutrition [6, 8–12]. Despite increasing recognition of the importance of PD illnesses, the patterns of occurrence, risk factors, causes, and impact in tropical, developing regions remain largely unknown. To elucidate these factors, we undertook a prospective cohort study of PD in an urban shantytown in northeastern Brazil. Studies of selected specific pathogens in this and nearby populations have been conducted for *Cryptosporidium* [13–15], enteroaggregative *Escherichia coli* (EAaggEC) [12], torovirus [16], and Norwalk virus [17] infections. The focus of this report is on overall illness rates, risk factors for PD, and the pivotal event that a PD illness represents in early childhood.

## Materials and Methods

**Study site and design.** The study was conducted in a 5-block area of a shantytown, Gonçalves Dias, located in Fortaleza, the capital city of the northeastern Brazilian state of Ceará. In 1994, Fortaleza's infant mortality was 59/1000 live births. The population of Fortaleza is ~2.3 million. Gonçalves Dias is a community of 1826 people (February 1993), 13.5% (247) of whom are children  $\leq 5$  years old.

Pregnant women in the community were identified by the study team (2 nurses and 1 health care worker) and were asked to enroll in the project. After providing informed consent, women choosing to participate completed a detailed demographic and socioeconomic questionnaire with the help of a study nurse.

The house of each newborn child in the study was visited 3 times weekly (Monday, Wednesday, and Friday) by a study nurse who recorded diarrheal illnesses and dietary information. Mothers (or guardians) of children with diarrhea were asked to provide detailed clinical information about the illness, including other symptoms (fever, vomiting, dehydration), stool consistency and character, and medication used. A study nurse visited children with diarrhea daily until 48 h after resolution of the illness. The duration of the study was 3 years and 9 months, from August 1989 through April 1993. Diarrhea attack rate and prevalence data and anthropometric data from this time period have been reported in a community secular-trends analysis of diarrhea burdens and malnutrition during 1989–1996 [18].

Diarrhea was defined as  $\geq 3$  liquid stools in the preceding 24-h period. An episode of diarrhea was defined as diarrhea lasting  $\geq 1$  day and separated from another episode by  $\geq 48$  h without diarrhea. We defined acute diarrhea as an episode that lasted  $< 14$  days and PD as an episode that lasted  $\geq 14$  days. The study was approved by the human investigation committees at the Federal University of Ceará, the Johns Hopkins University School of Medicine, and the University of Virginia.

**Effects of first persistent illness on subsequent diarrhea burdens.** Diarrhea surveillance data were condensed into sequential, non-overlapping, child-quarters (90 days) of observation, with the number of episodes and total days of diarrhea recorded for each quarter.

Episodes that began in 1 quarter and extended to the next were attributed to the initial quarter, as were the total days for that episode. Of 189 children followed up in the cohort, 52 experienced at least 1 episode of PD during the study. Cases of PD were sex- and age-matched (to within 2 months) to controls (children with no PD recorded in current or previous quarters). When children designated as controls developed PD after their case-match, they were not analyzed as cases but were used only as controls, to avoid the potential bias of comparing diarrhea burdens between children with a history of PD and children with no occurrence of PD during their entire surveillance. Of the 52 children with PD, 3 could not be matched, and 11 had been designated as controls before onset of PD. This gave 38 case-control pairs for the analysis. Diarrhea burdens were compared for as long as 3 quarters before and 8 quarters after the PD illness. Only case-control pairs with  $\geq 80$  days of follow-up during the quarter were included in the analysis of a particular quarter.

**Nutritional assessment.** The length and weight of all children were measured at 3-month intervals. Clothed weights to the nearest 0.1 kg were obtained by use of a calibrated-sling Salter scale. Length was measured supine to the nearest centimeter. Weight-for-age, height-for-age, and weight-for-height Z scores (WAZ, HAZ, and WHZ, respectively) were calculated by use of nutrition software (EPINUT, World Health Organization, Geneva; Epi Info version 6.0, Centers for Disease Control and Prevention, Atlanta). These anthropometric Z scores are a measure of SDs above or below the median for the international reference population.

**Breast-feeding analysis.** At each visit, caretakers were asked to provide detailed information on the child's diet. The data were later classified into exclusive (only breast milk, with or without teas or juices), partial (breast milk plus other milks or foods), and no breast-feeding (no breast milk) categories. Observation days for children 0–6 months old were condensed into child-months containing both the number of diarrheal episodes and the feeding category for the month. Diarrheal incidence for different age groups by month was then compared across the 3 groups. No child  $> 6$  months old was exclusively breast-fed.

**Microbiologic studies.** During the study period, cups for the collection of stool specimens were distributed to the households of children with diarrhea. Caretakers were instructed to collect the first stool the following day. Every 3 months, specimen cups were also distributed to children who had no diarrhea for  $\geq 14$  days. At least 3 days separated antibiotic ingestion from all stool collections.

All stool specimens were collected, transported on ice, and processed within 4 h of collection. All specimens were examined initially by microscopy for parasites and leukocytes by use of iodine-stained and methylene blue-stained wet-mount preparations. Fecal smears were prepared on glass slides for modified acid-fast stain for *Cryptosporidium* and *Isospora belli* [13, 19, 20], and another slide was prepared for modified trichrome stain for microsporidium [20–23]. One aliquot of each fecal specimen was frozen and stored for testing for virus—including rotavirus, torovirus, and Norwalk virus—by use of ELISAs and polymerase chain reaction assays [16, 17, 24]. In addition, 1 fecal specimen was saved in polyvinyl alcohol fixative for trichrome staining to review protozoal morphology.

Initial bacterial cultures were done for *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* species by use of MacConkey's, xylose-lysine-deoxycholate, and Gram-negative broth enrichment and subcul-

ture, TCBS (thiosulfate, citrate, bile salt, and sucrose) agar, and ampicillin blood agar plates (with 10  $\mu\text{g}/\text{mL}$  ampicillin). Thereafter,  $\geq 4$  lactose-fermenting colonies were selected from MacConkey's agar plates (depending on the number of colony types) and saved for toxin, adherence, and serologic testing.

At least 1 coliform isolate was tested for heat-labile toxin, heat-stable toxin, and other enteric virulence traits by use of gene probes for heat-labile toxin and heat-stable toxin and for classic enteropathogenic *E. coli* (probes EAF and eae), EAggEC (probe AA), diffusely adherent *E. coli* (probe DA), enteroinvasive *E. coli* (probe EIEC), and Shiga-like toxin (probe ST) I and II. In addition, at least 1 *E. coli* strain from each child with PD and malnutrition was tested by use of a phenotypic bioassay for HEP-2 cell adherence [25–27].

**Statistical analyses.** Statistical analyses were performed by using SPSS software (version 7.5; SPSS, Chicago) and Epi-Info. Statistical tests included Spearman's rank correlation for PD incidence by socioeconomic factors; Wilcoxon signed-rank test for the analysis of diarrhea burdens among cases and age- and sex-matched controls before and after a first PD episode; paired *t* tests for comparison of nutritional status before and after a PD episode; Pearson's  $\chi^2$  and the Mantel-Haenszel technique for comparison of diarrhea rates among children with different feeding practices, at 0–6 months old; and  $\chi^2$  or Fisher's exact test for associations of pathogens with persistent, acute, and nondiarrheal stools. All tests were 2-tailed, and *P* values  $< .05$  were considered statistically significant.

## Results

**Population characteristics, illness rates, and risk factors for diarrhea.** Of the 421 households in Gonçalves Dias, 184 (44%) were enrolled in the study. Study households had a median of 2 rooms and a median of 5 persons who slept in the home. A total of 218 pregnancies were followed up in the cohort from August 1989 through April 1993. Of these, 29 were in women who never entered the study (3 women aborted, 4 had stillbirths or infant deaths, 4 elected not to participate, and 18 moved away before any surveillance data were obtained). Thus, we analyzed data from 189 children, of whom 129 remained under surveillance in April 1993 (50 moved away before April 1993, 8 elected to leave the study, and 2 died [1 perinatally from pneumonia and 1 at 7 months of age after extensive hospitalization for diarrhea]). This community infant mortality of 5.1/1000 per year contrasts with the infant mortality in Fortaleza of 59/1000 per year in 1994 (State Secretary of Health records). Birth weights (measured to the nearest 0.01 kg) were recorded for 180 of the children. Of these, 8.8% (16/180) were low birth weight ( $< 2500$  g).

One-third of the dwellings were temporary structures made of mud and wood, and two-thirds were permanent, built of brick and adobe. The main source of drinking water was piped city water (48% [75/155]). Forty-eight percent (75/155) of homes had running water, and 74% (120/161) had either an indoor pit or flush toilet. The survey of maternal education showed that

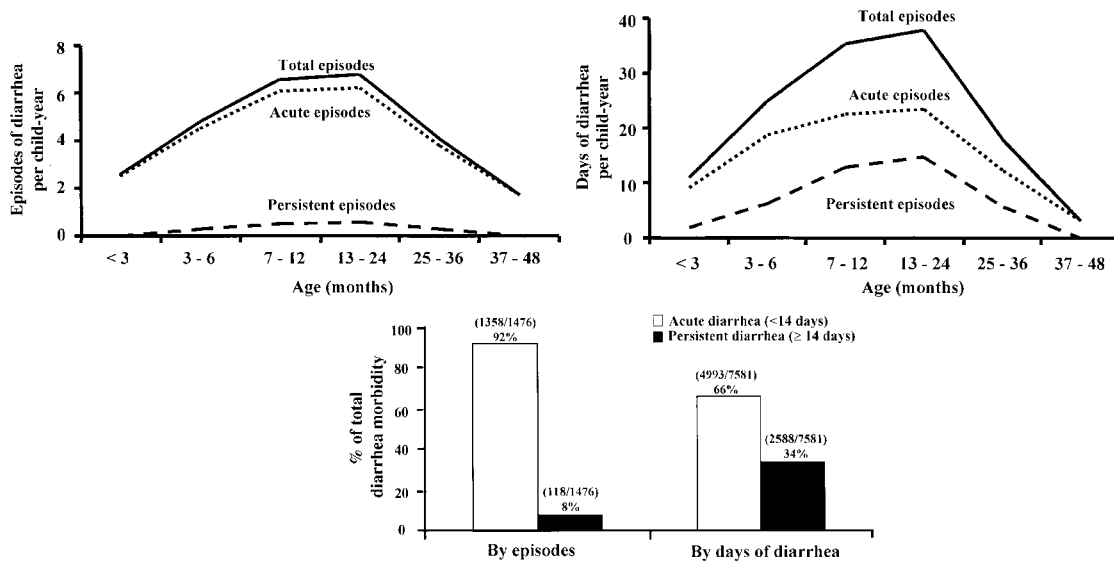
86% (139/162) had not completed primary school; 8% were illiterate. Of 126 households with income data, 43% received  $< 1$  minimum wage per month (US \$102); 71% received  $< 2$  minimum wages per month ( $< \$204$ ).

**Episodes and days of diarrheal diseases.** Overall, these children had 5.25 episodes of diarrhea per child-year (1476 episodes per 102,672 observation days, or 281 child-years), spending an average of 27 days per year with diarrhea (7581 days of diarrhea per 281 child-years of observation). We found an average of 4.83 acute episodes per child-year and 0.42 persistent episodes per child-year. Figure 1 (*top panels*) shows age-specific attack rates. Total, acute, and persistent episodes peaked at 13–24 months of age (6.8, 6.2, and 0.6 episodes per child-year, respectively). Days spent with diarrhea also peaked at 13–24 months of age for total, acute, and persistent episodes (37.9, 23.3, and 14.6 days per child-year, respectively). As shown in figure 1 (*bottom panel*), PD accounted for only 8% (118/1476) of all episodes but 34% (2588/7581) of days with diarrhea.

Presence or absence of a toilet (pit or flush) and number of persons sleeping in the home were significantly associated with PD burdens. Of the 70 study children with the most complete surveillance in their first 2 years of life (i.e.,  $\geq 96\%$ , or 700 days), the 29 with no toilet in the home had a higher incidence of PD (relative risk [RR], 4.79; 95% confidence interval [CI], 2.17–10.54) than did children with toilets. Among these same 70 children, the number of persons sleeping in the home was positively correlated with the number of PD illnesses during the first 2 years of life (Spearman's  $\rho$ , .298; *P* = .012). Birth weight (recorded for 69 of the 70 children) was not a risk factor for PD among infants  $\leq 2$  years old (Spearman's  $\rho$ , .032; *P* = .794).

The proportion of children who were exclusively breast-fed decreased from 52.3% (90/172) in the first month of life to 0.57% (8/139) at 6 months of age; the crude RR for diarrheal illnesses in non-breast-fed versus exclusively breast-fed infants was 7.96 (CI, 4.40–14.38; *P*  $< .00001$ ). The crude RR for partial versus exclusive breast-feeding was 4.31 (CI, 2.46–7.55; *P*  $< .00001$ ). The crude RR for non-breast-fed versus partially breast-fed infants was 1.85 (CI, 1.40–2.43; *P*  $< .00001$ ). Exclusive breast-feeding was protective, independent of age, and episode durations were slightly longer for non-breast-fed infants (5.7 days) than for partially breast-fed infants (4.5 days; *P* = .02, by analysis of variance).

The etiologic agents found in acute, persistent, and nondiarrheal stool samples are shown in table 1. Although Norwalk-like, calciviruses [17, 24], toroviruses [16], and *Shigella* were associated with acute diarrheal illnesses (*P* = .0021, *P* = .017, and *P* = .0035, respectively), associations with acute diarrhea and PD, respectively, included adenoviruses (*P* = .035 and *P* = .003), *Cryptosporidium* (*P* = .004 and *P* = .0002), localized (enteropathogenic) adherent *E. coli* (*P* = .035 and *P* = .0017), and fecal leukocytes and lactoferrin (*P*  $< .01$ ). *Giardia* (*P*  $< .001$ ) and heat-labile toxigenic *E. coli* (*P* = .013) were encoun-



**Figure 1.** *Top left*, Diarrhea attack rates per child-year, by age, among 189 children in Gonçalves Dias in Fortaleza, Brazil, from August 1989 through April 1993. *Top right*, Days with diarrhea per child-year, by age. Top panels show peaks at 13–24 months of age for episodes and days of acute and persistent diarrhea. *Bottom*, Proportions of total diarrhea morbidity accounted for by episodes and by days of illness.

tered in PD stools, whereas intestinal helminths, EAaggEC, and diffusely adherent *E. coli* were seen among control and case subjects.

**Effects of first PD illness on subsequent diarrhea burdens.** To evaluate the effects of the first PD illness on subsequent diarrhea burdens, we also examined children <3 years old with a first episode of PD and compared their diarrhea burdens (exclusive of their PD illness) with those of age- and sex-matched control children without concurrent or previous PD illnesses (i.e., the child of the same sex with the closest birthday and no prior PD who was not previously matched). Figure 2 shows previous and subsequent diarrhea burdens among case and control children. Until 3 months before the PD illness, case and control children had the same diarrhea burdens. Children who were about to develop a PD illness then had a 2-fold increase in their acute diarrhea burdens ( $P = .018$ ). After their PD illness, these children experienced a further increase in diarrhea burdens that remained significantly different from those of controls for as long as 18 months ( $P < .05$ ).

**Growth and nutritional impact of illness episodes.** Figure 3 shows the impact of a child's first PD episode on anthropometry. To determine the change in nutritional status after a PD illness, we identified children's first PD episode and compared their anthropometric Z scores 3 months before and after the episode. We found significant decreases in WAZ ( $-0.48$  to  $-0.62$ ,  $P < .05$ ) and WHZ ( $0.44$  to  $0.12$ ,  $P < .05$ ) but not HAZ ( $-1.23$  to  $-1.26$ ,  $P > .05$ ) in the period after the PD illness (figure 3).

## Discussion

In this long-term cohort study of children followed up from birth, we found an overall diarrhea-attack rate of 5.25 illnesses, with 27 days of diarrhea per child per year and peak rates in the second year of life. Although PD accounts for only 8% of episodes, it accounts for more than one-third of days with diarrhea and is associated with crowding and inadequate sanitary facilities (but not with low birth weight). Perhaps our most important finding, from a public health perspective, is that PD marks a critical period of increasing burdens of diarrhea for 18 months after the illness. The relationship between increased diarrhea burdens and a previous episode of PD may be one of cause or effect or both, or PD may be merely a signal. Regardless of its nature, the association clearly shows that a child with a PD illness warrants extended attention, to prevent further diarrhea and short-term growth deficits, as well as to avoid the potential long-term functional and growth consequences of heavy diarrhea burdens in the developmentally critical first years of life [28] (S. R. Moore and M. D. Niehaus, unpublished data).

The peak diarrheal illness attack rate was 6.8 episodes per child-year among children 13–24 months old. Diarrhea rates in this cohort are lower than those reported for 1984–1986 by Schorling et al. [3] for an earlier, different cohort of children in the same community. They are, however, comparable to rates from similar studies in other tropical areas, such as Bangladesh, Guatemala, Peru, Kenya, and Guinea-Bissau [29–33]. Our study's PD illness rates of 0.42 episodes per child-year (with

**Table 1.** Results of etiologic studies of diarrheal illness episodes (1091 specimens tested) among children in northeastern Brazil.

Etiologic agent	Acute diarrhea <sup>a</sup>		Persistent diarrhea <sup>b</sup>		Control, % (no. with agent/ no. tested) <sup>c</sup>
	% (no. with agent/ no. tested)	<i>P</i>	% (no. with agent/ no. tested)	<i>P</i>	
<b>Virus</b>					
Norwalk-like virus	52.0 (34/65)	.0021	12.5 (1/8)		24.0 (12/50)
Rotavirus	4.5 (6/133)		9.1 (6/66)		2.4 (2/82)
Adenovirus	5.3 (7/133)	.035	10.6 (7/66)	.0025	0 (0/82)
Torovirus	27.3 (9/33)	.017	20.0 (1/5)		0 (0/17)
<b>Parasite (all pathogens)</b>					
<i>Cryptosporidium</i> species	11.4 (46/402)	.0043	31.8 (28/88) <sup>d</sup>	.025	25.1 (111/442)
<i>Giardia lamblia</i>	7.7 (40/519)		23.9 (13/71)	.0002	5.2 (15/289)
<i>Microsporidium</i> species	0 (0/26)		20.5 (18/88) <sup>e</sup>	.0003	7.9 (35/442)
<i>Strongyloides</i> species	0 (0/26)		0 (0/75)		4.0 (1/25)
<i>Strongyloides</i> species	0.4 (2/519)		1.1 (1/88)		0.5 (2/442)
<i>Ascaris</i> species	8.3 (43/519)	.0061	10.2 (9/88)		13.8 (61/442)
<i>Trichuris</i> species	7.1 (37/519)		9.1 (8/88)		7.7 (34/442)
<i>Entamoeba histolytica</i>	0.8 (4/519)		0 (0/88)		1.1 (5/442)
<b>Bacteria (all pathogens)</b>					
<i>Shigella</i> species	6.3 (32/514)	.0007	2.3 (2/88)		1.8 (8/440)
<i>Salmonella</i> species	4.3 (22/514)	.0035	2.3 (2/88)		1.1 (5/440)
<i>Salmonella</i> species	0.2 (1/514)		0 (0/88)		0.2 (1/440)
<i>Vibrio cholerae</i>	0.2 (1/514)		0 (0/88)		0 (0/440)
<i>V. parahaemolyticus</i>	0.2 (1/514)		0 (0/88)		0 (0/440)
<i>Yersinia</i> species	0.4 (2/514)		0 (0/88)		0 (0/440)
LT or ST <i>Escherichia coli</i>	10.0 (39/389)		17.6 (12/68)	.0128	7.2 (14/195)
ST <i>E. coli</i>	5.1 (20/389)		5.9 (4/68)		2.1 (4/195)
LT <i>E. coli</i>	4.9 (19/389)		11.8 (8/68)		5.1 (10/195)
EPEC EAE probe	5.9 (23/389)		5.9 (4/68)		7.2 (14/195)
EAggEC (HEp-2)	38.0 (125/329)		34.8 (24/69)		34.8 (64/184)
AA probe	6.9 (27/389)		4.4 (3/68)		9.7 (19/195)
DAEC probe	7.7 (28/364)		11.5 (6/52)		8.0 (9/112)
EHEC probe	0 (0/344)		0 (0/51)		0 (0/112)
EIEC probe	0.3 (1/344)		2.0 (1/51)		0 (0/112)
Fecal leukocytes	33.0 (171/518)	<.00001	43.2 (38/88)	<.00001	6.3 (28/442)
<b>Lactoferrin</b>					
All	77.9 (95/122)	.00001	76.9 (20/26)	.013	49.5 (47/95)
Mild	51.6 (63/122)	.00008	42.3 (11/26)		25.3 (24/95)
High (1 : 400)	26.2 (32/122)		34.6 (9/26)		24.2 (23/95)
Any pathogen	81.8 (283/346)	.027	87.5 (63/72)	.017	73.0 (143/194)
No pathogen	18.2 (63/346)	.027	12.5 (9/72)	.017	27.0 (51/194)
2 Pathogens	39.0 (135/346)		50.0 (36/72)		40.7 (82/194)

NOTE. Data are for case or control fecal specimens with the specified etiologic agent. *P* values are for differences from controls. LT, heat labile; ST, heat stable; EPEC, enteropathogenic *E. coli*; EAggEC, enteroaggregative *E. coli*.

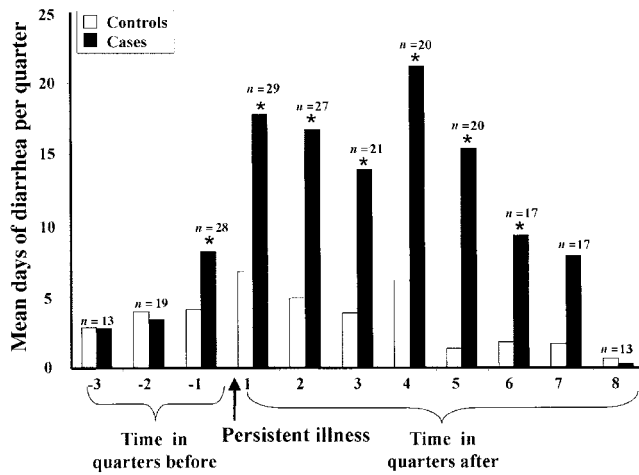
- <sup>a</sup> Includes 528 specimens representing 520 illnesses.
- <sup>b</sup> Includes 120 specimens representing 88 illnesses.
- <sup>c</sup> Includes 443 control specimens.
- <sup>d</sup> Significantly different from acute diarrhea.
- <sup>e</sup> Significantly different from acute diarrhea, *P* = .0002.

the peak of 0.6 at 13–24 months of age) are lower than rates reported by Schorling et al. [3]; are higher than those reported from Peru, India, Guatemala, Indonesia, and Kenya [31, 32, 34–37]; and are similar to or lower than rates in studies from Bangladesh [38], Ethiopia [39], and Guinea-Bissau [33]. We had previously documented a secular decline in diarrhea incidence (especially PD incidence) in this cohort, with both age-specific attack rates and malnutrition decreasing with time [3, 40].

This pattern may not have extended to nearby shantytowns (A.A.M.L., unpublished data). Our findings that absence of a toilet in the home and number of persons sleeping in the home were risk factors for PD show that unsanitary, crowded home

conditions increase the likelihood of PD in early childhood, probably by increasing fecal-oral and person-to-person transmission of diarrheal pathogens to young children.

We consider 2 new findings about PD in this cohort study to be particularly provocative. First, we found that children who developed PD (figure 2) were not different from matched PD (figure 2) were not different from matched controls 6–9 months before the PD illness, suggesting they were not initially different either as hosts (genetically or nutritionally) or in exposure to pathogens. A critical occurrence then resulted in a 2-fold increase in diarrhea burdens in the 3 months before the PD illness. Whether this key antecedent event was a specific infection or a shortfall in micronutrients is not clear from this study and warrants further investigation. The critical



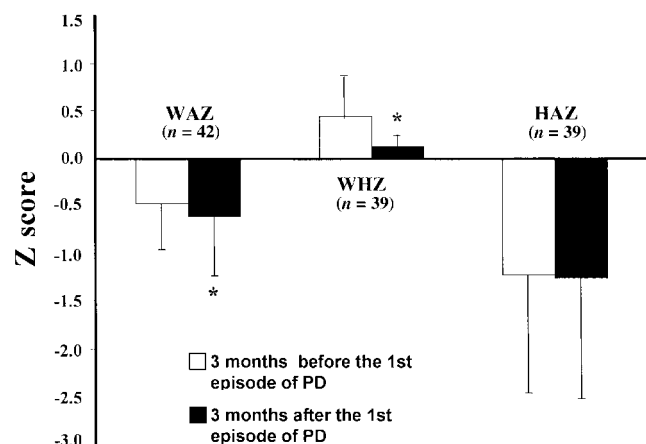
**Figure 2.** Diarrhea burdens among children <3 years old with a first episode of persistent diarrhea (PD). Until 3 months before the PD illness, case and control children had the same diarrhea burdens. Children who were about to develop a PD illness then had a 2-fold increase in their total diarrhea burdens ( $P = .018$ ). After their PD episodes, these children experienced a further 2.64–3.46-fold increase in diarrhea burdens for 18 months, when compared with age- and sex-matched controls ( $P < .05$ ). Numbers above bars indicate the number of matched case-control pairs available for each time period relative to a PD episode.

nature of the event(s) preceding PD is further emphasized by our second provocative finding. Despite the 2-fold increase in diarrhea burdens just before the PD illness, a further 2.6–3.5-fold increase in diarrhea burdens, compared with age- and sex-matched controls, followed the PD illness and continued for 18 months thereafter. These temporal relationships clearly show that PD represents a pivotal event with profound implications in the lives of young children in tropical, developing nations. This nested case-control analysis strongly supports and substantially extends the first descriptive observations by McAulliffe et al. [5], who found that PD defined a subpopulation of high-risk children who spent >16% of their days with diarrhea. A similar observation was noted by Schorling et al. [3] for a cohort of 61 children (not the children in the present study) <5 years of age followed up for 2 years in the same geographic area as the current study. Further attention is warranted for children whose high-risk status is signaled by a PD illness, to attempt to define potentially treatable causes of PD and to assure appropriate nutritional support.

The present study evaluated whether an episode of PD contributes to a worsening of nutritional status. We and others have shown the influence of nutritional status on diarrheal duration [8, 33, 41] and incidence [8, 31, 42]. The present study shows a negative impact of PD on nutritional status, as measured by WAZ and WHZ. Sharp reductions in WHZ and WAZ are markers of wasting [43], a disorder that can manifest itself

quickly in young children. We saw no reduction in HAZ, which is a marker of long-term malnutrition [43]. Although the immediate impact of PD illnesses is on WHZ and WAZ, detailed long-term analyses reported elsewhere show an impact on stunting (i.e., HAZ) as well [18]. There are convincing data showing that (1) diarrhea exacerbates malnutrition [8, 44–46] and (2) malnutrition predisposes to increases in both incidence and duration of diarrhea [8, 45, 47–51]. Few studies have found an association between malnutrition and PD [52, 53]. A case-control study of 756 children 3–69 months old who were followed up for 18 months in rural areas of northern India showed that WAZ at or below the 70th percentile was associated with PD, compared with control children who had either acute or no diarrhea [52]. A hospital-based study to identify risk factors for PD among 307 children presenting at the University Hospital at Ibadan, Nigeria, also found that malnutrition was associated with PD [53].

Finally, we found that exclusive or partial breast-feeding protected against diarrhea in the first 6 months of life. Several studies have shown that infants who are exclusively breast-fed have less frequent episodes of diarrhea than do those who are partially breast-fed or fully weaned [41, 54, 55]. Partial breast-feeding also is protective but to a much lesser extent than exclusive breast-feeding [56]. Exclusive breast-feeding probably protects against diarrhea in the early months of life by enhancing the infant’s resistance to infection and minimizing exposure to waterborne and foodborne pathogens [54, 57, 58]. Two recent studies showed that breast-feeding protects against PD [32, 33]. Early studies, in which breast-feeding continued during the rehydration phase of acute diarrhea, showed an improvement in stool consistency, a reduction in the number



**Figure 3.** The impact of persistent diarrhea (PD) on anthropometry. Anthropometric measurements were compared 3 months before and after a child’s first PD episode. PD was associated with significant decreases in weight-for-age Z scores (WAZ) and weight-for-height Z scores (WHZ) but not in height-for-age Z scores (HAZ) in the period after the episode.

of stools, and a tendency toward lower fecal output and improved rehydration [59–61]. We observed a low prevalence of exclusive breast-feeding, and we noted that mothers often withheld breast-feeding or other food when a child began a diarrheal episode. These factors may partly explain why exclusive breast-feeding did not change the duration of episodes. However, we did find that partially breast-fed infants had significantly shorter illnesses, compared with weaned children. Although further studies are needed, promotion of breast-feeding is an essential element in preventing acute diarrhea and PD and the malnutrition associated with them.

Earlier etiologic studies showed that *Cryptosporidium*, *Giardia*, enteric adenoviruses, and enterotoxigenic *E. coli* are significantly associated with 23.9%, 20.5%, 10.6%, and 17.6%, respectively, of cases of PD and are potential causes of PD among children in tropical, developing nations [62]. *Cryptosporidium*, *Shigella*, Norwalk virus, enteric adenoviruses, and toroviruses are associated with 11.4%, 4.3%, 52.0%, 5.3%, and 27.3%, respectively, of acute diarrhea [13].

Effective treatments for infections caused by *Cryptosporidium* species and torovirus are not presently available. Toroviruses, which we have reported to be associated with PD [16], were sought in only a relatively small number of these children. We have shown that infections with *Cryptosporidium* species are associated with an increase in diarrhea burdens and growth faltering [15]. In a cohort of Peruvian children, asymptomatic and symptomatic cryptosporidiosis infections caused short-term weight loss [11] and had a lasting adverse impact on linear growth as well [63].

EAggEC has been shown to be associated with PD in several settings, including India, Mexico, and Brazil [27, 62]. We and others have identified several isolates that exhibit morphologic evidence of HEp-2 cell aggregative adherence and were negative with the currently available AA gene probe [25–27]. In a previous report from this cohort, symptomatic and asymptomatic EAggEC infections were associated with lactoferrin and impaired growth [64]. Although EAggEC was found as frequently in controls as in cases, growth shortfalls were also seen in the control children [12]. Thus, EAggEC may be a widespread, potentially treatable cause of malnutrition among children in impoverished, tropical urban settings. The mechanisms by which these infections trigger a subsequent increased diarrhea burden and impair growth are unknown and deserve further study.

We conclude that PD (1) accounts for a substantial portion of diarrhea burdens in this impoverished, tropical urban setting, (2) marks a critical period of a substantial increase in diarrhea burdens, and (3) is associated with nutritional shortfalls. Although viruses or *Cryptosporidium* species that may be associated with PD are not specifically treatable, attention to breast-feeding, sanitation, and the improvement of nutritional status

is crucial to prevention of potentially lasting sequelae of the vicious cycle of diarrhea and malnutrition.

#### Acknowledgments

We thank the field workers Sayonara S. B. Alencar, Maria de Lourdes P. Rodrigues, Maria Luzia F. Melo, and Rosania Silva; research technologist Nancy A. Nelson; laboratory technicians Maria do Carmo, N. Pinho, Maria Isabel L. McAuliffe, Jania Teixeira, Conceição Nogueira Raulino, and Leah Barrett; and the participating children and their families. We also thank Dr. Ann Hill for her thoughtful review of this manuscript.

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