

## Fecal Markers of Environmental Enteropathy Are Associated with Animal Exposure and Caregiver Hygiene in Bangladesh

Christine Marie George,\* Lauren Oldja, Shwapon K. Biswas, Jamie Perin, Gwenyth O. Lee, Shah Nawaz Ahmed, Rashidul Haque, R. Bradley Sack, Tahmina Parvin, Ishrat J. Azmi, Sazzadul Islam Bhuyian, Kaiser A. Talukder, and Abu G. Faruque

Johns Hopkins University, Baltimore, Maryland; International Center for Diarrhoeal Disease Research, Bangladesh (icddr), Dhaka, Bangladesh

**Abstract.** Undernutrition is estimated to be an underlying cause of over half of all deaths in young children globally. There is a growing body of literature suggesting that increased exposure to enteric pathogens is responsible for environmental enteropathy (EE), a disorder associated with impaired growth in children. To determine if household unsanitary environmental conditions were significantly associated with EE and stunting in children, we conducted a cohort of 216 children ( $\leq 30$  months) in rural Bangladesh. Stool was analyzed for four fecal markers of EE: alpha-1-antitrypsin, myeloperoxidase, and neopterin combined to form an EE disease activity score, and calprotectin. We observed a significant association between having an animal corral in a child's sleeping room and elevated EE scores (1.0 point difference, 95% confidence interval [CI]: 0.13, 1.88) and a two times higher odds of stunting (height-for-age z-score  $< -2$ ) (odds ratio [OR]: 2.53, 95% CI: 1.08, 5.43) after adjusting for potential confounders. In addition, children of caregivers with visibly soiled hands had significantly elevated fecal calprotectin ( $\mu\text{g/g}$ ) (384.1, 95% CI: 152.37, 615.83). These findings suggest that close contact with animals and caregiver hygiene may be important risk factors for EE in young children. These findings are consistent with the hypothesis that unsanitary environmental conditions can lead to EE in susceptible pediatric populations.

### INTRODUCTION

In 2010, 171 million children were estimated to be stunted globally, with nearly 98% of these children from low-income countries.<sup>1</sup> Undernutrition is estimated to be an underlying cause of 53% of all deaths in young children globally.<sup>2</sup> This decline in nutritional status is thought to be greatest during the first 2 years of life.<sup>3</sup> A study of infants in Bangladesh found a 50% increase in stunting for children from 5 to 12 months of age.<sup>4</sup> Therefore, identifying risk factors for stunting in susceptible pediatric populations is of high priority.

There is a growing evidence base demonstrating an association between stunting and environmental enteropathy (EE).<sup>5–13</sup> This disorder is defined by abnormal intestinal morphology, including villous atrophy and crypt hyperplasia, which leads to reduced intestinal barrier function and increased inflammation.<sup>7,8,14</sup> EE is thought to arise from unsanitary environmental conditions that lead to repeated exposure to enteric pathogen causing chronic infections.<sup>15–18</sup> There is a growing body of literature suggesting that these chronic enteric infections alter intestinal structure and function in a manner that is suboptimal for child growth.<sup>7–9</sup> It is suspected that many of these infections in children are subclinical and that diarrhea only accounts for a small proportion of EE.<sup>9,16,19</sup> Consistent with the hypothesis that subclinical infections are an important contributor to growth deficits in children, Checkley and others found that asymptomatic cryptosporidiosis infections, although resulting in less weight loss in the month post infection than symptomatic infections, occurred twice as much, and therefore likely contributed greater overall to child growth deficits.<sup>19</sup> This is also consistent with findings from Lee and others who reported that asymptomatic *Campylobacter* infections were associated with significant reductions

in weight gain over a 3-month period.<sup>20</sup> In rural Gambia infants had diarrhea 7.3% of the time, however, elevated lactulose:mannitol (L:M) ratios in urine, a measure of impaired intestinal barrier function, were observed in infants 76% of the time. Furthermore, it was estimated that L:M ratios in this population could predict 43% of observed variation in length growth and 39% of weight growth.<sup>9</sup> These findings suggest that EE may be representative of long periods of intestinal damage from assaults by enteric pathogens that cannot be explained by diarrhea episodes alone.

The assessment of biopsies from endoscopy are considered to be the gold standard to assess EE.<sup>21</sup> However, because this method is invasive and often not feasible in the context of a research study in a low-income setting, surrogate measures of EE, which assess intestinal absorption and barrier function, are typically used.<sup>7,9,22</sup> There is a growing body of literature validating the use of fecal markers of EE.<sup>8,23–27</sup> Fecal alpha-1-antitrypsin, myeloperoxidase, and calprotectin have all been found to be noninvasive tests of intestinal inflammation when compared with biopsies from endoscopy in studies of inflammatory bowel disease and human immunodeficiency virus (HIV)-associated enteropathy.<sup>23,27,28</sup> A recent multisite study of pediatric populations found a significant association between fecal myeloperoxidase, alpha-1-antitrypsin, and neopterin concentrations and declines in length-for-age z-scores. In addition, in this study a novel method was developed for combining these three fecal markers to form an EE disease activity score to account for the correlation between markers. This allowed the EE score to account for a greater degree of linear growth deficit than any marker alone.<sup>5</sup>

Despite the growing literature demonstrating that EE is associated with impaired growth in children, there is limited information on environmental risk factors for EE.<sup>5–13</sup> A previous study in Zimbabwe found that individuals of a lower economic status excreted significantly less xylose, a measure of intestinal absorption, than those individuals of a higher economic status.<sup>18</sup> A more recent study in rural Bangladesh found that children residing in “contaminated” environments

\*Address correspondence to Christine Marie George, Department of International Health, Johns Hopkins University, 615 N. Wolfe Street, Room E5535, Baltimore, MD 21205-2103. E-mail: cmgeorge@jhsph.edu

defined by water quality, improved sanitation, and hygiene conditions had significantly higher L:M ratios in urine compared with children in “clean” environments.<sup>15</sup> However, these studies looked at two extremes and neither evaluated the impact of a single environmental condition such as caregiver hygiene on the risk of EE.

Our primary objective in conducting this study was to determine if household level unsanitary environmental conditions were associated with elevated fecal markers of EE and stunting in children less than 5 years of age. We hypothesized that modifiable unsanitary environmental conditions are responsible for EE and stunting in pediatric populations in low-income countries through increased exposure to enteric pathogens.

## METHODS

**Ethical approval.** Informed consent was obtained from a parent or guardian of all study participants, and study procedures were approved by the research ethics committees of the International Center for Diarrheal Disease Research, Bangladesh (icddr,b) and an exemption was obtained from the ethical review board at the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

**Study site and design.** This cross-sectional study of 216 randomly selected children 6–30 months of age was conducted in Mirzapur upazila in the Tangail district of Bangladesh at the site of the Global Enteric Multicenter Study (GEMS) demographic surveillance system (DSS). The GEMS DSS covers a population of 240,000. This study was nested within a larger investigation of the association between geophagy (mouthing of soil), EE, and stunting.<sup>51</sup> The sample size was based on the number of study participants that could be recruited from February to April 2014. The eligibility criteria for study households were that they had live chickens present on their compound. This was included to evaluate if toddlers consumed chicken feces, as previously reported.<sup>29,30</sup> Study participants 6–30 months of age were selected to target children most susceptible to growth faltering.<sup>3</sup> If the selected study child was reported by a caregiver to be sick during the initial household visit, a return visit was made once the child recovered. A total of three attempts were made to follow-up with each study participant.

**Measurements of environmental conditions in household.** Trained research assistants administered a questionnaire tool to caregivers on household demographic characteristics and conducted a spot check of household environmental conditions according to previously published methods.<sup>31</sup> This spot check included observing if soap was present at the household water source as a proxy measure of hand washing with soap practices (typically a tube well), the sleeping room floor type, presence of animals in and around the home, location of an animal corral (shelter where animals are held), and sanitation option type. An unimproved sanitation option was defined as no sanitation option, an open-pit latrine, a pit latrine with broken slab, a bucket toilet, or a hanging toilet.

**Hand cleanliness check.** A check of child and caregiver hand cleanliness was conducted, according to previously published methods.<sup>31</sup> This indicator was used as a proxy measure of child and caregiver hygiene practices. For the hand cleanliness check, research assistants assessed the respondent’s fingernails, finger pads, and palms on both the left and right hand for cleanliness and assigned one of the following

codes for each part of the hand: visible dirt, unclean appearance, and clean appearance. An intensive training was conducted on how to assess hand cleanliness before the study was conducted. For this analysis, a child or caregiver with “visibly soiled hands” was defined as an individual with a code of visible dirt for all parts of the hand (e.g., finger pads, nails, and palms).

**Stool collection and anthropometric measurements.** Research assistants also collected each child’s stool and measured their weight once and height three times, measurements were averaged for standardization. These measurements were used to calculate z-scores according to the World Health Organization (WHO) child growth standards.<sup>32</sup>

**Laboratory analysis.** All stool samples collected were transported in cooler boxes to the Enteric Microbiology Laboratory at icddr,b in Dhaka, Bangladesh, and stored at  $-80^{\circ}\text{C}$  until analysis. Alpha-1-antitrypsin (Biovendor, Asheville, NC), neopterin (Genway, San Diego, CA), and calprotectin (ALPCO, Salem, NH) enzyme-linked immunosorbent assay (ELISA) kits were run for sample analysis according to the package insert. Myeloperoxidase (ALPCO) ELISA kits were also run according to the manufacturer specified instructions, except for a 1:500 dilution used for initial runs.

The EE disease activity score was calculated using fecal myeloperoxidase, alpha-1-antitrypsin, and neopterin, according to previously published methods.<sup>5</sup> For each of these three markers the following categories were assigned: 0 points for concentrations < 25th percentile, 1 point for a concentrations between the 25th and 75th percentile, and 2 points for a value > 75th percentile. The EE score was then calculated using the following formula:  $2 \times (\text{alpha-1-antitrypsin category}) + 2 \times (\text{myeloperoxidase category}) + 1 \times (\text{neopterin category})$ . Percentiles for fecal markers were calculated based on the collected study data. Possible EE disease activity scores can range from 0 to 10 points.

**Statistical analysis.** Our primary objective in conducting this study was to determine if unsanitary environmental conditions were significantly associated with elevated fecal markers of EE and stunting in children less than 5 years of age. Therefore, our primary study outcomes were calprotectin, EE disease activity score, and low height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) z-scores, using the WHO Global Database on Child Growth and Malnutrition z-score cutoff point of <  $-2$  standard deviations (SDs).<sup>33</sup> Our measurements of unsanitary environmental conditions were the presence of an animal corral in the sleeping room of the household, unimproved sanitation (defined as no sanitation option, open-pit latrine, latrine with broken slab, bucket, or hanging toilet), no soap near the tube well (as a proxy measure of hand washing with soap behavior), caregivers and study children with visibly soiled hands based on the hand cleanliness check, and an earth floor in the study child’s sleeping room. To assess the association between unsanitary environmental conditions and the selected fecal makers of EE, linear regression models were used with calprotectin and EE disease activity score as the outcomes, and our measures of unsanitary environmental conditions as predictors. To assess the association between unsanitary environmental conditions and HAZ, WAZ, and WHZ, logistic regression models were conducted where the binary outcomes were the proportion of children with low HAZ, WAZ, and WHZ values, <  $-2$  SDs, and our measures of unsanitary environmental conditions as

predictors.<sup>33</sup> A sub-analysis was also conducted where each of the markers comprising the EE disease activity score were examined individually for each outcome of interest. All models were adjusted for age based on previous studies that found significant associations between EE markers and age.<sup>5,6</sup> For our adjusted models, covariates were selected if their association with the outcome had significance < 0.2. Age, age squared, caregiver educational level, and family size all met these criteria.

## RESULTS

A total of 216 children were included in this analysis. Of the 324 children screened for eligibility, 99 children were excluded because they were not available, one child died, one child was ill and couldn't participate, and one child was excluded because the caregiver refused to participate in the study. Of the 222 children enrolled in the study, six were excluded from this analysis because their caregiver interview was incomplete. Fifty four percent of children were female, and the median age was 17 months (Table 1).

**Environmental conditions.** Seventy-six percent of the study households had an earth floor in their sleeping room and 23% had a concrete floor. Seventy eight percent of household reported having a cow on their compound, 50% had ducks, 24% had pigeons, 21% had goats, and all households had chickens. All households had an animal corral. Fourteen percent of households had animal corrals in their sleeping room, 52% in a space adjacent to their sleeping room, and 34% in a separate structure from their sleeping room. For

households that reported having animals corrals in their sleeping room, 61% reported having chickens, 39% cows, 32% ducks, 25% pigeons, and 7% goats. All households except for one that reported a corral animal in their sleeping room had an earth floor. The majority of households (72%) had no soap next to their tubewell during a spot check of their home. Sixteen percent of households had an unimproved sanitation option. Twenty one percent of caregivers and 34% of children had visibly soiled hands. None of the Pearson correlation coefficients between household characteristics exceeded 0.40. The largest three correlations were between caregiver and child visibly soiled hands (0.33,  $P < 0.05$ ), no soap present near the household tube well and having an earth floor in ones sleeping room (0.25,  $P < 0.05$ ), and caregiver educational level and having an earth floor in ones sleeping room ( $-0.28$ ,  $P < 0.05$ ). All correlations can be found in Supplemental Table 1.

**Associations between environmental conditions and environmental enteropathy markers.** The median concentration for each EE marker was the following: 1,505.50 nmol/L for neopterin, 3,576.75 ng/mL for myeloperoxidase, 402.67  $\mu$ g/g for calprotectin, and 0.26 mg/g for alpha-1-antitrypsin. The median value for the EE disease activity score was 5. Children in households with an animal corral in the sleeping room had significantly higher EE scores (1.0 point difference, 95% confidence interval [CI]: 0.13, 1.88), after adjustment for age, age squared, caregiver educational level, and family size in the fully adjusted models (Table 2). Furthermore, children in households with caregivers found to have visibly soiled

TABLE 1  
Study population characteristics

Characteristics	%	N
Number of children	–	216
Female	54%	116
Age (months) (median $\pm$ SD [min–max])	17 $\pm$ 5.8 [6–30]	216
Anthropometric measurements		
Proportion WAZ < –2	22%	47
Proportion HAZ < –2	26%	56
Proportion WHZ < –2	6%	14
Fecal environmental enteropathy markers (median [25th, 75th percentile])		
Calprotectin ( $\mu$ g/g)	402.67 [193.37, 822.30]	216
EE score	5.00 [3.00, 7.00]	216
Alpha-1-antitrypsin (mg/g)	0.26 [0.16, 0.51]	216
Myeloperoxidase (ng/mL)	3,576.75 [1,969.50, 5,998.00]	216
Neopterin (nmol/L)	1,505.50 [572.00, 3,011.00]	216
Number of individuals living in household (median $\pm$ SD [min–max])	5 $\pm$ 1.9 [1–12]	216
Age of caregiver (years) (median $\pm$ SD [min–max])	25 $\pm$ 6.2 [17–52]	216
Caregiver educational level		
No formal education	10%	22
Primary school education	26%	57
Secondary education or higher	64%	137
Location of animal corral in household		
No animal corral	0%	0
Connected to sleeping room	14%	31
Adjacent to sleeping room	52%	113
Separated from house	34%	72
Floor type in sleeping room		
Earth	76%	164
Concrete	23%	49
Other	1%	3
No soap present near household tube well	72%	155
Unimproved household sanitation option*	16%	34
Caregiver with visibly soiled hands†	21%	45
Child with visibly soiled hands†	34%	74

HAZ = length/height-for-age z-score; WAZ = weight-for-age z-score; WHZ = weight-for-length/height z-score.

\*Unimproved sanitation (defined as no sanitation option, open-pit latrine, latrine with broken slab, bucket, or hanging toilet).

†Visibly soiled hands was defined as an individual with a code of visible dirt for all parts of the hand (e.g., finger pads, nails, and palms).

TABLE 2  
Association between environmental conditions and fecal EE markers

Environmental conditions	%	Total N	EE score coefficient (95% CI)		Calprotectin ( $\mu\text{g/g}$ ) coefficient (95% CI)	
			Age adjusted	Fully adjusted†	Age adjusted	Fully adjusted†
Animal corral in sleeping room	14	216	1.0 (0.14, 1.88)*	1.0 (0.13, 1.88)*	-168.35 (-446.56, 109.86)	-162.65 (-441.31, 116.01)
Caregiver with visibly soiled hands‡	21	216	0.01 (-0.74, 0.77)	0.05 (-0.70, 0.80)	385.74 (154.18, 617.30)*	384.10 (152.37, 615.83)*
Child with visibly soiled hands‡	34	216	-0.06 (-0.71, 0.58)	-0.09 (-0.73, 0.56)	101.01 (-102.11, 302.13)	106.92 (-96.29, 310.13)
Unimproved sanitation option§	16	216	-0.50 (-1.33, 0.34)	-0.52 (-1.37, 0.33)	-116.85 (-381.19, 147.49)	-116.77 (-385.02, 151.48)
No soap present near tubewell¶	72	216	-0.02 (-0.69, 0.66)	-0.03 (-0.72, 0.66)	19.01 (-195.09, 233.10)	-7.99 (-227.77, 211.79)
Earth floor in sleeping room	76	216	0.06 (-0.65, 0.77)	0.05 (-0.70, 0.81)	35.42 (-190.03, 260.88)	34.09 (-203.52, 271.70)

CI = confidence interval; EE = environmental enteropathy.

\*P value < 0.05.

†Fully adjusted models adjust for age, age squared, caregiver educational level, and family size.

‡Visibly soiled hands was defined as an individual with a code of visible dirt for all parts of the hand (e.g., finger pads, nails).

§Unimproved sanitation (defined as no sanitation option, open-pit latrine, latrine with broken slab, bucket, or hanging toilet).

¶Near is defined as within ten steps of the tubewell.

hands during the hand cleanliness check had significantly higher calprotectin concentrations (384.1  $\mu\text{g/g}$ , 95% CI: 152.37, 615.83) in the fully adjusted model. There were no other significant associations found between environmental conditions and fecal markers of EE.

**Associations between environmental conditions and anthropometric measurements.** Of the 216 study participants, 22% had low HAZ, 26% had low WAZ, and 6% had low WHZ. The odds of being stunted (HAZ < -2 SDs) was significantly higher for children in households with an animal corral in the sleeping room (odds ratio [OR]: 2.53, 95% CI: 1.08, 5.43) in the fully adjusted model and for children in households with an earth floor in their sleeping room in the age adjusted model (OR: 2.33, 95% CI: 1.01, 5.33) (Table 3). The odds of being underweight (WAZ < -2 SDs) were significantly higher for children with an earth floor in their sleeping room (OR: 3.07, 95% CI: 1.13, 8.34) in the fully adjusted model. There were no other significant associations found between environmental conditions and anthropometric measurements.

## DISCUSSION

To our knowledge, this is the first study to show a significant association between fecal markers of EE and household environmental conditions. We found a significant association between children with animal corrals in their sleeping room

and elevated EE disease activity scores and stunting, after adjusting for potential confounders. In addition, caregiver hand cleanliness was significantly associated with calprotectin concentrations. We also found the odds of being underweight were significantly higher for children with earth floors in their sleeping rooms. These findings suggest that close contact with animals and poor caregiver hygiene are significant risk factors for EE in young children. This result is consistent with the hypothesis that unsanitary environmental conditions can potentially lead to EE and stunting in susceptible pediatric populations.

Children with animal corrals in their sleeping room had significantly elevated EE scores and an increased odds of stunting, even after adjustment for potential confounders such as caregiver educational level and number of individuals living in the household. This is consistent with a recent study in Malawi that found sleeping with animals was associated with impaired growth in young children.<sup>10</sup> We suspect that corralling animals in the sleeping space of the home resulted in increased exposure to enteric pathogens through increased direct contact with these animals and through fecal contamination on the household floor from animal feces. Previous studies have demonstrated that contact with domestic animals can be a transmission route for enteric pathogens.<sup>34-37</sup> These pathogens include *Campylobacter jejuni*, *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC), *Cryptosporidium parvum*, and *Salmonella enterica*. The most common animals found in the

TABLE 3  
Association between environmental conditions and anthropometric measurements

Outcome	%	Total N	Proportion WAZ < -2 OR (95% CI)		Proportion HAZ < -2 OR (95% CI)		Proportion WHZ OR (95% CI)	
			Age adjusted	Fully adjusted†	Age adjusted	Fully adjusted†	Age adjusted	Fully adjusted†
Animal corral in sleeping room	14	216	2.14 (0.93, 4.94)	2.14 (0.92, 4.98)	2.53 (1.14, 5.63)*	2.43 (1.08, 5.43)*	3.16 (0.90, 11.32)	3.63 (0.96, 13.66)
Caregiver with visibly soiled hands‡	21	216	1.03 (0.46, 2.27)	1.03 (0.46, 2.29)	1.38 (0.67, 2.85)	1.44 (0.69, 3.00)	1.57 (0.47, 5.30)	1.63 (0.47, 5.71)
Child with visibly soiled hands‡	34	216	0.96 (0.49, 1.91)	0.96 (0.48, 1.91)	1.17 (0.62, 2.21)	1.18 (0.62, 2.25)	0.78 (0.24, 2.60)	0.76 (0.22, 2.56)
Unimproved sanitation option§	16	216	1.11 (0.46, 2.65)	1.11 (0.46, 2.72)	1.97 (0.90, 4.29)	1.82 (0.82, 4.04)	1.53 (0.40, 5.84)	1.82 (0.45, 7.39)
No soap present near tubewell¶	72	216	1.11 (0.54, 2.26)	1.03 (0.49, 2.17)	0.71 (0.35, 1.45)	0.73 (0.35, 1.53)	1.48 (0.47, 4.63)	1.20 (0.36, 3.98)
Earth floor in sleeping room	76	216	2.59 (1.03, 6.51)*	3.07 (1.13, 8.34)*	2.33 (1.01, 5.33)*	2.18 (0.91, 5.19)	1.89 (0.41, 8.79)	2.76 (0.51, 14.85)

CI = confidence interval; HAZ = length/height-for-age z-score; OR = odds ratio; WAZ = weight-for-age z-score; WHZ = weight-for-length/height z-score.

\*P value < 0.05.

†Fully adjusted models adjust for age, age squared, caregiver educational level, and family size.

‡Visibly soiled hands was defined as an individual with a code of visible dirt for all parts of the hand (e.g., finger pads, nails, palms).

§Unimproved sanitation (defined as no sanitation option, open-pit latrine, latrine with broken slab, bucket, or hanging toilet).

¶Near is defined as within ten steps of the tubewell.

sleeping rooms of study households were chickens followed by cows, both of which have been implicated in zoonotic disease outbreaks, most commonly *Campylobacter* and diarrheagenic *E. coli*.<sup>37</sup>

A previous study in a Peruvian slum area found that young children living in households with domestic chickens frequently consumed animal feces and that this feces was a potential exposure route for *Campylobacter jejuni*.<sup>29,38</sup> Consistent with this finding another study in Peru found that children living in households with live chickens had significantly increased odds of having *Campylobacter jejuni*-associated diarrhea.<sup>39</sup> Furthermore, a later study in the same slum communities found that corralling these chickens, as was done in the sleeping space of our study households, lead to higher rates of *Campylobacter*-related diarrhea than households with free-ranging chickens.<sup>38</sup> These findings support our hypothesis that children living in households with animal corrals in their sleeping room had greater exposure to enteric pathogens through close contact with these animals. Future studies should analyze child and animal stool for the presence of enteric pathogens.

The significant association found between caregivers with visibly soiled hands and elevated EE in children is consistent with a recent study conducted in rural Bangladesh. This study found that children living in "contaminated" households had significantly higher L:M ratios compared with children in "clean" households with higher water quality and improved sanitation and hygiene conditions.<sup>15</sup> Furthermore, the finding is consistent with the larger body of literature indicating that improved hygiene practices can lower exposure to enteric pathogens, evidenced by reductions in diarrheal disease in children.<sup>40,41</sup> Hand cleanliness as a proxy measure of hand washing with soap behavior was evaluated in a previous study in rural Bangladesh. In this study, both caregiver and child hand cleanliness was significantly associated with the availability of water and spare soap at a hand washing location and the use of an improved sanitation option.<sup>31</sup> Our findings suggest that household hygiene practices may be an important determinant of EE in young children.

The lack of a significant association between environmental conditions and alpha-1-antitrypsin, myeloperoxidase, and neopterin is likely a reflection of our small sample size, and the EE score representing a more comprehensive measure of intestinal inflammation. Furthermore, Pickering and others found that bacterial hand contamination among mothers in Tanzania varied temporally based on household activities, therefore collecting information on hand cleanliness at a single time point likely limited our ability to fully assess caregiver and child hygiene in our study population.<sup>42</sup> Future studies should measure hand cleanliness at multiple time points, and collect measurements of bacterial hand contamination.

A previous study in an urban slum area of Nepal evaluated the impact of a hand washing with soap intervention on EE markers and growth among children 3–12 months of age. Although there was a 41% reduction in diarrhea morbidity over the 6-month intervention period when compared with the control arm, there was no significant change in growth or lactose:creatinine ratios, a measure of mucosal damage.<sup>43</sup> This finding suggests that hand washing with soap alone may not be sufficient to reduce EE markers in pediatric populations and that more comprehensive interventions are likely needed that include water treatment and improved sanitation.

In this study, we found that having an earth floor was significantly associated with being underweight. Earth floors, because they are made of soil and cannot be easily cleaned, can be a potential reservoir for enteric pathogens.<sup>44</sup> A study in Eritrea found that children living in households with earth floors had 30% more diarrhea than those with non-earth floors, after adjusting for socioeconomic status of the household.<sup>45</sup> Furthermore, there is a growing evidence base demonstrating that soil is a direct exposure route for pathogens that can increase the risk of enteric infections in young children.<sup>46–51</sup> In Kenya, young children putting soil into their mouth was significantly associated with helminth infections and diarrhea.<sup>47,52</sup> However, in this study, we did not find a significant association between earth floors and elevated EE markers, which would be expected if earth floors contributed to an increased risk of enteric infections. In rural Bangladesh, floor type is often a reflection of the socioeconomic status of the household, wealthier households typically have concrete floors compared with earth floors used by the majority of households (Christine Marie George, personal communication). Therefore it is possible that our observed association between earth floors and being underweight is a reflection of the lower socioeconomic status of these households. Future studies should investigate the association between the presence of enteric pathogens on earth floors and EE prospectively.

Our study had several important limitations. First, the cross-sectional study design prevented us from establishing causality. Second, we relied on information at one time point as a proxy measure of study children's exposure history. Third, we only obtained growth measurements at one time point. Therefore, we cannot make conclusions on the impact of environmental conditions on changes in growth over time. Fourth, we did not collect samples of animal feces or culture human feces samples collected. Therefore, we cannot determine if domestic animals on household compounds carried enteric pathogens that could pose a risk to human health. Finally, we lacked comparison data on established markers of intestinal inflammation such as the L:M test.

Animal corrals in the household sleeping room and caregiver hygiene were significantly associated with elevated EE markers in children in rural Bangladesh. These findings build on a recent study from this cohort that found a significant association between EE disease activity score and impaired growth.<sup>51</sup> Therefore, together these findings provide preliminary evidence to support the hypothesis that unsanitary environmental conditions are responsible for EE and stunting in pediatric populations in low-income countries. Future studies should evaluate the impact of environmental conditions on EE and growth prospectively. Furthermore, intervention approaches that target multiple routes of fecal oral transmission are needed to reduce exposure to enteric pathogens in young children who are most susceptible to growth faltering.

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Authors' addresses: Christine M. George, Lauren Oldja, Jamie Perin, Gwenyth O. Lee, and R. Bradley Sack, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, E-mails: cmgeorge@jhsph.edu, loldja@jhsph.edu, jperin@jhu.edu, glee35@jhu.edu, and rsack1@jhu.edu. Shwapon K. Biswas and Sazzadul Islam Bhuyian, Center for Communicable Diseases, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, E-mails: drskbiswas2004@yahoo.com and sazzadulislam@icddr.org. Shah Nawaz Ahmed and Tahmina Parvin, Centre for Nutrition and Food Security (CNFS), icddr,b, Dhaka, Bangladesh, E-mails: shahnawaz@icddr.org and tparvin@icddr.org. Rashidul Haque, icddr,b, Dhaka, Bangladesh, E-mail: rhaque@icddr.org. Ishrat J. Azmi and Kaisar A. Talukder, Enteric Microbiology Unit, Centre for Health and Population Research, icddr,b, Dhaka, Bangladesh, E-mails: ishratazmi@icddr.org and kaisar@icddr.org. Abu G. Faruque, Clinical Sciences Division, icddr,b, Dhaka, Bangladesh, E-mail: gfaruque@icddr.org.

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