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Geophagy Is Associated with Environmental Enteropathy and Stunting in Children in Rural Bangladesh

Christine Marie George,* Lauren Oldja, Shwapon Biswas, Jamie Perin, Gwenyth O. Lee, Margaret Kosek, R. Bradley Sack, Shahnawaz Ahmed, Rashidul Haque, Tahmina Parvin, Ishrat J. Azmi, Sazzadul Islam Bhuyian, Kaisar A. Talukder, Shahnaij Mohammad, and Abu G. Faruque

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

Abstract. There is a growing body of literature indicating an association between stunting and environmental enteropathy (EE), a disorder thought to be caused by repeated exposures to enteric pathogens. To investigate the relationship between exposure to enteric pathogens through geophagy, consumption of soil, EE, and stunting, we conducted a prospective cohort study of 216 children under 5 years of age in rural Bangladesh. Geophagy was assessed at baseline using 5 hour structured observation and caregiver reports. Stool was analyzed for fecal markers of intestinal inflammation: alpha-1-antitrypsin, myeloperoxidase, neopterin (all three combined to form an EE disease activity score), and calprotectin. Eighteen percent of children had observed geophagy events by structured observation and 28% had caregiver reported events in the past week. Nearly all households had *Escherichia coli* (97%) in soil, and 14% had diarrheagenic *E. coli*. Children with caregiver-reported geophagy had significantly higher EE scores (0.72 point difference, 95% confidence interval [CI]: 0.01, 1.42) and calprotectin concentrations (237.38 μg/g, 95% CI: 12.77, 462.00). Furthermore, at the 9-month follow-up the odds of being stunted (height-for-age z-score < –2) was double for children with caregiver-reported geophagy (odds ratio [OR]: 2.27, 95% CI: 1.14, 4.51). These findings suggest that geophagy in young children may be an important unrecognized risk factor for EE and stunting.

INTRODUCTION

Recent estimates by the World Health Organization (WHO) report that a quarter of children under 5 years of age are stunted globally.[1] There is a growing body of literature indicating an association between stunting and environmental enteropathy (EE), a disorder defined by abnormal intestinal morphology, reduced intestinal barrier function, and increased inflammation.[2–10] Although its etiology is not fully defined, EE is thought to be caused by unsanitary environmental conditions leading to repeated exposures to enteric pathogens.[11]

The gold standard to measure EE is endoscopy and biopsy, which is impractical in most low-income settings.[12] Therefore, generally markers of intestinal barrier function and absorptive capacity of the small intestine are used as surrogate measures.[4,6,13] Dual sugar permeability tests such as lactulose and mannitol are one such indicator of intestinal barrier disruption and absorptive capacity.[13] Two studies of infants in rural Gambia have found a significant association between growth faltering and lactulose and mannitol.[4,6] In Lunn and others, it was estimated that the lactulose: mannitol (L:M) ratio of infants could predict 43% of observed variation in length growth and 39% of weight growth.[6]

Fecal markers of EE have the advantage of being easier to collect in comparison to the 5-hour urine collection typically used for the dual sugar permeability tests.[6] Fecal calprotectin is one such marker that has been found in several studies to be a noninvasive means to assess intestinal inflammation.[14–17] Fecal alpha-1-antitrypsin and myeloperoxidase have also been found to be significantly higher in patients with inflammatory bowel disease confirmed by endoscopy.[18,19] In Campbell and others, fecal neopterin, thought to occur from cell-mediated inflammation in the intestinal tract, was found to be negatively associated with height and weight gains in infants.[5] Consistent with these findings, a recent multisite study of eight countries found a significant association between fecal myeloperoxidase, alpha-1-antitrypsin, and neopterin and declines in length-for-age z-scores.[2] In this study, these three markers were also combined to form an EE disease activity score (0–10 points). The EE disease activity score was formed to account for correlation between the fecal markers and was able to explain a greater degree of linear growth deficit than any marker alone.[2]

In the scientific literature, most studies focus on the fecal oral pathways for enteric infections described in the F Diagram (fluids, fingers, fields, flies, and food).[20] However, recent studies suggest that geophagy, defined as the consumption of soil, dirt, or mud, is frequent among young children and a potential risk factor for enteric infections.[21,22] A recent study in rural Zimbabwe found that infants observed using structured observation frequently consumed soil and feces, and that the majority of soil samples collected from child outdoor play areas had detectable *Escherichia coli*.[21] In Shivoga and others, geophagy was significantly associated with diarrhea in children under 5 years of age in rural Kenya. In this study, 37% of children had caregiver reports of geophagy. In two studies also conducted in Kenya, geophagy was associated with helminth infections in children.[23,24] This is consistent with the growing body of literature indicating that soil is an exposure route for enteric pathogens.[23–26] Furthermore, these findings suggest that geophagy may be a potential risk factor for EE and stunting in pediatric populations. However, there are very few published studies assessing geophagy in pediatric populations,[23–27] and none assessing the role of this behavior in EE or stunting.

We hypothesize that geophagy leads to impaired growth through increased childhood exposure to enteric pathogens from soil causing EE. In an initial attempt to test our hypothesis and to determine the extent of geophagy in pediatric populations in rural Bangladesh, we have conducted the first prospective cohort study to assess the relationship between geophagy, EE, and stunting in children under 5 years of age.

*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

METHODS

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

Study site. This study was conducted in rural Mirzapur subdistrict in the Tangail district of Bangladesh, located 60 km northwest of Dhaka, Bangladesh. Mirzapur is the Bangladesh site of the Global Enteric Multicenter Study (GEMS) demographic surveillance system (DSS). This DSS was started in 2007, and has a population size of approximately 240,000.

Study design. A prospective cohort study was conducted of 216 randomly selected children 6–30 months of age residing in the Mirzapur GEMS DSS area from February 2014 to November 2014. This study was a pilot investigation, therefore our sample size was based on the number of children we were able to recruit between February and April 2014. We focused on this age group to target the time when children are most susceptible to growth faltering.[28] In addition, a previous study found that children < 6 months of age were mostly inactive during structured observation of geophagy events.[21] The eligibility criteria for study households were that they had chickens present in their compound. We included this criteria to evaluate if small children in this population consumed chicken feces, as recently reported in rural Zimbabwe and previously in Peru.[21,29]

At baseline, a 5-hour structured observation session was conducted by a trained research assistant between 8:00 AM and 1:00 PM in the household of each enrolled child. A structured questionnaire tool was used to collect information on whether the child touched or mouthed soil, mud, clay, sand, or feces during the structured observation period. A geophagy event during the structured observation period was defined as a child putting soil, mud, clay, or sand directly into his/her mouth. Information was also collected on whether this soil was spit out by the child, the quantity of soil consumed, and the caregiver's response to the child's geophagy event. If a child put an object or food in his/her mouth *with* visible soil this was *not* recorded as a geophagy event. Two soil samples were also collected in the outdoor courtyard area where the enrolled child was observed playing in a subset of 128 randomly selected households.

A questionnaire was administered to the child's caregiver to obtain information on household demographic characteristics. Caregivers were also asked if they had observed their child putting soil, mud, clay, or sand directly into his/her mouth, a geophagy event, in the past week. In addition, information was recorded on caregiver reports of the daily frequency of geophagy events, the quantity of soil consumed, their response to the child's geophagy event, if they considered eating soil to be good for their child's health, and if feces was consumed by their child.

A stool sample was collected from each child at baseline, and research assistants trained in standardized anthropometry measured the child's weight once and height three times. Height and weight measurements were used to calculate z-scores according to the WHO child growth standards at a 9-month follow-up visit.[30] We defined low height-for-age z-scores (HAZ), weight-for-age z-scores (WAZ), and weight-for-height z-scores (WHZ) using the WHO Global Database on Child Growth and Malnutrition z-score cutoff point of < −2 standard deviations (SD).[31] A child was considered stunted if his/her HAZ was more than 2 SDs below the WHO growth standard.[31,32]

Laboratory analysis Stool samples were stored in cooler boxes upon collection, and transferred to the Enteric Microbiology Laboratory at icddr,b in Dhaka, where they were stored at −80°C until processing. Calprotectin (ALPCO, Salem, NH), alpha-1-antitrypsin (Biovendor, Asheville, NC), and neopterin (Genway, San Diego, CA) enzyme-linked immunosorbent assay (ELISA) kits were run for sample analysis according to the package insert. Myeloperoxidase ELISA kits (ALPCO) were also run according to the manufacturer specified instruction, except for a 1:500 dilution used for initial runs.

Fecal myeloperoxidase, alpha-1-antitrypsin, and neopterin results were combined to form an EE disease activity score (0–10 points) for each study participant, according to previously published methods.[2] For each of these three EE 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× (alpha-1-antitrypsin category) + 2× (myeloperoxidase category) + 1× (neopterin category).

Soil samples were stored in cool boxes upon collection and transported to the Enteric Microbiology Laboratory, where total *E. coli* counts were conducted immediately by bacterial culture, and diarrheagenic *E. coli* was detected using multiplex polymerase chain reaction (PCR), according to previously published methods.[33,34]

Statistical analysis. Our primary objective in conducting this study was to determine if geophagy was significantly associated with elevated markers of EE and stunting in children under 5 years of age. Therefore our primary study outcomes were calprotectin, EE disease activity score, and low HAZ (stunting), WAZ, and WHZ at follow-up. Geophagy was defined in two ways: 1) geophagy observed during the structured observation period (observed geophagy) and 2) a caregiver-reported geophagy event in the past week (caregiver-reported geophagy). To assess the association between geophagy and the selected fecal makers of EE, linear regression models were used with calprotectin and EE disease activity score as the outcomes and caregiver-reported and observed geophagy as the predictors. To assess the association between geophagy and HAZ, WAZ, WHZ at the 9-month follow-up, logistic regression models were used, where the binary outcomes were the proportion of children with low HAZ, WAZ, and WHZ values (< −2 SDs), and the predictors were observed and caregiver-reported geophagy.[31]

Our secondary objective of this analysis was to determine the association between elevated markers of EE and low HAZ, WAZ, WHZ at the 9-month follow-up. To assess this association, we conducted logistic regression models with the proportion of children with low HAZ, WAZ, and WHZ at follow-up as the binary outcomes and calprotectin and EE activity score as the predictors. 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 because of previous significant associations found between EE markers and age.[2,3] Covariates for the fully adjusted models 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. Spearman correlations were calculated between all EE markers. χ^2 tests were used to compare categorical variables between children with and without observed and caregiver-reported geophagy.

RESULTS

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, 6 were excluded from this analysis because their structured observation session and caregiver interview was incomplete. Therefore the total sample size for analysis was 216 children. The median age of study children was 17 months (range: 7–30), and 54% were female. The median number of individuals living in a household was 5 (range: 2–12). Ten percent of caregivers had no formal education and 63% had a secondary level of education or higher. Seventy-two percent of study households has an earth floor and 23% had a concrete floor. Eighty-eight percent of children were partially breast-fed and 12% were not breast-fed. All households had visible fecal matter present in the outdoor areas of their compound. At the 9-month follow-up, 11 out of 216 children (5%) were reported to have relocated outside the GEMS DSS area.

Geophagy events. Eighteen percent of children (N = 38) were observed to have at least one geophagy event during the 5-hour structured observation period (observed geophagy), and 28% (N = 60) were reported by a caregiver to have at least one geophagy event in the past week (caregiver-reported geophagy) (Table 1). Seventy-one percent (27/38) of children with observed geophagy events during the structured observation period also had caregiver-reported geophagy events in the past week. For children with more than one geophagy event during the structured observation period (N = 9), all observed geophagy events were also caregiver-reported geophagy events in the past week. Of the 38 children with an observed geophagy event, 21% were observed spitting out this soil. There was no significant difference in geophagy events by gender for observed (P = 0.39) or caregiver-reported (P = 0.33) geophagy events. Observed and caregiver-reported geophagy was the highest for children in the 6- to 12-month of age category, 34% and 50%, respectively. When study children 6–24 months of age were compared with children > 24 months of age, their rates of both observed geophagy (21% versus 5% [P = 0.015]) and caregiver-reported geophagy (33% versus 7% [P = 0.0009]) were significantly higher. All geophagy events, observed and caregiver reported, involved mouthing less than a child's handful of soil. Seventy-two percent of caregivers reported stopping their child if they tried to eat soil, while only 14% of caregivers were actually observed stopping their child from eating soil during the structured observation period. Nine percent of caregiver's reported that eating soil was good for their child's health. Only one child was observed consuming feces during the structured observation period, and 7% of children had caregiver reports of consuming feces in the past week.

Out of the 128 households where soils samples were collected in the study child's outdoor play area, 97% (124) had at least one sample with detectable *E. coli*, and 14% (N = 18) had at least one sample with detectable diarrheagenic *E. coli*. Out of the 20 soil samples found to have diarrheagenic *E. coli*: 20% (N = 4) were enteropathogenic (EPEC), 60% (N = 12) were enteroaggregative (EAEC), 10% (N = 2) were enterotoxigenic (ETEC), 10% (N = 2) enterohemorrhagic (EHEC),

TABLE 1
Characteristics of geophagy events*

	Total children (N)	Observed† N = 216 (%)	Caregiver reported‡ N = 216 (%)
Number of children with a geophagy event	216	18	28
Children with geophagy events by gender			
Female	100	16	25
Male	116	20	31
Children with geophagy events by age category			
6–12 Months	32	34	50
12–18 Months	84	24	32
18–24 Months	58	9	24
24–30 Months	42	5	7
Frequency of geophagy events during 5-hour structured observation period			
0 Events	178	83	–
1 Event	27	12	–
2–5 Events	11	5	–
> 5 Events	0	0	–
Caregiver reported daily frequency of child geophagy events in past week			
0 Events	156	–	72
< 1 Event daily	20	–	9
1 Event daily	8	–	4
2–5 Events daily	24	–	11
> 5 Events daily	8	–	4
Caregiver response during geophagy event§			
Stop the child from eating the soil	–	14	72
Wash child's hands with water	–	0	23
Nothing	–	83	0
Other	–	6	5

*Children with at least one reported geophagy event.
†Observed geophagy: geophagy observed during 5-hour structured observation.
‡Caregiver-reported geophagy: caregiver reporting a geophagy event in the past week.
§Observed geophagy (Total N = 38) and caregiver-reported geophagy (Total N = 60).

TABLE 2
Association between geophagy* and Fecal EE markers

Outcome	Median (25th, 75th percentile)	Total *N*	Observed geophagy† coefficient (95% CI)		Caregiver-reported geophagy‡ coefficient (95% CI)	
			Age adjusted	Fully adjusted§	Age adjusted	Fully adjusted§
Calprotectin (μg/g)	402.67 (193.37, 822.30)	216	12.87 (−250.56, 276.29)	17.98 (−246.88, 282.84)	226.67 (3.55, 449.79)‖	237.38 (12.77, 462.00)‖
EE score	5.00 (3.00, 7.00)	216	−0.45 (−1.28, 0.38)	−0.42 (−1.25, 0.42)	0.66 (−0.05, 1.37)	0.72 (0.01, 1.42)‖
Alpha-1-antitrypsin (mg/g)	0.26 (0.16, 0.51)	216	−0.01 (−0.16, 0.14)	−0.01 (−0.16, 0.14)	0.06 (−0.07, 0.19)	0.06 (−0.07, 0.19)
Myeloperoxidase (ng/mL)	3,576.75 (1,969.50, 5,998.00)	216	−1,798.66 (−3,910.48, 313.16)	−1,857.88 (−3,989.53, 273.77)	1,390.04 (−417.54, 3,197.63)	1,353.39 (−474.74, 3,181.52)
Neopterin (nmol/L)	1505.00 (572.00, 3,011.00)	216	−347.10 (−1,245.90, 551.69)	−398.11 (−1,294.70, 498.48)	−14.74 (−784.17, 754.69)	−72.83 (−841.61, 695.95)

CI = confidence interval; EE = environmental enteropathy.
* Children with at least one reported geophagy event.
† Observed geophagy: geophagy observed during 5-hour structured observation.
‡ Caregiver-reported geophagy: caregiver reporting a geophagy event in the past week.
§ Fully adjusted models adjust for age, age squared, caregiver educational level, and family size.
‖ *P* value < 0.05.

and 1 was enteroinvasive (EIEC). Of the 252 soil samples found to be positive for *E. coli*, the geometric mean was 7,028 colony forming unit (CFU)/g (range: 50–12,800,000 CFU/g).

Associations between geophagy and EE markers. The median concentration for each marker was the following: 402.67 μg/g for calprotectin, 0.26 mg/g for alpha-1-antitrypsin, 3,576.75 ng/mL for myeloperoxidase, and 1,505.00 nmol/L for neopterin (Table 2). The median value for the EE disease activity score was 5. Significant spearman correlation coefficients were found between alpha-1-antitrypsin and calprotectin (0.21), myeloperoxidase and calprotectin (0.55), and myeloperoxidase and alpha-1-antitrypsin (0.27) (Table 3). Children with observed geophagy during structured observation had no significant difference in their calprotectin concentrations (17.98 μg/g, 95% confidence interval [CI]: −246.88, 282.84) or EE scores (−0.42 point difference, 95% CI: −1.24, 0.42) when compared with children without observed geophagy, after adjustment for age, age squared, caregiver educational level, and family size in the fully adjusted models. However, there was a significant association found between caregiver-reported geophagy and elevated calprotectin concentrations (237.38 μg/g, 95% CI: 12.77, 462.00), and EE scores (0.72 point difference, 95% CI: 0.01, 1.42) in the fully adjusted models. There was no significant association found between observed or caregiver-reported geophagy and myeloperoxidase, alpha-1-antitrypsin, or neopterin concentrations. The presence of diarrheagenic *E. coli* in 18 out of 128 households where soil was collected was found to be significantly associated with elevated EE scores in study children in the age adjusted model (1.08 point difference, 95% CI: 0.002, 2.15), however only marginally significant for the fully adjusted model (1.08 point difference, 95% CI: −0.004, 2.16) (Supplemental Table 1). In addition, the presence of diarrheagenic *E. coli* in household soil was also significantly associated with elevated myeloperoxidase (4324.24 ng/mL, 95% CI: 1215.51, 7432.97) and neopterin concentrations (2165.63 nmol/L, 95% CI: 917.23, 3414.02) in the fully adjusted models (Supplemental Table 1). There was no significant association between calprotectin and the presence of diarrheagenic *E. coli* or *E. coli* counts and the four EE fecal markers measured.

Associations between follow-up anthropometric measurements and geophagy. Of the 205 study participants located for the 9-month follow-up, 34% had low HAZ, 27% had low WAZ, and 7% had low WHZ defined a score of < −2 SD (Table 4). The odds of being stunted (HAZ < −2 SDs) was not significantly higher for those children with observed geophagy (odds ratio [OR]: 1.81, 95% CI: 0.85, 3.89) in the fully adjusted model. However, the odds of being stunted was more than double for children with caregiver-reported geophagy (OR: 2.27, 95% CI: 1.14, 4.51)) in the fully adjusted model. There was no significant association found between observed or caregiver-reported geophagy and low WAZ or WHZ.

TABLE 3
Spearman correlation coefficients of CAL, AAT, MPO, NEO

	CAL	AAT	MPO	NEO
CAL	1	–	–	–
AAT	0.21*	1	–	–
MPO	0.55*	0.27*	1	–
NEO	−0.10	−0.04	0.08	1

CAL = calprotectin; AAT = alpha-1-antitrypsin; MPO = myeloperoxidase; NEO = neopterin.
* *P* value < 0.05.

TABLE 4
Association between geophagy and growth measurements at 9-month follow-up (N = 205)

		Observed geophagy OR (95% CI)†		Caregiver-reported geophagy OR (95% CI)‡	
Outcome	%	Age adjusted	Fully adjusted*	Age adjusted	Fully adjusted*
Proportion WAZ < –2	24	1.15 (0.49, 2.72)	1.14 (0.48, 2.71)	1.19 (0.56, 2.51)	1.17 (0.55, 2.49)
Proportion HAZ < –2	34	1.81 (0.85, 3.89)	1.89 (0.87, 4.09)	2.23 (1.23, 4.38)†	2.27 (1.14, 4.51)†
Proportion WHZ < –2	7	1.57 (0.45, 5.48)	1.59 (0.45, 5.64)	2.38 (0.76, 7.44)	2.22 (0.70, 7.08)

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.
*Fully adjusted models adjust for age, age squared, caregiver educational level, and family size.
†Observed geophagy: geophagy observed during 5-hour direct observation.
‡Caregiver-reported geophagy: caregiver reporting a geophagy event in the past week.

Associations between follow-up anthropometric measurements and EE markers. There was no significant association observed between baseline fecal markers of EE in the highest versus lowest quartile and stunting or wasting (WHZ < –2 SDs) at follow-up (Tables 5 and 6). However, the odds of being underweight (WAZ < –2 SD) was more than three times higher for children with EE scores in the highest versus lowest quartile (OR: 3.73, 95% CI: 1.38, 10.12), and three times higher for children with alpha-1-antitrypsin concentrations in the highest versus lowest quartile (OR: 3.17, 95% CI: 1.19, 8.43) in the fully adjusted models. There was no significant association observed between being underweight and calprotectin, myeloperoxidase, or neopterin concentrations. The regression models for the associations between fecal markers of EE by each quartile and anthropometric measurements are given in Supplemental Table 2.

DISCUSSION

To our knowledge this is the first study to assess the association between geophagy, EE, and stunting. We found a significant association between caregiver-reported geophagy in the past week and elevated EE disease activity scores and calprotectin. Furthermore, the odds of being stunted at our 9-month follow-up was more than double for children with caregiver-reported geophagy. Consistent with the hypothesis that geophagy can be a exposure route to enteric pathogens, all study households had visible fecal matter present in the outdoor areas where study children were observed playing, and 14% of soil samples collected had diarrheagenic *E. coli*, one of the most common enteric pathogens found in children under 5 years of age globally.[35] In addition, the presence of diarrheagenic *E. coli* was significantly associated with elevated EE scores, and myeloperoxidase and neopterin concentrations in study children. These study findings provide preliminary evidence to support the hypothesis that geophagy leads to impaired growth through increased childhood exposure to enteric pathogens causing EE.

The significant association we observed between EE disease activity score and fecal alpha-1-antitrypsin and being underweight is consistent with the growing body of literature demonstrating that EE is associated with impaired growth in children.[2–10] Furthermore, these findings provide further validation of the use of the EE score as composite marker of EE in children. The lack of a significant association between geophagy 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.

There were no significant associations found between geophagy observed during the 5-hour structured observation period and EE markers and stunting. We suspect this was due to the short duration of the 5-hour structured observation period only capturing a small fraction of actual geophagy events, and the infrequent occurrence of geophagy events in the population. Of the 60 children with caregiver-reported geophagy events in the past week, only half were observed by caregivers to have more than one geophagy event per day. Consistent with this finding, less than one-third of children with observed geophagy during the structured observation period exhibited this behavior more than once. Therefore, it is likely that a large proportion of children who regularly exhibit geophagy were missed during the 5-hour structured observation period.

Structured observation as a measure of geophagy in pediatric populations is cost and time intensive. Therefore, in this study, we investigated the use of caregiver reports of geophagy in the past week as a proxy measure of actual geophagy behavior. If caregivers were able to give accurate reports of child geophagy events in the past week, we expect that events identified by 5 hour structured observation would be included in caregiver-reported events in the past week in the majority of cases. Consistent with this, we found that 71% (27/38) of children with observed geophagy events during the structured observation period also had caregiver reports of this behavior. Furthermore, for children with more than one geophagy event during the structured observation period, all observed geophagy events during the structured observation period were also caregiver reported (9/9). These findings suggest that caregivers can give fairly accurate reports of child

TABLE 5
Association between anthropometric measurements and fecal markers of EE at 9-month follow-up (N = 205)

		Calprotectin OR (95% CI)*		EE score OR (95% CI)*	
Outcome	%	Age adjusted (Q1 vs. Q4)	Fully adjusted† (Q1 vs. Q4)	Age adjusted (Q1 vs. Q4)	Fully adjusted† (Q1 vs. Q4)
Proportion WAZ < –2	24	1.21 (0.43, 3.40)	1.27 (0.45, 3.59)	3.74 (1.38, 10.15)‡	3.73 (1.38, 10.12)‡
Proportion HAZ < –2	34	2.02 (0.77, 5.27)	2.25 (0.83, 6.10)	1.30 (0.54, 3.13)	1.32 (0.54, 3.22)
Proportion WHZ < –2	7	2.12 (0.20, 22.77)	2.56 (0.24, 27.81)	3.13 (0.57, 17.25)	3.18 (0.57, 17.73)

CI = confidence interval; EE = environmental enteropathy; HAZ = length/height-for-age z-score; OR = odds ratio; WAZ = weight-for-age z-score; WHZ = weight-for-length/height z-score.
*OR compares the first to the fourth quartile.
†Fully adjusted models adjust for age, age squared, caregiver educational level, and family size.
‡P value < 0.05.

TABLE 6

Association between anthropometric measurements and alpha-1-antitrypsin, myeloperoxidase, and neopterin at 9-month follow-up (N = 205)

		Alpha-1- antitrypsin OR (95% CI)*		Myeloperoxidase OR (95% CI)*		Neopterin OR (95% CI)*	
Outcome	%	Age adjusted (Q1 vs. Q4)	Fully adjusted† (Q1 vs. Q4)	Age adjusted (Q1 vs. Q4)	Fully adjusted† (Q1 vs. Q4)	Age adjusted (Q1 vs. Q4)	Fully adjusted† (Q1 vs. Q4)
Proportion WAZ < –2	24	3.11 (1.18, 8.17) ‡	3.17 (1.19, 8.43)‡	1.93 (0.70, 5.37)	1.92 (0.69, 5.32)	2.07 (0.86, 5.00)	2.17 (0.87, 5.38)
Proportion HAZ < –2	31	1.44 (0.62, 3.37)	1.42 (0.59, 3.39)	1.08 (0.44, 2.66)	1.16 (0.46, 2.88)	1.86 (0.83, 4.14)	1.57 (0.69, 3.59)
Proportion WHZ < –2	7	6.66 (0.76, 58.12)	7.41 (0.83, 66.49)	2.98 (0.30, 30.08)	3.21 (0.32, 32.35)	2.02 (0.47, 8.66)	1.91 (0.43, 8.56)

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.
*OR compares the first to the fourth quartile.
†Fully adjusted models adjust for age, age squared, caregiver educational level, and family size.
‡P value < 0.05.

geophagy events, particularly when these events are frequent. We recommend future studies use structured observation during multiple visits or over a longer duration to validate the use of caregiver reports of geophagy events.

Nearly one-third of study children were reported by caregivers to have consumed soil in the past week. Furthermore, 7% of children were reported by caregivers to be directly consuming fecal matter. Our findings are consistent with two previous studies that assessed child mouthing events using structured observation. An earlier study in Kenya found that 37% of children less than five years of age had caregiver reports of geophagy, and a recent study in rural Zimbabwe that used structured observation found that 13% of children 3–18 months of age had geophagy events and 9% consumed chicken feces.[21,22]

In our study, rates of caregiver-reported geophagy were the highest for children 6–24 months of age. We suspect that the high prevalence of geophagy behavior in this age group was attributed to mouthing behavior, which has been found to be most frequent in children < 2 years of age.[36,37] Mouthing behavior in children during the first 2 years of life is considered a normal part of the exploratory stage of child development when children put their hands, objects, and substances they have contact with into their mouths.[37–40] Consistent with this literature, study children 6–24 months of age had significantly higher caregiver reports of geophagy than children > 24 months of age (33% versus 7% [P = 0.0009]). Therefore, geophagy behavior in this age group is likely attributed to normal child exploratory development, rather than children intentionally seeking out soil as geophagy is typically defined for adults.[41]

Fourteen percent of households had detectable diarrheagenic *E. coli* in the soil where children were observed playing, and all study households had visible fecal matter in their household compound. This finding is consistent with those of Pickering and others who found diarrheagenic *E. coli* in the soil of household compounds in rural Tanzania.[42] We suspect this fecal contamination was from domestic animals that were present in household compounds and from improper disposal of feces from child defecation events. In a nested sub-study, we found that only 14% of feces from study children was disposed of in the toilet and that the majority (74%) was disposed of in a nearby field or waste ditch (Christine Marie George, personal communication). The EPEC, EHEC, EAEC, and ETEC pathotypes found in this study can all cause disease in both animals and humans, with EPEC and ETEC being most common in children under 5 years of age in low-income settings.[35,43] EAEC, the most common pathotype found in soil in our study, was also found to be a leading cause of moderate to severe diarrhea in children 12–23 months of age at our present field site in Mirzapur, in the recent GEMS study.[35] Furthermore, the presence of diarrheagenic *E. coli* in soil was significantly associated with an elevated EE score and elevated myeloperoxidase and neopterin concentrations. These study findings demonstrate that geophagy can be a direct exposure route for EAEC, EPEC, EHEC, and ETEC, and provides support for our proposed casual pathway by which fecal contamination in soil can lead to EE in children.

Our findings add to the growing body of literature demonstrating that soil is a direct exposure route for fecal pathogens, which can increase the risk for enteric infections in susceptible pediatric populations.[23–26,42] However, despite this growing evidence base, interventions to intervene upon this exposure route for pediatric populations are nonexistent. Furthermore, we found that in our study population that caregivers do little to prevent children from putting soil in their mouth. While 72% of caregivers reported stopping their child from eating soil, only 14% were actually observed doing so during the structured observation period. Interventions are urgently needed to stop young children from ingesting fecal bacteria from contaminated soils, particularly because geophagy is found to be highest during the stage of life when children are most susceptible to growth faltering.[28] One potential intervention that has been piloted in rural Zimbabwe is the use of playpens that provide a hygienic play space, which reduces child contact with microbiological contamination in their environment.[44] Future studies should evaluate the efficacy of this intervention in reducing exposure to enteric pathogens, EE, and stunting in susceptible pediatric populations.

Micronutrient deficiencies have previously been found to be associated with pica, craving and consumption of nonfood items in adult populations.[45,46] A recent meta-analysis found that pica was significantly associated with an increased risk for anemia and low hemoglobin, hematocrit, and plasma zinc.[27] However, there are no published studies that we are aware of that have been able to establish a causal relationship between geophagy and micronutrient status. Two randomized controlled trials conducted to determine the impact of iron supplementation on geophagy in children found no significant reduction in geophagy behavior among study children with supplementation.[47,48]

There have been a few studies assessing the association between EE and micronutrient status.[49–51] A cross-sectional study in Brazil found that children which received vitamin A and zinc supplementation had lower L:M ratios and higher HAZ scores.[49] Consistent with this finding, two studies in Malawi and Bangladesh found that zinc supplementation in children resulted in lower L:M ratios.[50,51] A cross-sectional

study in Guatemala found that children with low serum iron (< 40 μg/dL) had higher L:M ratios.[13] However, in contrast, a later randomized controlled trial in Zambia found that children who received iron supplementation had significantly higher L:M ratios.[52] Unfortunately, the present study did not include measures of micronutrient status, therefore we could not assess these associations in our study population. Future studies should evaluate the association between geophagy, EE, and micronutrient status prospectively.

This study has several limitations. First, we only have cross-sectional data on the relationship between geophagy and EE, and therefore we cannot demonstrate the causality of this association. Future studies should investigate causality through measuring growth and collecting EE markers at multiple time points. Second, we lack detailed histories of geophagy behaviors for children enrolled in the study. Instead we rely on structured observation at one time point and caregiver-reported geophagy events in the past week as a proxy measure of previous geophagy behavior. Therefore we may have misclassified the exposure histories of some study children, particularly children in the older age groups when geophagy is less common. Third, we lack comparison data on more established markers of intestinal inflammation such as the lactulose and mannitol test.[13] Finally, we lack data on the micronutrient status of study children, which has previously been found to be associated with pica.[27] Future studies should include hemoglobin, hematocrit, and plasma zinc to investigate the relationship between micronutrient status, geophagy, and EE.

Geophagy was significantly associated with EE and stunting a pediatric population in rural Bangladesh. These results provide preliminary evidence to support the hypothesis that geophagy leads to stunting through increased childhood exposure to enteric pathogens causing EE. Future studies are urgently needed to evaluate intervention approaches that can be used to prevent exposure to enteric pathogens through geophagy in susceptible pediatric populations.

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Authors' addresses: Christine M. George, Lauren Oldja, Jamie Perin, Gwenyth O. Lee, Margaret Kosek, and R. Bradley Sack, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, E-mails: cmgeorge@jhsph.edu, loldja@gmail.com, jperin@jhu.edu, golee@jhsph.edu, makosek@jhmi.edu, and rsack1@jhu.edu. Shwapon Biswas, Rashidul Haque, and Sazzadul Islam Bhuyian, Center for Communicable Diseases, icddr,b, Dhaka, Bangladesh, E-mails: drskbiswas2004@yahoo.com, rhaque@icddrb.org, and sazzadul.islam@icddrb.org. Shahnawaz Ahmed and Tahmina Parvin, Centre for Nutrition and Food Security (CNFS), icddr,b, Dhaka, Bangladesh, E-mails: shahnawz@icddrb.org and tparvin@icddrb.org. Ishrat J. Azmi and Kaisar A. Talukder, Enteric Microbiology Unit, Centre for Health and Population Research, icddr,b, Dhaka, Bangladesh, E-mails: ishratazmi@icddrb.org and kaisar@icddrb.org. Shahnaij Mohammad, Enteric Bacteriology and Epidemiology Unit, icddr,b, Dhaka, Bangladesh, E-mail: shahnawajbmb@gmail.com. Abu G. Faruque, Clinical Sciences Division, icddr,b, Dhaka, Bangladesh, E-mail: gfaruque@icddrb.org.

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