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Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children

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Abstract

Background & Aims—Prolonged episodes of acute diarrhea (ProD, duration 7–13 days) or persistent diarrhea (PD, duration ≥14 days) are important causes of undernutrition, yet the epidemiology and nutritional impact of ProD are poorly understood.

Methods—We conducted a 10-year cohort study of 414 children from a Brazilian shantytown who were followed from birth; data were collected on diarrhea, enteric pathogens, and anthropometry.

Results—During 1,276 child-years of observation, we recorded 3,257 diarrheal episodes. ProD was twice as common as PD (12% and 5% of episodes, respectively); ProD and PD together accounted for 50% of all days with diarrhea. ProD was more common in infants whose mothers had not completed primary school (relative risk [RR]=2.1; 95% confidence interval=1.02–2.78). Early weaning was associated with earlier onset of ProD (Spearman's ρ, 0.309; P=0.005). Infants with ProD were twice as likely to develop PD in later childhood (log rank P=0.002) compared to infants with only acute diarrhea (AD, duration <7 days), even after controlling for confounders. Children's growth was more severely stunted before their first episode of ProD, compared with AD (mean height-for-age Z score (HAZ) -0.81 vs. -0.51, P<0.05, unpaired *t* test). Following ProD, HAZ (ΔHAZ=-0.232) and weight-for-age (ΔWAZ=-0.26) significantly decreased (P<0.005 in paired *t* tests). ProD was associated with Cryptosporidium and Shigella infections.

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Conclusions—ProD accounts for significant morbidity and identifies children at risk of a vicious cycle of diarrhea and malnutrition. Further studies are needed to address the recognition and control of ProD and its consequences in resource-limited settings and assess its role in PD pathogenesis.

Keywords

Longitudinal study; chronic diarrhea; cryptosporidiosis; shigellosis

Introduction

Despite substantial reductions in diarrhea mortality over recent decades, diarrhea and its reciprocal relationship to undernutrition remain leading causes of global disability-adjusted life years (DALYs).[1] Diarrhea causes 1 to 2.5 billion illnesses and 1.5 to 2.5 million deaths per year in children under the age of 5 in developing countries.[2, 3] Furthermore, heavy burdens of diarrhea and undernutrition in early childhood profoundly impair growth and development.[4]

The majority of diarrheal illnesses are acute in duration (AD, <14 days); however, a subset of illnesses—known as persistent diarrhea (PD)—begin acutely then persist 14 days or longer, thereby resulting in a disproportionate share of diarrhea-related morbidity and mortality.[5] Moreover, PD illnesses are associated with growth faltering,[6] micronutrient deficiencies,[7, 8] impaired neurodevelopment,[9] and increased morbidity and mortality from other childhood diseases.[10]

Advances in the prevention and treatment of PD were the recent focus of an international PD Working Group who identified several research priorities for PD.[11] Chief among these was the need to characterize “prolonged” episodes of acute diarrhea (ProD, duration 7–13 days) in order to further understand the pathogenesis and classification of PD, as well as the potential impact of interventions to halt the progression to persistent diarrhea. Although a number of studies have addressed the epidemiology of AD and PD, no studies have yet examined ProD as a distinct category of diarrhea. Thus, ProD's specific risk factors, etiologies, nutritional impact, and relationship to PD are unknown. To the extent that ProD represents an intermediate stage in the continuum from acute to persistent diarrhea, such studies would greatly enhance our understanding of PD's evolution and potential strategies for its control.

To elucidate the epidemiology and impact of ProD in a highly endemic setting, we analyzed diarrheal episodes from a 10-year prospective birth cohort study of persistent diarrhea in Gonçalves Dias, an urban shantytown in Brazil's developing Northeast region. During the first 4 years of surveillance, we reported on PD and its associated pathogens,[6] followed by an analysis of community-wide improvements in the incidence of diarrhea and prevalence of malnutrition, due, in part, to intense surveillance and an increase in exclusive breastfeeding.[12] Now, with over a decade of cohort data collected, we report herein that ProD accounts for significant morbidity and is a key predictor of PD, with distinct risk factors, etiologies, and associations with undernutrition.

Methods and Materials

Study site and design

The study was conducted from August 1989 to March 2000 in a 5-block area of a shantytown, Gonçalves Dias; located in Fortaleza, Brazil. Fortaleza (population 3.5 million) is the capital city of the northeastern state of Ceará (population 8.2 million). In 1994, Fortaleza's infant mortality was 59/1,000 live births. At the study's midpoint, Gonçalves Dias was a community of 1,826 people, 13.5% (247) of whom were children <5 years old. The study team identified

all pregnant women in the community and invited them to enroll in the project. Women choosing to participate provided informed consent and completed a detailed demographic and socioeconomic questionnaire with the assistance of a study nurse. In the first 45 months of the study, nurses visited the home of each newborn child three times weekly to record diarrheal illnesses. Thereafter, nurses visited homes twice weekly. At each visit, the study team also asked detailed information on the child's diet, including breastfeeding. Mothers (or guardians) of children with diarrhea were asked to give detailed clinical information about each illness, including accompanying symptoms and stool consistency and character. Study nurses visited children with diarrhea daily until 48 h after resolution of the illness.

We used World Health Organization (WHO) guidelines to define diarrhea as ≥3 looser than normal stools in the preceding 24-hr period.13 An episode of diarrhea was defined as lasting ≥1 day and separated from another episode by 2 or more days without diarrhea. Discrete episodes of diarrhea were classified by their total duration. Acute diarrhea (AD) was defined as an episode that lasted <7 days, a prolonged episode of acute diarrhea (ProD) as an episode that lasted ≥7 and <14 days, and persistent diarrhea (PD) as an episode that lasted ≥14 days. During the time period of the study, rotavirus vaccine was not yet available in Northeast Brazil, 14 nor had WHO recommendations for zinc supplementation during diarrhea been published; thus, participants did not receive these interventions. When children developed severe or bloody diarrhea (or other worrisome conditions), they were promptly referred by a study nurse to a pediatrician, who prescribed antimicrobials when appropriate. We did not; however, collect detailed data on children's use of medications. Human investigation committees at the Federal University of Ceará, the Johns Hopkins University School of Medicine, and the University of Virginia approved the study protocol.

Nutritional assessment

We measured weights and lengths of all children at 3-month intervals. A calibrated-sling Salter scale was used to obtain lightly-clothed weights to the nearest 0.1 kg. Lengths were measured supine to the nearest centimeter. We used NutStat (Epi Info version 3.5.1, Center for Disease Control and Prevention, Atlanta) to calculate weight-for-age, height-for-age, and weight-for-height Z scores (WAZ, HAZ, and WHZ, respectively); sex- and age-adjusted measures of the number of standard deviations above or below the median for the international reference population.[15]

Microbiologic studies

Detailed methods and materials for stool collection and examination have been described elsewhere.[6] Briefly, specimen cups were distributed to homes of children with diarrhea and caretakers were instructed to collect the first stool the following day. To provide controls, specimens were also obtained every 3 months from children who had no diarrhea for at least 14 days. Control specimens were not matched by age or season to diarrheal specimens. Stools were not collected from children who had ingested antibiotics in the previous 3 days. Specimens were transported on ice and processed within 4 h of collection.

All specimens were examined by microscopy for parasites and leukocytes. One aliquot from each specimen was frozen and stored for viral testing by use of ELISA and PCR assays. Stool samples were screened for Norwalk-like viruses by RT-PCR; rotavirus antigen by ELISA (Rotaclone; Cambridge Biotech, Worcester, MA); torovirus antigen by ELISA after sucrose partial purification of virus from stool by sucrose gradient purification; and adenovirus by ELISA (Adenoclone, Cambridge Biotech). Initial bacterial cultures were done by use of MacConkey's, xylose-lysinedeoxycholate, and Gram-negative broth enrichment and subculture, TCBS (thiosulfate, citrate, bile salt, and sucrose) agar, and ampicillin blood agar

plates (with 10 mg/mL ampicillin). Lactose-fermenting colonies were selected from MacConkey's agar plates and saved for further toxin, adherence, and serologic testing.

Statistical analyses

Statistical analyses were performed using SPSS software (version 17.0; SPSS, Chicago) and Epi-Info. Statistical tests included Spearman's rank correlation for age of first ProD episodes with age at weaning; paired *t*-tests for comparison of nutritional status before and after a ProD episode; X^2 or Fisher's exact test for associations of pathogens with prolonged, persistent, acute, and non-diarrheal stools. All tests were 2-tailed, and *P* values <0.05 were considered statistically significant.

To determine whether early childhood ProD is a risk factor for subsequent PD, we used Kaplan-Meier analysis. Children were classified by whether or not they experienced ProD in their first year of life. Age in days at the time of a child's first episode of PD was used as the time variable. Children without PD were censored as they left the study or at the end of the surveillance period. The 8 children who developed PD prior to their first episode of ProD were excluded, giving a total of 406 children in the overall analysis. To account for possible confounders, we performed Cox regression to control for covariates associated ($P<0.20$) with persistent diarrhea. Covariates were identified by univariate analyses.

Variables considered for inclusion in the multivariate model included: birthweight, child gender, type of house, number of people in household, type of sanitation, mother's level of education, age at weaning from exclusive breastfeeding, and date of birth (day, month, and year). Of these, number of people in household, type of sanitation, mother's years of education, age at weaning from exclusive breastfeeding and birth date were associated at $P<0.20$ using appropriate univariate analyses. These variables were subsequently included as covariates in a series of Cox regression equations.

Results

We enrolled 414 children (193 males, 47%) during the study period. Children were followed a mean of 1,125 days, a median of 803.5 days (range, 10–3,769), and the cumulative number of days of observation was 465,953 (1,276 child-years). Overall, these children had 2.55 episodes of diarrhea per child-year (3,257 episodes per 1,276 child-years), spending an average of 10.4 days per year with diarrhea (13,724 days per 1,276 child-years). Of the 414 children in the cohort, 68 (16.4%) had no diarrhea recorded during the study; however, this subset was followed a median of only 83 days (range, 10 to 2194) compared to the median of 1,094 days (range, 24 to 3,769) for children with at least one recorded episode of diarrhea. The mean duration of a diarrheal episode was 4.2 days (SD, 5.2); the median duration was 3 days. Of 3,257 episodes, 2,722 (83.6%) were AD, 380 (11.7%) were ProD, and 155 (4.7%) were PD illnesses.

Figure 1 shows age-specific attack rates and days with diarrhea for total, AD, ProD, and PD episodes. Total, AD, and PD all peaked at 6-12 months of age (5.15, 4.22, and 0.68 episodes per child-year, respectively). PD peaked at 12-24 months of age (0.26 episodes per child-year). As shown in Figure 2, ProD accounted for only 11.7% (380/3,257) of diarrheal episodes but 25.2% (3463/13,724) of all days of diarrhea. Similarly, PD accounted for only 4.7% (155/3257) of episodes but 24.5% (3368/13,724) of days of diarrhea. Importantly, PD episodes accounted for 155 (29%) of the 535 diarrheal episodes ≥7 days in duration in our study. Thus, when a child's diarrheal episode progressed from acute to prolonged, the overall risk that the episode would evolve into PD increased from 4.8% to 29% (RR=6.09; 95% CI, 4.96-7.45).

Maternal education and early weaning were significant risk factors for ProD. Of the 210 children with >11 months of surveillance data in the first year of life, the 157 whose mothers did not complete primary school had a 2-fold higher incidence of prolonged diarrhea (relative risk [RR], 2.1; 95% confidence interval [CI] 1.02-2.78) than children whose mothers finished primary school. In the 112 infants with complete follow-up and ProD the first year of life, age at weaning from exclusive breastfeeding was positively correlated with age at first ProD episode (Spearman's ρ=0.309; P=0.005), i.e., early weaning was associated with earlier onset of ProD. Birthweight, family income, crowding, and lack of a toilet in the home were not significantly associated with ProD.

Table 1 shows etiologic agents found in stools collected from children with ProD and controls in the first four years of the study. We obtained 132 samples from a total of 130 ProD episodes (34% of all ProD episodes), as well as 442 control samples from children without diarrhea. *Cryptosporidium* and *Shigella* species were isolated more frequently from ProD than control specimens (P=0.0178 and P=0.0223, respectively). *Ascaris* ova were found more frequently in controls than in ProD specimens (P=0.008). To further identify etiologies specific to ProD, we compared ProD data with previous results for AD and PD from this same cohort of children.[5] ProD episodes were delineated from AD episodes to create four categories of specimens: AD (<7 days), ProD (≥7 and <14 days), PD (≥ 14 days), and control. *Cryptosporidium* was seen more frequently in ProD than AD specimens (P=0.023). *Giardia* was more common in PD than ProD episodes (P=0.012). No other significant differences between ProD and other categories were detected.

To evaluate the effects of ProD on subsequent risk for PD we used Kaplan-Meier analysis and Cox regression. Time to first PD episode was compared for 112 children with ProD before age 1 year and 294 children with no ProD before age 1 year. Prior to the analysis, we noted that 57 (80.3%) of the 71 children who developed PD during the study also experienced ProD. Of these 57 children, 30 (52.6%) had ProD in their first year of life. Kaplan-Meier analysis showed that children with ProD before age 1 year were significantly more likely to develop PD later in childhood compared to those without ProD (Log Rank P=0.002). As shown in Figure 3, children who experienced at least one ProD episode of diarrhea before age 1 were nearly twice (15.5% (s.e.=2.5%)) versus 29.9% (s.e.=5.0) as likely to experience PD by age 2 years. Cox Regression showed (OR 2.2, 95% CI; [1.32–3.54], P=0.002) a similar overall increased risk of PD by age 6 years. ProD before age 1 year remained a significant (P=0.025) independent predictor of later PD even after number of people in household, type of sanitation, mother's years of education, age at weaning from exclusive breastfeeding, and birth date were included in the equation. Only date of birth was a significant (P=0.004) independent predictor of PD due to previously documented improvements over time in the incidence of PD in this cohort.[11]

The incidence of AD was significantly higher among the 195 (47%) children who experienced at least one episode of ProD or PD (mean incidence of 3.12 (s.d=2.3) AD episodes/child-year) versus children who experienced only AD or no diarrhea (mean incidence 1.91 (s.d.=2.7) AD episodes/child-year; P<0001, unpaired *t* test). Only 11 children experienced ProD and/or PD with no episodes of AD.

Figure 4 shows the impact of AD (duration, <7 days), ProD, and PD illnesses on a child's anthropometry. To determine changes in nutritional status after a diarrheal illness, we identified children's first AD, ProD, and PD episodes and compared anthropometric Z scores 3 months prior to and following the episode. We found significant decreases in WAZ (-0.19 to -0.45, P=0.0001) and HAZ (-0.81 to -1.40, P=0.0002) in the period after children's first ProD illness. Similarly, first AD episodes were followed by declines in WAZ (0.02 to -0.28, P<0·0001) and

HAZ (-0.51 to -0.82, $P<0.0001$). Furthermore, children's first PD illnesses were accompanied by declines in both WAZ (-0.46 to -0.74, $P=0{\cdot}0012$) and WHZ (0.44 to 0.17, $P=0.0015$).

We also noted striking downward trends in WAZ and HAZ as children progressed from first AD to ProD and PD episodes. Children's HAZ scores (Figure 4C) prior to ProD or PD episodes were significantly worse than children's HAZ before their first AD episode ($P<0{\cdot}05$). Likewise, WAZ prior to PD was significantly worse than WAZ prior to AD episodes (Figure 4B).

Discussion

In this decade-long cohort study of children followed from birth in an urban Brazilian shantytown, we found that ProD accounts for significant morbidity and is associated with early weaning, low maternal schooling, and undernutrition. Furthermore, we showed that ProD in infancy is a strong indicator of children's future risk of PD. To our knowledge, this is the first study to examine ProD as a distinct class of diarrhea, thus our findings have several important public health implications that merit further consideration.

First, although ProD and PD comprise only a small portion of diarrheal episodes (12% and 5%, respectively), together they account for half of all days of diarrhea. In addition, once an acute episode of diarrhea progresses to ProD, the relative risk that the episode will evolve into PD is 6-fold higher. These two findings suggest that targeted interventions to halt the progression from AD to ProD would not only mitigate PD and its consequences, but have a substantial impact on overall diarrhea burdens as well.

Our second key finding is that infants with ProD have a 2.2-fold higher risk of developing PD in later childhood. In our analysis, ProD remains a significant risk factor for PD even after controlling for a number of confounders. ProD may therefore identify infants whose pathogens, nutritional status, diet, environment, or genes predispose them to PD. Alternatively, ProD may play a direct role in the evolution of PD via effects on growth, innate immunity, disruption of intestinal barrier function, or alteration of gut flora. Regardless of its nature, ProD clearly highlights infants at risk of both PD and growth faltering who therefore warrant increased vigilance and support in order to prevent a vicious cycle of diarrhea and malnutrition.

Third, we find that lack of a primary school education and early weaning from exclusive breastfeeding are significant maternal risk factors for infantile ProD. Several studies from developing countries indicate that children of uneducated mothers are at increased risk of diarrhea.[16-18] Despite limited schooling, a number of educational campaigns have been shown to reduce the incidence of childhood diarrhea in these settings, e.g., handwashing interventions in Pakistan,[19, 20] and exclusive breastfeeding campaigns in India.[21] Our present study indicates that early weaning from exclusive breastfeeding was associated with earlier onset of ProD. This supports previous findings by our group and others that breastfeeding is highly protective against diarrhea and limits the duration of diarrheal illnesses.[6] Thus, promotion of breastfeeding must remain an essential component of diarrheal control programs.

Fourth, we demonstrate that a first episode of ProD is associated with undernutrition both before and after the illness. Prior to ProD, children's mean HAZ was already significantly below the WHO median. Following ProD, we see further declines in both HAZ (indicating stunting, a marker of chronic undernutrition) and WAZ (indicating underweight, a marker of more acute malnutrition). In addition, we find baseline decrements in HAZ and WAZ prior to both ProD and PD versus AD episodes. This trend is not seen for low WHZ (an indicator of wasting), which was less common in our study due to the number of children who were both underweight and stunted. We interpret these results as further evidence of a “vicious cycle” of diarrhea and malnutrition in which diarrhea causes undernutrition and, in turn, poor nutritional status predisposes to further, lengthier episodes of diarrhea. Importantly, these acute effects of ProD

on nutritional status likely have chronic implications for children's growth. Despite catch-up growth following diarrheal illnesses, our studies and a more recent multi-country analysis have shown that early childhood diarrhea remains highly predictive of stunting at age 2 years and beyond.[22, 23]

Finally, we found that *Shigella* and *Cryptosporidium* species were etiologies significantly associated with ProD episodes. *Shigella* is well-recognized as a major cause of dysentery in developing countries and has consistently been shown to associate with growth faltering, as well as ProD and PD.[24] Because of this impact and the worldwide emergence of antibiotic-resistant strains, WHO has targeted the development of a *Shigella* vaccine as a high priority.[25] Similarly, *Cryptosporidium* is increasingly recognized as an important cause of severe diarrhea and undernutrition in tropical, developing regions; however, existing therapies are only partially effective.[26] The antiparasitic drug nitazoxanide has demonstrated efficacy for childhood cryptosporidiosis,[27] however it is has not been shown effective for cryptosporidiosis in undernourished children or patients with HIV/AIDS.[28]

In addition to the pressing need for effective vaccines and therapies for *Shigella* and *Cryptosporidium*, recent WHO reports highlight more immediate approaches to control childhood diarrhea in developing countries, which are of course relevant to ProD. These include: 1) improved outcomes through rotavirus and measles vaccination; promotion of early breastfeeding and Vitamin A supplementation; promotion of handwashing with soap; improved water supply and quality, including household water treatment and safe storage of household water; and community-wide sanitation, and 2) improved management of diarrhea by scaling up implementation of low-osmolarity ORS and zinc supplementation worldwide.[2, 29]

Of note, *Ascaris* was found more frequently in control stools than in ProD specimens, but not AD specimens. We speculate that *Ascaris* might have a mitigating effect on the duration of intestinal infections by altering intestinal immune responses to diarrheal pathogens. Alternatively, ProD may purge *Ascaris* ova from the digestive tract, making them more difficult to identify in stools.

The major strengths of our study include a large sample of children followed intensively from birth for diarrheal, nutritional, and microbiologic surveillance over multiple seasons in a highly endemic setting. Our study was limited by several factors. First, we recorded substantial improvements over time in the incidence of diarrhea and undernutrition in this community—changes not seen in nearby shantytowns.12 Second, loss to follow-up was common and previous analyses suggest that dropouts were more likely to be underweight.[22] Therefore, our current results may represent a “best-case scenario”, which underestimates the true impact of ProD in communities where undernutrition and diarrhea morbidity remain endemic. Third, stools were collected only during the first four years of the study and only 34% of ProD episodes were sampled. Fourth, all stools obtained from children with ProD were examined for bacterial and parasitic pathogens; however only 26% of samples were tested for viral pathogens. Previous findings from our study site (in a different cohort of children) revealed that the use of antibiotics is very common, often inappropriate, and may be a risk factor for diarrhea.[30, 31] Lack of adequate data on antibiotic use in the current study prevented us from defining the extent to which antibiotics or other drugs may influence the risk of ProD or PD. Lastly, our study represents results from only one community and may not be generalizable across multiple settings. Several of these limitations will be addressed by the recently launched Global Enterics Multi-Center Study and MAL-ED Network, multi-country investigations of the complex interrelationships of malnutrition and a broader range of enteric infections.[32, 33]

There is limited consensus between pediatric and adult subspecialties on definitions of acute, prolonged, persistent, and chronic episodes of diarrhea. In general, infectious disease and pediatric GI texts use the WHO cutoff of ≥2 weeks to delineate persistent from acute episodes.[34,35] Some experts use "chronic" for illnesses lasting >30 days.36 Conversely, adult GI texts use chronic to describe episodes lasting >2 or >4 weeks.[37, 38] Because our data indicate that ProD identifies children at increased risk of PD and the vicious cycle of diarrhea and malnutrition, we propose that the current WHO classification of acute diarrhea as <14 days be revised as follows: 1) acute diarrhea, <7 days and 2) prolonged episodes of acute diarrhea, ≥7 and <14 days.

We conclude that ProD: 1) accounts for a substantial portion of diarrhea burdens in this tropical, developing setting; 2) robustly predicts persistent diarrhea; and 3) associates with undernutrition, growth faltering, early weaning, and low maternal educational status. Further studies are needed to assess the role of ProD in the pathogenesis of persistent diarrhea, as well as the recognition, prevention and treatment of ProD in resource-limited settings, with the overall goal of preventing persistent diarrhea, as well as long-term growth and neurodevelopmental deficits in at-risk children.

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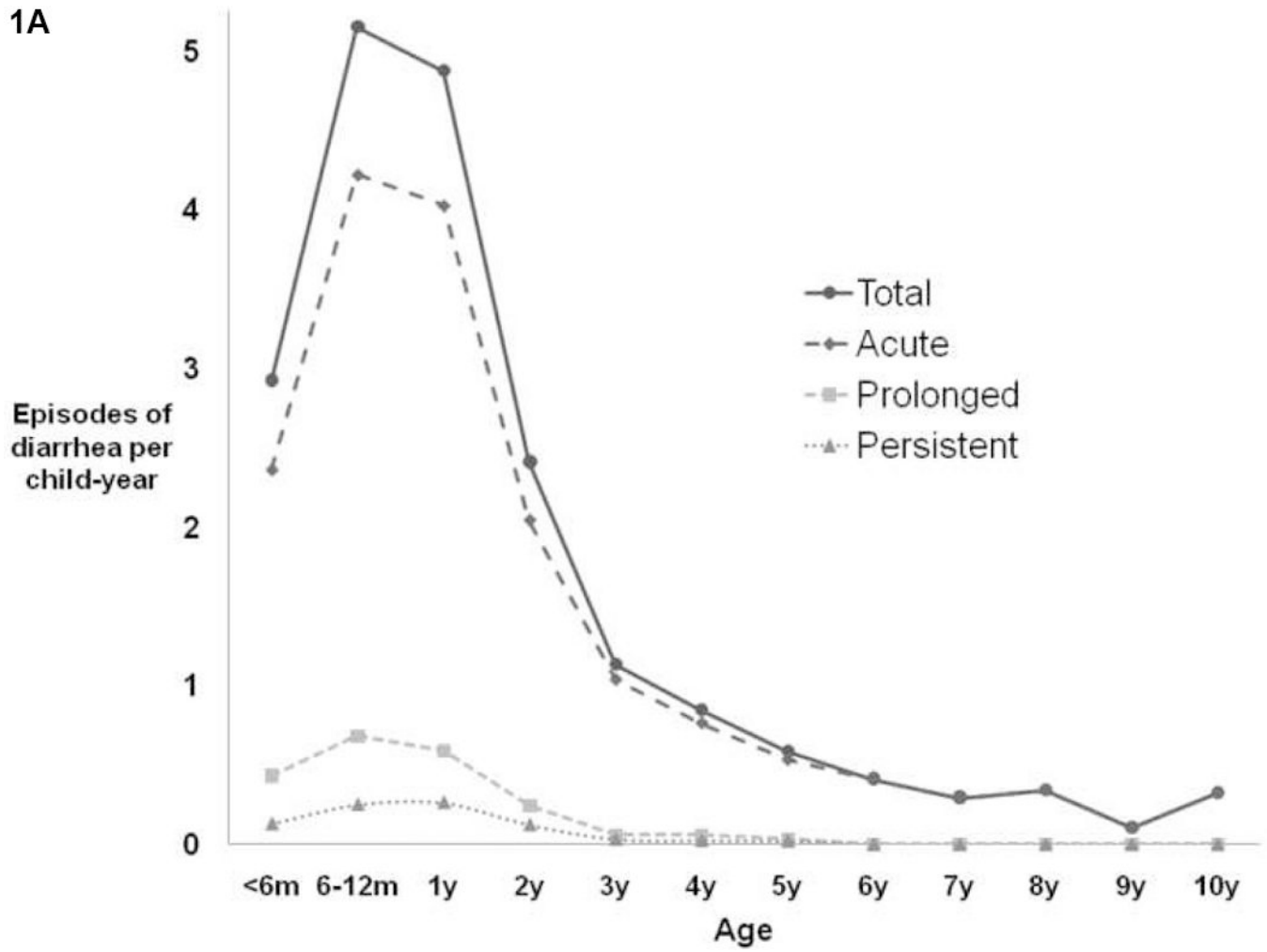
Abbreviations

AD	acute diarrhea
ProD	prolonged episodes of acute diarrhea
PD	persistent diarrhea
HAZ	height-for-age Z score
WAZ	weight-for-age Z score
WHZ	weight-for-height Z score
LT	heat labile
ST	heat stable
EPEC	enteropathogenic E. coli
EAggEC	enteroaggregative E. coli
EIEC	enteroinvasive E. coli



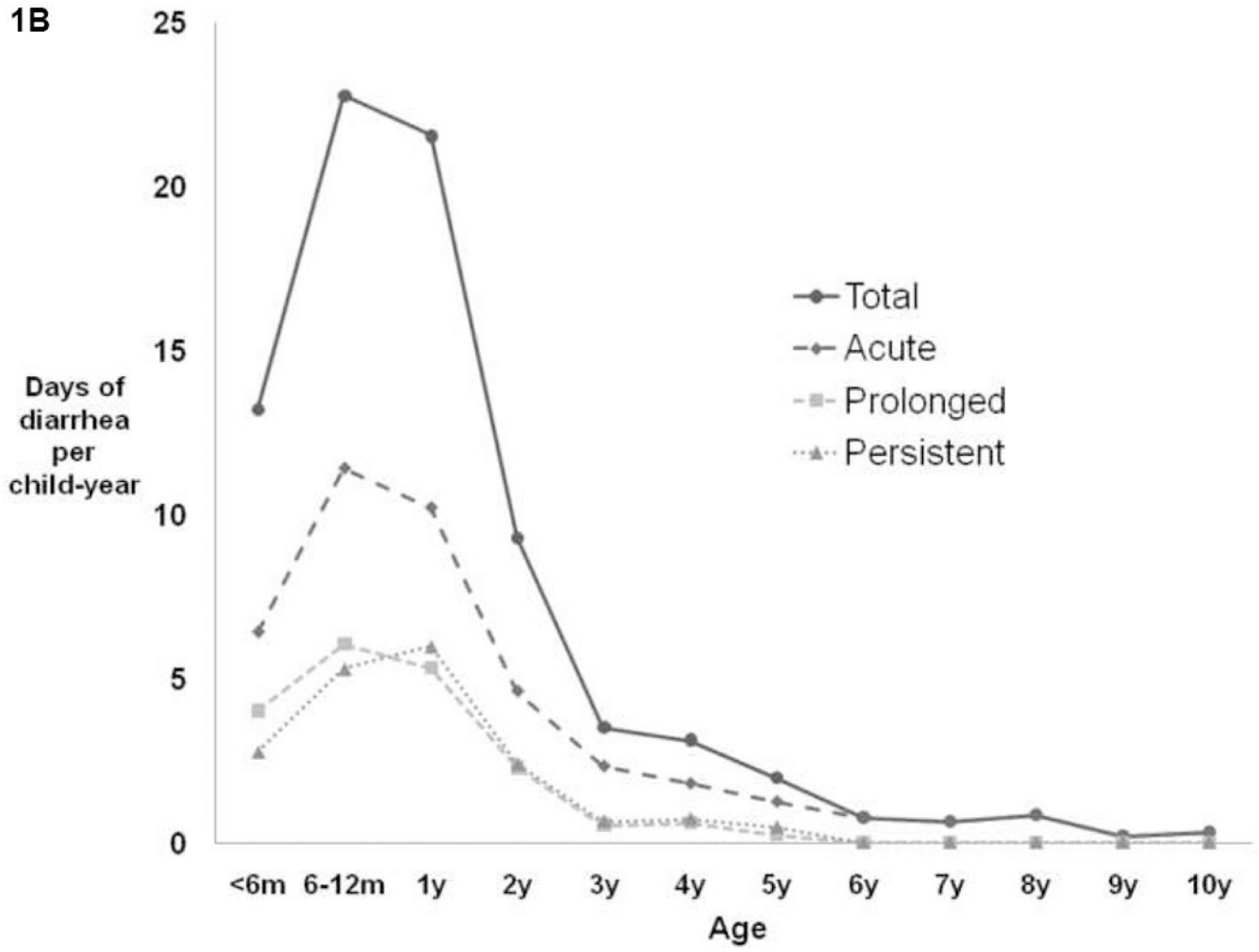


Figure 1.

A. Diarrhea attack rates of acute (<7 days), prolonged (≥7 and <14 days), and persistent (≥14 days) episodes per child-year, by age, among 414 children in Gonçalves Dias in Fortaleza, Brazil, from August 1989 through March 2000. **B.** Days with diarrhea per child-year, by age. Episodes and days of diarrhea for prolonged and persistent diarrhea peaked at 6-12 and 12–24 months of age, respectively.

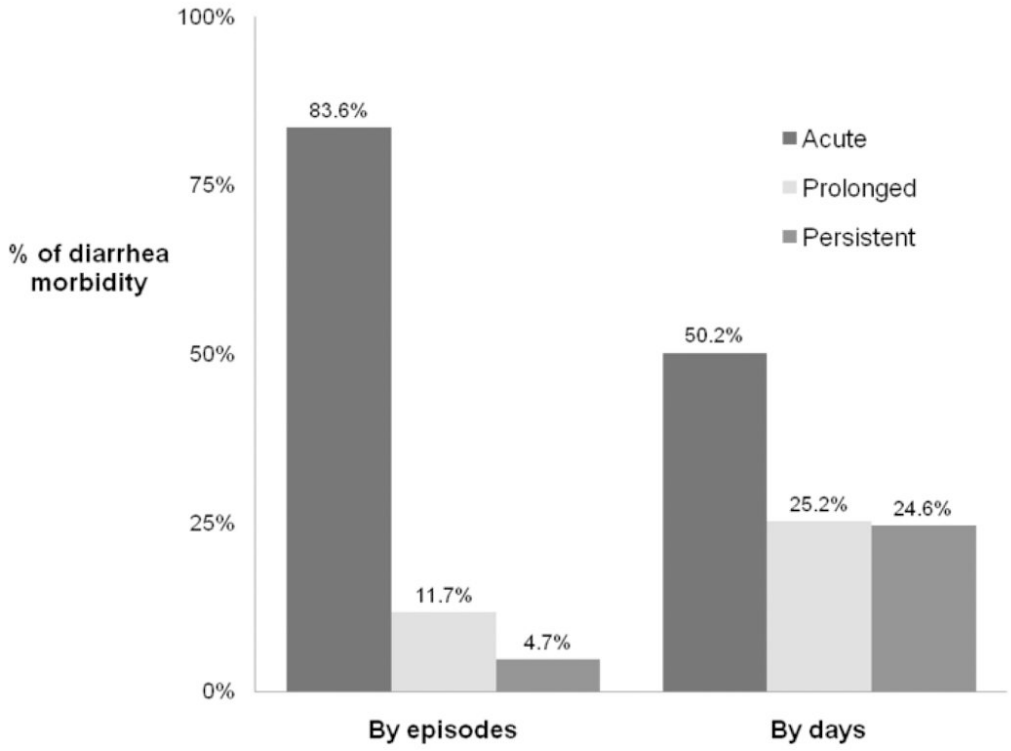


Figure 2.
Proportions of total diarrhea morbidity accounted for by acute (<7 days), prolonged (≥7 and <14 days), and persistent (≥14 days) episodes and by days of illness.

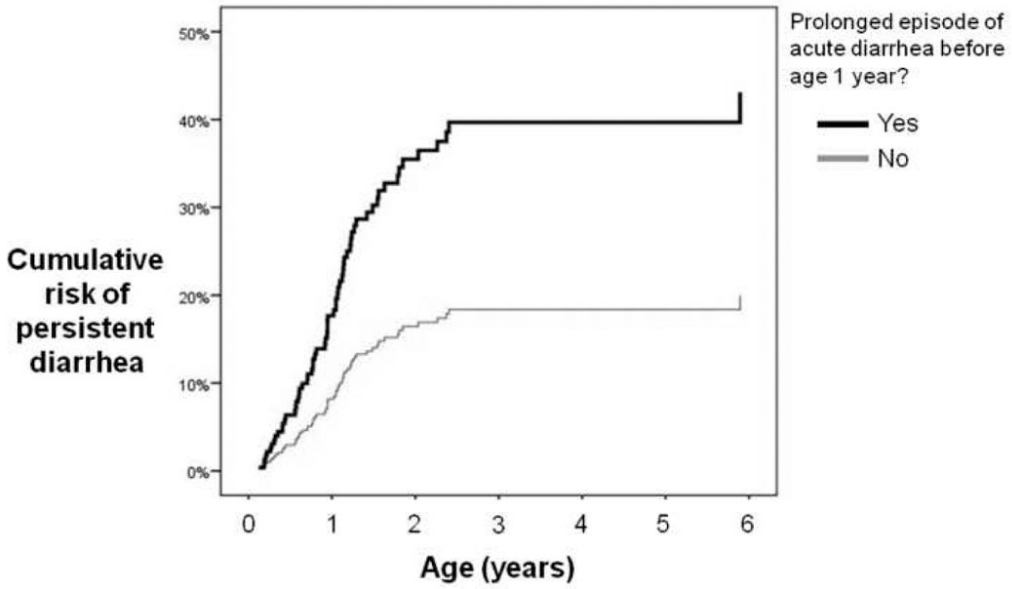


Figure 3.

Proportional hazard curves of time to first persistent diarrheal (PD, duration ≥14 days) episode in children with (n=112) and without (n=294) a prolonged episode of diarrhea (ProD, duration ≥7 and <14 days) in the first year of life. Eight children who developed PD prior to their first episode of ProD were excluded. Children who experienced at least one ProD episode of diarrhea before age 1 were nearly twice (15.5% (s.e.=2.5%) versus 29.9% (s.e.=5.0) as likely to experience a PD episode by age 2 years. Cox Regression showed (OR 2.2, 95% CI; [1.32-3.54], *P*=0.002) a similar overall increased risk of PD by age 6 years. ProD before age 1 year remained a significant (*P* =0.025) independent predictor of later PD even after controlling for household crowding, type of sanitation, maternal education, age at weaning from exclusive breastfeeding, and birth date.

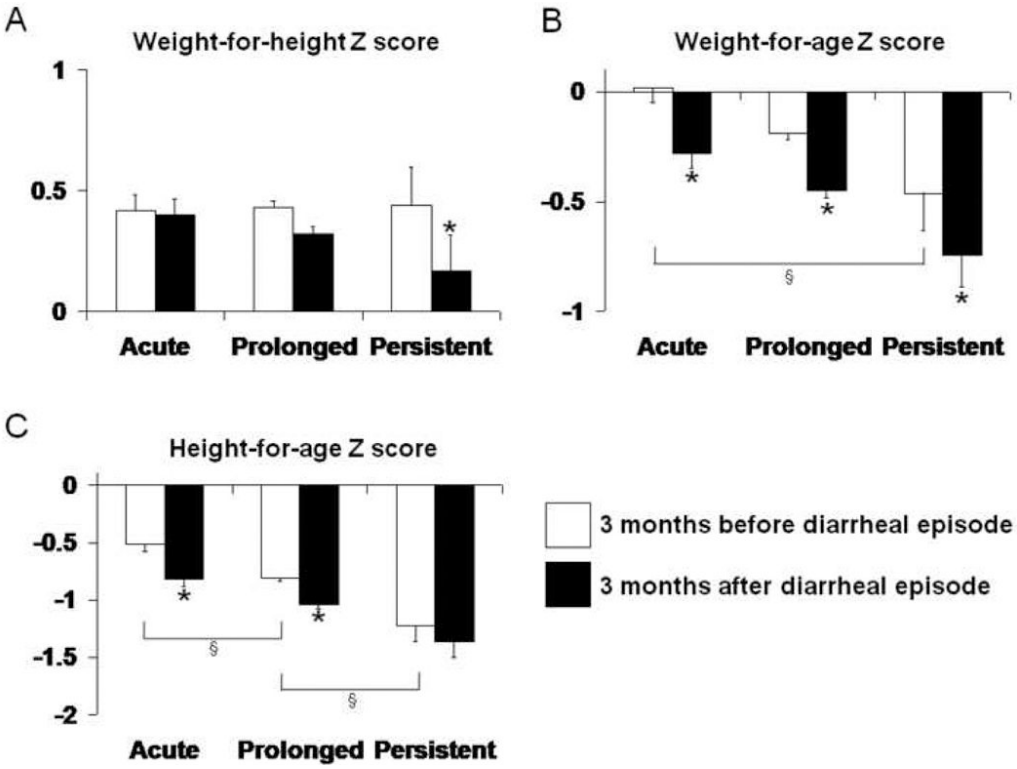


Figure 4.

Impact of acute (<7 days), prolonged (≥7 and <14 days), and persistent (≥14 days) diarrhea on anthropometry. Nutritional Z-scores were compared 3 months before and after children's first acute (n=308), prolonged (n=145), and persistent (n=62) episodes. **A**. Weight-for-height Z (WHZ) scores declined significantly following persistent episodes, but not acute or prolonged episodes. **B.** Weight-for-age Z (WAZ) scores decreased with all episodes types and mean WAZ prior to acute diarrhea was significantly greater than mean WAZ prior to persistent diarrhea. **C.** Height-for-age Z (HAZ) scores declined following acute and prolonged episodes, but not persistent episodes. HAZ prior to acute diarrhea was greater than HAZ prior to prolonged or persistent diarrhea. (Error bars indicate SEM; **P*<0.005, paired *t*-test of Z scores before and after diarrhea; §*P*<0.05, unpaired *t*-test of Z scores prior to acute vs. prolonged, acute vs. persistent, or prolonged vs. persistent episodes.)

Table 1

Etiologic studies of 130 prolonged episodes of acute diarrhea (duration ≥7 and <14 days) among children in Fortaleza, Brazil.

	Prolonged Diarrhea[a]		Control[b]
Etiologic Agent	**% (no. with agent/no. tested)**	***P***	**% (no. with agent/no. tested)**
Virus			
Norwalk-like virus	38.5% (5/13)		24.0% (12/50)
Rotavirus	2.9% (1/34)		2.4% (2/82)
Torovirus	0.0% (0/5)		0.0% (0/17)
Adenovirus	5.9% (2/34)		0.0% (0/82)
Parasite (all pathogens)	21.2% (28/132)		25.1% (111/442)
Cryptosporidium species	12.2% (12/98)	0.0178	5.2% (15/289)
Giardia lamblia	6.8% (9/132)		7.9% (35/442)
Ascaris species	5.3% (7/132)	0.008	13.8% (61/442)
Trichuris species	4.5% (6/132)		7.7% (34/442)
Entamoeba histolytica	0.8% (1/132)		1.1% (5/442)
Strongyloides species	0.0% (0/132)		0.5% (2//442)
Microsporidium species	0.0% (0/8)		4.0% (1/25)
Bacteria (all pathogens)	12.1% (16/132)	<0.0001	1.8% (8/440)
Shigella species	4.5% (6/132)	0.0223	1.1% (5/440)
Salmonella species	0.0% (0/132)		0.2% (1/440)
Vibrio cholera	0.0% (0/132)		0.0% (0/440)
Vibrio parahemolyticus	0.8% (1/132)		0.0% (0/440)
Yersinia species	0.8% (1/132)		0.0% (0/440)
LT or ST *E. Coli*	6.1% (8/132)		7.2% (14/195)
LT *E. Coli*	3.0% (4/132)		5.1% (10/195)
ST *E. Coli*	3.0% (4/132)		2.0% (4/195)
EPEC EAE probe	4.4% (4/90)		7.0% (14/195)
EAggEC (Hep-2)	31.1% (23/74)		35.0% (64/184)
AA probe	5.6% (5/90)		10.0% (19/195)
DAEC	6.7% (6/90)		8.0% (9/112)
EHEC probe	0.0% (0/90)		0.0% (0/112)
EIEC	1.2% (1/84)		0.0% (0/112)
Fecal leukocytes	50.8% (67/132)	<0.0001	6.3% (28/442)
Lactoferrin			
All	77.1% (27/35)	0.0047	49.5% (47/95)
Mild	45.7% (16/35)	0.0102	25.3% (24/95)
High	31.4% (11/35)		24.2% (23/95)
Any pathogen	74.5% (73/98)		73.0% (143/194)
Multiple pathogens	26.5% (26/98)	0.0058	40.7% (82/194)

Data represent the presence or absence of specified etiologies in case or control fecal specimens. *P* values are for differences between controls and cases. LT, heat labile; ST, heat stable; EPEC, enteropathogenic E. coli; EAggEC, enteroaggregative E. coli, EIEC, enteroinvasive E. coli.

[a] Includes 132 specimens representing 130 illnesses.

[b] Includes 442 control specimens.