Mouthing of Soil Contaminated Objects is Associated with Environmental Enteropathy in Young Children

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Abstract

OBJECTIVE To characterise childhood mouthing behaviours and to investigate the association between object-to-mouth and food-to-mouth contacts, diarrhoea prevalence and environmental enteropathy.
METHODS A prospective cohort study was conducted of 216 children ≤30 months of age in rural Bangladesh. Mouthing contacts with soil and food and objects with visible soil were assessed by 5-h structured observation. Stool was analysed for four faecal markers of intestinal inflammation: alpha-1-antitrypsin, myeloperoxidase, neopterin and calprotectin.
RESULTS Overall 82% of children were observed mouthing soil, objects with visible soil, or food with visible soil during the structured observation period. Sixty percent of children were observed mouthing objects with visible soil, 63% were observed mouthing food with visible soil, and 18% were observed mouthing soil only. Children observed mouthing objects with visible soil had significantly elevated faecal calprotectin concentrations (206.81 μg/g, 95% confidence interval [CI]: 6.27, 407.36). There was also a marginally significant association between *Escherichia coli* counts in soil from a child's play space and the prevalence rate of diarrhoea (diarrhoea prevalence ratio: 2.03, 95% CI 0.97, 4.25).
CONCLUSION These findings provide further evidence to support the hypothesis that childhood mouthing behaviour in environments with faecal contamination can lead to environmental enteropathy in susceptible paediatric populations. Furthermore, these findings suggest that young children mouthing objects with soil, which occurred more frequently than soil directly, was an important exposure route to faecal pathogens and a risk factor for environmental enteropathy.

keywords mouthing, non-dietary ingestion, environmental exposure, child behaviour, diarrhoea, environmental enteropathy

Introduction

Exploratory behaviours play an important role in motor, perceptual, and cognitive development in infancy [1]. Mouthing is one of the primary exploratory behaviours that emerges during the first months of life [1, 2]. Sucking during breastfeeding or bottle-feeding is necessary for essential nutrient intake [3]. Infants explore their surroundings through placing objects in their mouth [2], which has been linked with the emergence of vocalisation [4]. While mouthing is crucial to early child development, it is an important pathway for paediatric exposures to environmental contaminants, rendering infants and young children vulnerable to exposure to pesticides [5–8], heavy metals [9–11], brominated flame retardants [12–14] and enteric pathogens [15–25].

The faecal-oral route as described in the F Diagram (fluids, fingers, fields, flies and food) is thought to be an important pathway for enteric infections [26]. However, for infants and young children, their unique activity patterns may contribute to additional exposures to enteric pathogens. In many low-income countries, infants and young children frequently come into contact with animal or human faeces and contaminated soil while crawling and playing outdoors [27–29]. A structured observation study conducted in rural Zimbabwe found that infants frequently ingested chicken faeces and soil through exploratory mouthing and that soil had high counts of *Escherichia coli* [28]. Another study estimating the relative contributions of hand-to-mouth contacts to faecal ingestion reported that more than 90% of faeces ingested daily by Tanzanian children were from mouthing behaviors [30].

There is a growing evidence base on the health implications of mouthing fomites (soil and objects). Studies have found geophagy, defined as the consumption of soil, dirt or mud, to be a risk factor for intestinal helminth infections in children [15–25]. In rural Kenya, caregiver-reported geophagy was significantly associated with increased incidence of diarrhoea [24], which remains one of the leading causes of death among children under 5 years of age globally [31]. In peri-urban Peru, caregiver-reported child ingestion of faeces and soil was significantly associated with diarrhoea incidence in children under 3 years of age [32]. In rural Bangladesh, geophagy was significantly associated with environmental enteropathy (EE) or environmental enteric dysfunction (EED) [33], and growth faltering in young children [34, 35]. Environmental enteropathy is a subclinical disorder of the small intestine marked by chronic inflammation, villous atrophy and crypt hyperplasia and impaired intestinal barrier function [36–45]. Chronic exposure to faecal pathogens is hypothesised to cause EE, and these morphological and functional changes of the small intestine are thought to result in malabsorption of nutrients, leading to growth failure [36–46].

This study was conducted to characterise childhood mouthing behaviours and to assess the association between fomite-to-mouth and food-to-mouth contacts, diarrhoea prevalence and EE in young children in rural Bangladesh.

Materials and methods

Study design

This prospective cohort study of 216 randomly selected children 6–30 months of age was conducted in Mirzapur subdistrict in the Tangail district of Bangladesh from February to November 2014. Our sample size was based on the number of children we were able to recruit between February and April 2014. Mirzapur is the Bangladesh site of the Global Enteric Multicenter Study (GEMS) demographic surveillance system (DSS), covering a population of approximately 240 000 [47]. Study participants 6–30 months of age were enrolled to target children most susceptible to growth faltering [48]. These children were randomly selected from our demographic surveillance area. At baseline, a trained research assistant conducted a 5-h structured observation session between 8:00 AM and 1:00 PM in the household of each enrolled child. A structured questionnaire tool was used to obtain information on whether the child touched or mouthed specific fomites and foods during the structured observation period and on demographic and environmental conditions in the household. Hand-to-object contact was defined as a child touching: (i) soil only, (ii) an object with visible soil, (iii) food with visible soil, (iv) food with visible faeces, (v) animal faeces, or (vi) human faeces during the structured observation period. 'Soil' refers to any unconsolidated earth material including soil, dirt, mud, sand, silt, and clay. Objects included sticks, leaves, plastic bottles, and garbage such as discarded wrappers, paper, and medicine droppers. An object-to-mouth contact was defined as a child putting any of these fomites or foods directly into his/her mouth after a hand-to-object contact during the structured observation period. The research assistants recorded the time the child spent sleeping. Information was also collected on whether the fomite or food was spit out by the child, and the caregiver's response to the child's mouthing event.

Information on diarrhoea morbidity among study children was collected bi-weekly from baseline to 3 months. Diarrhoea was defined as three or more loose stools in a 24-h period. In a subset of 128 randomly selected households, two soil samples were also collected in the outdoor courtyard areas where enrolled children were observed playing.

Laboratory analysis

Collected stool samples were stored in cooler boxes and transferred to the Enteric Microbiology Laboratory at the International Center for Diarrheal Disease Research, Bangladesh (icddr,b) in Dhaka, where they were stored at −80 °C until processing, on average within 6 h after defecation events. We evaluated faecal markers of intestinal inflammation: alpha-1-antitrypsin, myeloperoxidase, neopterin and calprotectin using enzyme-linked immunosorbent assays (ELISA) according to previously published methods [34]. All dilutions were performed according to package inserts except for a 1:500 dilution used for initial runs for myeloperoxidase. Faecal myeloperoxidase, alpha-1-antitrypsin and neopterin results were then combined to form an EE disease activity score (0–10 points) for each study participant, using previously published methods [39].

Soil samples were stored in cooler boxes upon collection and transported to the Enteric Microbiology Laboratory where total *E. coli* counts were measured immediately by bacterial culture, and diarrheagenic *E. coli* was detected using multiplex polymerase chain reaction (PCR) according to previously published methods [28, 49].

Statistical analysis

For each child, the total number of hand-to-object and object-to-mouth contacts observed was divided by the total observation time to calculate the frequency of contacts per hour by fomite type. The time when the child was sleeping was excluded from this calculation. The Mann–Whitney *U*-test was used to compare the median hand-to-object and object-to-mouth frequencies by gender of children.

Our primary study outcomes were the number of visits in which caregivers reported diarrhoea among study children, faecal calprotectin concentrations and the EE disease activity score. To investigate the association between childhood mouthing behaviours and diarrhoea prevalence, Poisson regression models were used with caregiver-reported diarrhoea as an outcome and the presence of fomite- or food-to-mouth contacts as the predictor. The models included the natural log of the total number of diarrhoea surveillance time points as an offset to account for the different follow-up periods among children. To assess the association between childhood fomite or food mouthing behaviours and the selected faecal markers of EE, linear regression models were run with calprotectin concentration and EE disease activity score as the outcomes, and the frequency of object-to-mouth contacts as the predictor.

In all regression analyses, the frequency of object-to-mouth contacts was defined as a binary variable and was coded as 0 when the child did not have object-to-mouth contacts and as 1 when the child had at least one object-to-mouth contact. This was performed because of the rarity of events. All models were adjusted for age based on previous studies that found significant associations between age and diarrhoea [50, 51] as well as EE [39, 40]. For the adjusted models, covariates were selected if their association with the outcome had significance <0.2. All analyses were performed using Stata, version 13 (Stata Corporation, College Station, TX).

Ethical approval

Informed consent was obtained from a parent or guardian of all study participants. The study procedures were approved by the research ethical review committee of icddr,b, and an exemption was obtained from Johns Hopkins Bloomberg School of Public Health.

Results

Study population

Structured observation data were collected for 216 children. Their median age was 17 ± 5.8 months, and 54% (N = 116) were female. Eighty eight percent (N = 190) of children were partially breast-fed and 12% (N = 26) were not breast-fed. The median number of individuals living in a household was 5 ± 1.9 (standard deviation) (range, 1–12). Ten percent of caregivers (N = 22) had no formal education, 26% (N = 57) had completed primary school and 64% (N = 137) had secondary or higher education. The median age of caregivers was 25 ± 6.2 years.

Hand-to-object contacts with fomite or food

Children slept for an average of 1 h and 13 min during the 5-h structured observation period. All 216 children had at least one hand-to-object contact with fomites or food with visible soil. By category of fomite or food, 99% of children (N = 214) touched soil only, 99% (N = 214) touched objects with visible soil, 64% (N = 138) touched food with visible soil, 12% (N = 26) touched animal faeces, 2% (N = 4) touched human faeces and <1% (N = 1) touched food with visible faeces. Male sex was significantly associated with a higher median frequency of hand-to-object contacts with fomites or food with visible soil (6.0 *vs.* 5.4 events per hour, P = 0.047). The median frequency of hand-to-object contacts with soil was 4.7 contacts per hour for children 6–12 months of age, 6.4 contacts per hour for children 12–18 months of age, 6.3 contacts per hour for children 18–24 months of age and 4.8 contacts per hour for children 24–30 months of age (Figure 1).

Object-to-mouth contacts with fomites or food

Eighty two percent of children (N = 178) had at least one object-to-mouth contact with fomites or food with visible during the structured observation period. By category of fomite or food, 18% (N = 38) mouthed soil only, 60% (N = 129) mouthed objects with visible soil and 63% (N = 137) mouthed foods with visible soil. Only one child mouthed animal faeces, and no children were observed mouthing human faeces or food with visible faeces. When total mouthing events are considered, children mouthed fomites more often than food (54% *vs.* 46%), and the median frequency of mouthing non-dietary objects was higher than that of food in children 6–12 months of age (0.8 *vs.* 0.0 contacts per hour). In all observed mouthing events, children mouthed very small quantities of soil or faeces (less than a child's handful). Sex was not significantly associated with the median frequency of object-to-mouth contacts with fomites or food (P = 0.32). The median frequency of object-to-mouth contacts with soil was 1.0 contact per hour for children 6–12 months of age, 0.8 contact per hour for children 12–18 months of age, 0.6 contact per hour for

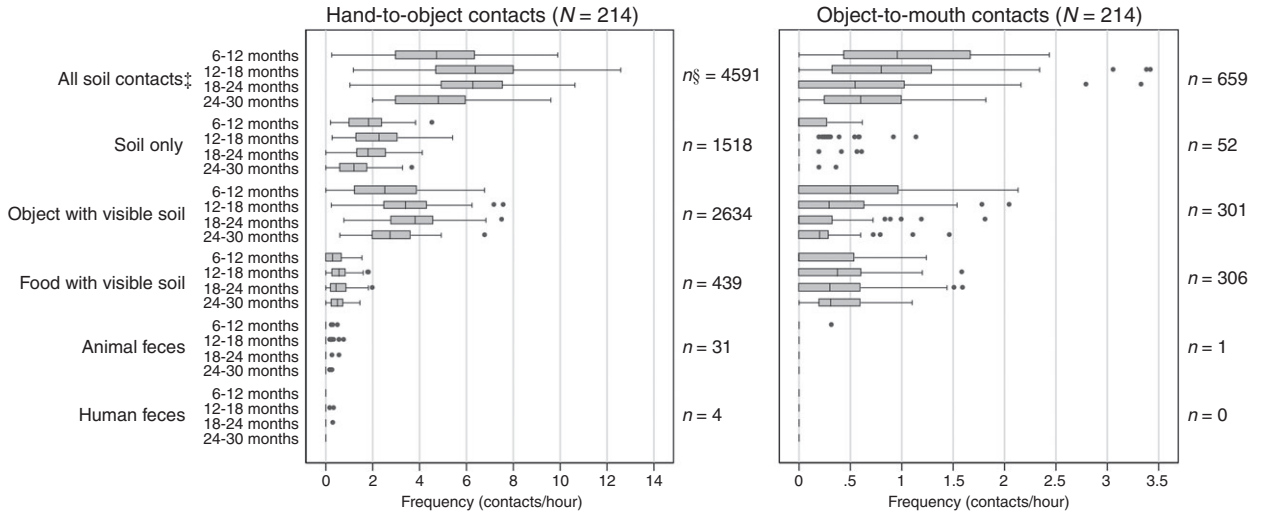

Figure 1 Frequency of hand-to-object and object-to-mouth contacts*†. *The frequency of hand-to-object and object-to-mouth contacts (contacts per hour) excludes the sleeping time of children during the structured observation period. †Two children were from the analysis because their sleeping time was not observed. *N* refers to the number of children. ‡All soil contacts include all types of soil contacts (soil only, fomite with visible soil and food with visible soil). §*n* refers to the number of contacts.

children 18–24 months of age and 0.6 contact per hour for children 24–30 months of age (Figure 1).

Children were observed spitting out soil during 6% (39/665) of object-to-mouth contacts with fomites or food (Table 1). Caregivers stopped children from mouthing fomites with soil in only 3% (18/665) of object-to-mouth contacts (0% for food with soil and 5% for objects with soil) and removed the soil from the child's mouth or hands in 4% (27/665) of contacts. Seventeen percent of object-to-mouth events ($N = 53$) involved sticks or leaves, 10% ($N = 30$) bottles, 8% ($N = 25$) toys or balls, 8% ($N = 25$) garbage (e.g. discarded wrappers or papers), 3% ($N = 10$) footwear, 2% ($N = 5$) medicine droppers and 1% ($N = 4$) pens.

Prevalence of diarrhoea

We were able to collect information on diarrhoea prevalence for 99.5% of children ($N = 215$). The median number of time points for diarrhoea surveillance was 5 (range: 1–5). The prevalence rate of diarrhoea was not significantly higher for children in contact with soil (of any type) (prevalence ratio: 1.14, 95% CI: 0.62, 2.11), or for children in contact with soil only, objects with visible soil or food with visible soil after controlling for age, gender, caregiver educational level and family size (Table 2). There was a marginally significant association between *E. coli* counts in soil from a child's play space and the prevalence rate of diarrhoea

Table 1 Child and caregiver response to fomite- or food-to-mouth contacts during 5-h structured observation

	Frequency (%)*		
Characteristic of child and caregiver response	Object with visible soil ($n = 304$)	Animal faeces ($n = 1$)	Food with visible soil ($n = 308$)
Child response after mouthing fomite or food			
Spit out	21 (7)	0 (0)	15 (5)
Nothing	277 (91)	1 (100)	293 (95)
Not observed by study personnel	6 (2)	0 (0)	0 (0)
Caregiver response during or after event			
Stopped the child from mouthing or handling the fomite or food	15 (5)	0 (0)	0 (0)
Removed the fomite or food from the child's mouth or hands	22 (7)	0 (0)	2 (1)
Washed the child's hands with water	0 (0)	0 (0)	2 (1)
Did nothing	127 (42)	0 (0)	214 (68)
Unaware (Not observing the child when event occurred)	123 (40)	1 (100)	88 (29)
Not observed by study personnel	17 (6)	0 (0)	2 (1)

*Number of fomite- or food-to-mouth contacts during 5-h structured observation.

(diarrhoea prevalence ratio: 2.03, 95% CI 0.97, 4.25) (Table S1). Adding breastfeeding to the adjusted models did not alter these findings (Table S1 and Table S2).

Association between mouthing behaviours and environmental enteropathy markers

The median concentration for calprotectin was 402.67 mg/g, 0.26 mg/g for alpha-1-antitrypsin, 3576.75 ng/ml for myeloperoxidase and 1505.00 nmol/L for neopterin. The median value for the EE disease activity score was 5. Children observed mouthing an object with visible soil during the structured observation period had significantly higher faecal calprotectin concentrations after controlling for age, gender, caregiver educational level and family size (206.81 μg/g, 95% CI: 6.27, 407.36) (Table 3). There were no other significant associations found between mouthing a fomite and faecal EE markers. Addition of breastfeeding to the adjusted models did not alter the observed associations (Table S3).

Discussion

We found that the prevalence of mouthing fomites or food contaminated with soil or faeces was high (82%) and that mouthing of objects with visible soil was significantly associated with elevated calprotectin concentrations in young children. Our previous work among children in this cohort found that the soil in outdoor play spaces was an exposure route for pathogenic *E. coli* and that all households had visible faecal matter present in the outdoor areas where children were observed playing [34]. Therefore, these findings provide evidence to support the hypothesis that childhood mouthing of soil-contaminated objects, which occurred more frequently than mouthing of soil (60% *vs.* 18%), is an important exposure route to enteric pathogens that can lead to environmental enteropathy in young children. Furthermore, caregivers do little to prevent this high-risk behaviour, demonstrating the urgent need for interventions to protect susceptible paediatric populations.

In this study, more than half of children mouthed food (60%) and/or objects (63%) with visible soil, whereas 18% ingested soil alone and only one child (<1%) mouthed animal faeces. Forty nine percent ($N = 106$) of children had multiple types of object-to-mouth contacts, and 85% ($N = 90$) of these children mouthed both food and objects with visible soil during the structured observation period. These findings demonstrate the importance of considering objects and food as exposure routes for contaminated soil that can lead to exposure to enteric pathogens and intestinal inflammation in susceptible paediatric populations. Our finding is consistent with those of previous studies that found unsanitary environmental conditions to be associated with markers of EE [52, 53]. Dual sugar permeability tests such as lactulose and mannitol are used as an indicator for intestinal barrier disruption and absorptive capacity [54]. In rural Bangladesh, children from 'contaminated' households had significantly higher lactulose: mannitol (L:M) ratios in urine, indicator of intestinal barrier disruption and absorptive capacity, compared to those from 'clean' households defined by water quality and sanitation and hygiene conditions [53]. Soil in the domestic environment and on children's toys

Table 2 Association between fomite- or food-to-mouth contacts and diarrhoea prevalence

		Children with exposure*		Children without exposure†		Age and gender adjusted prevalence ratio‡ (95% CI)	Fully adjusted prevalence ratio‡§ (95% CI)
Type of event	Total *N*	*N* (%)	Diarrhoea prevalence (%)	*N* (%)	Diarrhoea prevalence (%)		
All soil contacts‖	215	177 (82)	9.3	38 (18)	7.7	1.13 (0.61, 2.09)	1.14 (0.62, 2.11)
Soil only	215	178 (83)	9.8	37 (17)	8.9	1.08 (0.62, 1.91)	1.09 (0.62, 1.93)
Object with visible soil	215	128 (60)	9.9	87 (40)	7.7	1.24 (0.78, 1.97)	1.30 (0.81, 2.07)
Food with visible soil	215	137 (64)	8.9	78 (36)	9.2	0.96 (0.61, 1.50)	0.94 (0.60, 1.48)

CI, confidence interval.
*Children with at least one fomite- or food-to-mouth contact.
†Children without fomite- or food-to-mouth contacts.
‡Diarrhoea prevalence in children with at least one fomite- or food-to-mouth contact/diarrhoea prevalence in children without object-to-mouth contacts.
§Fully adjusted models adjust for age, age squared, gender, caregiver educational level and family size.
‖All soil contacts include all types of soil contacts (soil only, object with visible soil and food with visible soil).

Table 3 Association between fomite- or food-to-mouth contacts* and faecal environmental enteropathy markers

Outcome	Coefficient (95% CI)						
	All soil contacts† ($N = 216$)		Object with visible soil ($N = 216$)		Food with visible soil ($N = 216$)		
	Gender and age adjusted	Fully adjusted‡	Gender and age adjusted	Fully adjusted‡	Gender and age adjusted	Fully adjusted‡
Calprotectin (μg/g)	220.35 (−34.19, 474.89)	212.60 (−41.93, 467.14)	217.41 (18.34, 416.47)§	206.81 (6.27, 407.36)§	64.72 (−138.77, 268.22)	61.43 (−142.28, 265.15)
EE score	−0.22 (−1.03, 0.59)	−0.21 (−1.02, 0.61)	−0.56 (−1.19, 0.07)	−0.55 (−1.18, 0.09)	0.42 (−0.22, 1.06)	0.39 (−0.26, 1.03)
Alpha-1-antitrypsin (mg/g)	0.03 (−0.12, 0.17)	0.03 (−0.12, 0.18)	−0.10 (−0.21, 0.01)	−0.10 (−0.21, 0.19)	0.07 (−0.05, 0.19)	0.07 (−0.05, 0.19)
Myeloperoxidase (ng/ml)	1359.29 (−699.29, 3417.88)	1339.90 (−734.08, 3413.87)	746.13 (−873.55, 2365.81)	786.38 (−855.99, 2428.75)	464.55 (−1176.86, 2105.95)	490.26 (−1165.43, 2145.95)
Neopterin (nmol/l)	−109.53 (−984.52, 765.47)	−133.51 (−1004.34, 737.32)	−539.57 (−1222.82, 143.67)	−496.96 (−1182.18, 188.26)	140.21 (−555.04, 835.47)	158.94 (−533.95, 851.83)

CI, confidence interval; EE, environmental enteropathy.
*Children with at least one fomite- or food-to-mouth contact.
†All soil contacts include all types of soil contacts (soil only, object with visible soil and food with visible soil).
‡Fully adjusted models adjust for age, age squared, gender, caregiver educational level and family size.
§P value < 0.05.

and plates can also lead to exposure to faecal pathogens in low-income settings [55–57].

We found that non-dietary ingestion was more frequent than dietary ingestion (54% *vs*. 46%), and the median frequency of mouthing fomites was higher than that of food in children 6–12 months of age (0.8 *vs*. 0.0 contacts per hour). This result is consistent with those of a recent study in Taiwan which reported that children 7–12 months of age had higher median frequency of indoor mouthing of non-dietary objects than that of food (60.7 *vs*. 48.1 contacts per hour) [58]. In Black *et al*., the median frequency of mouthing non-dietary objects was also higher than that of mouthing food (18.1 *vs*. 10.0 contacts per hour) in children 7–12 months of age in the United States and Mexico border area [59]. In rural Bangladesh, Kwong *et al*. reported that the median mouthing frequency was 29.6 contacts per hour for non-dietary objects and 12.8 contacts per hour for dietary objects among children 6–12 month of age [60]. These findings support the hypothesis that non-dietary mouthing behaviour is an important route for childhood exposure to faecal pathogens, with infants being particularly vulnerable.

There was no significant association between caregiver-reported child diarrhoea and object-to-mouth contacts for fomites with visible soil. This result is in contrast to findings in Peru and Kenya, where geophagy was associated with childhood diarrhoea [24, 32]. In the present study, all children with object-to-mouth events consumed very small amounts of soil during the structured observation period. In Kenya, the prevalence of diarrhoea rose with increasing amounts of soil ingested by children [24]. Therefore, the small quantity of soil consumed in our study may not have been sufficient for a symptomatic infection. This association needs to be further investigated with a better quantification of the amount of soil ingested.

We found that childhood mouthing of objects with visible soil was significantly associated with elevated calprotectin concentration, while there was no significant association between soil mouthing and diarrhoea prevalence. Furthermore, detectable diarrheagenic *E. coli* in the soil were not significantly associated with diarrhoea prevalence, providing further evidence to support the hypothesis that chronic exposure to faecal pathogens can lead to EE and impaired growth through subclinical infections, with diarrhoea only contributing to a small portion of EE [61]. In rural Gambia, infants 3–15 months of age had diarrhoea 7.3% of the time but elevated L:M ratios in urine 76% of the time [46]. A study in peri-urban Peru found that while symptomatic cryptosporidiosis had a greater adverse effect on child

growth than asymptomatic cryptosporidiosis, the latter was twice as prevalent and thus may have had a greater effect on growth faltering [62]. Donowitz *et al.* reported that recent and frequent diarrhoea episodes were not significant predictors of small intestine bacterial overgrowth (SIBO), a condition in which excessive amounts of bacteria are present in the small intestine, while SIBO was associated with intestinal inflammation and linear growth faltering among Bangladeshi children [63].

There are several limitations to this study. First, we observed children's mouthing behaviours at a single time point and therefore could not have captured seasonal or day-to-day changes. Second, we measured faecal EE markers only at baseline. Therefore, we cannot investigate the causality of the association between children's mouthing behaviours and EE. Third, we only observed object-to-mouth contacts with visible soil or faeces and thus do not know what proportion of the total object-to-mouth events they comprise. Previous studies have reported that children put their hands in their mouths frequently and that hands themselves have faecal contamination [28, 59]. Therefore, mouthing contacts with hands alone may be an exposure route to faecal pathogens. Future studies should employ hand rinses to investigate this potential exposure and observe all types of mouthing events. Finally, we did not quantify the amount of soil mouthed by children, which may modify the effect of children's mouthing behaviours on diarrhoea and EE. This should be investigated in future work.

Conclusion

Soil mouthing behavior was common in young children, and mouthing of objects with visible soil was significantly associated with elevated fecal calprotectin. These findings support the hypothesis that childhood mouthing behaviours are an important pathway for exposure to faecal pathogens and asymptomatic infections leading to EE. Future studies are needed to investigate the association between mouthing behaviors, diarrhea, and EE among pediatric populations. Furthermore, interventions are needed to prevent pediatric exposures to fecal pathogens through childhood mouthing behaviors.

References

1. de Campos AC, Savelsbergh GJ, Rocha NA. What do we know about the atypical development of exploratory actions during infancy? *Res Dev Disabil* 2012: **33**: 2228–2235.
2. Moya J, Bearer CF, Etzel RA. Children's behavior and physiology and how it affects exposure to environmental contaminants. *Pediatrics* 2004: **113**: 996–1006.
3. Juberg DR, Alfano K, Coughlin RJ, Thompson KM. An observational study of object mouthing behavior by young children. *Pediatrics* 2001: **107**: 135–142.
4. Iverson JM. Developing language in a developing body: the relationship between motor development and language development. *J Child Lang* 2010: **37**: 229–261.
5. Beamer PI, Canales RA, Bradman A, Leckie JO. Farmworker children's residential non-dietary exposure estimates from micro-level activity time series. *Environ Int* 2009: **35**: 1202–1209.
6. Beamer PI, Canales RA, Ferguson AC, Leckie JO, Bradman A. Relative pesticide and exposure route contribution to aggregate and cumulative dose in young farmworker children. *Int J Environ Res Public Health* 2012: **9**: 73–96.
7. Freeman NC, Hore P, Black K *et al.* Contributions of children's activities to pesticide hand loadings following residential pesticide application. *J Expo Anal Environ Epidemiol* 2005: **15**: 81–88.
8. Zartarian V, Xue J, Glen G, Smith L, Tulve N, Tornero-Velez R. Quantifying children's aggregate (dietary and residential) exposure and dose to permethrin: application and evaluation of EPA's probabilistic SHEDS-Multimedia model. *J Expo Sci Environ Epidemiol* 2012: **22**: 267–273.
9. Lar UA, Agene JI, Umar AI. Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implications in mostly women and children who practice it. *Environ Geochem Health* 2015: **37**: 363–375.
10. Rahbar MH, White F, Agboatwalla M, Hozhabri S, Luby S. Factors associated with elevated blood lead concentrations in children in Karachi, Pakistan. *Bull World Health Organ* 2002: **80**: 769–775.
11. Wang Z, Chai L, Yang Z, Wang Y, Wang H. Identifying sources and assessing potential risk of heavy metals in soils from direct exposure to children in a mine-impacted city, Changsha, China. *J Environ Qual* 2010: **39**: 1616–1623.
12. Chen SJ, Ma YJ, Wang J, Chen D, Luo XJ, Mai BX. Brominated flame retardants in children's toys: concentration, composition, and children's exposure and risk assessment. *Environ Sci Technol* 2009: **43**: 4200–4206.
13. Harrad S, Hazrati S, Ibarra C. Concentrations of polychlorinated biphenyls in indoor air and polybrominated diphenyl ethers in indoor air and dust in Birmingham, United Kingdom: implications for human exposure. *Environ Sci Technol* 2006: **40**: 4633–4638.
14. Jones-Otazo HA, Clarke JP, Diamond ML *et al.* Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. *Environ Sci Technol* 2005: **39**: 5121–5130.
15. Alonso JM, Bojanich MV, Chamorro M, Gorodner JO. Toxocara seroprevalence in children from a subtropical city in Argentina. *Rev Inst Med Trop Sao Paulo* 2000: **42**: 235–237.
16. Cassenote AJ, Lima AR, Pinto Neto JM, Rubinsky-Elefant G. Seroprevalence and modifiable risk factors for Toxocara spp. in Brazilian schoolchildren. *PLoS Negl Trop Dis* 2014: **8**: e2830.

17. Geissler PW, Mwaniki D, Thiong F, Friis H. Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary schoolchildren. *Trans R Soc Trop Med Hyg* 1998: **92**: 7–11.
18. Glickman LT, Camara AO, Glickman NW, McCabe GP. Nematode intestinal parasites of children in rural Guinea, Africa: prevalence and relationship to geophagia. *Int J Epidemiol* 1999: **28**: 169–174.
19. Iddawela DR, Kumarasiri PV, de Wijesundera MS. A seroepidemiological study of toxocariasis and risk factors for infection in children in Sri Lanka. *Southeast Asian J Trop Med Public Health* 2003: **34**: 7–15.
20. Julian TR, Canales RA, Leckie JO, Boehm AB. A model of exposure to rotavirus from nondietary ingestion iterated by simulated intermittent contacts. *Risk Anal* 2009: **29**: 617–632.
21. Manini MP, Marchioro AA, Colli CM, Nishi L, Falavigna-Guilherme AL. Association between contamination of public squares and seropositivity for Toxocara spp. in children. *Vet Parasitol* 2012: **188**: 48–52.
22. Roldan WH, Cavero YA, Espinoza YA, Jimenez S, Gutierrez CA. Human toxocariasis: a seroepidemiological survey in the Amazonian city of Yurimaguas, Peru. *Rev Inst Med Trop Sao Paulo* 2010: **52**: 37–42.
23. Saathoff E, Olsen A, Kvalsvig JD, Geissler PW. Geophagy and its association with geohelminth infection in rural schoolchildren from northern KwaZulu-Natal, South Africa. *Trans R Soc Trop Med Hyg* 2002: **96**: 485–490.
24. Shivoga WA, Moturi WN. Geophagia as a risk factor for diarrhoea. *J Infect Dev Ctries* 2009: **3**: 94–98.
25. Yentur Doni N, Gurses G, Simsek Z, Yildiz Zeyrek F. Prevalence and associated risk factors of intestinal parasites among children of farm workers in the southeastern Anatolian region of Turkey. *Ann Agric Environ Med* 2015: **22**: 438–442.
26. Wagner EG, Lanoix JN. Excreta disposal for rural areas and small communities. *Monogr Ser World Health Organ* 1958: **39**: 1–182.
27. Mbuya MN, Tavengwa NV, Stoltzfus RJ *et al.* Design of an intervention to minimize ingestion of fecal microbes by young children in rural Zimbabwe. *Clin Infect Dis* 2015: **61** (Suppl 7): S703–9.
28. Ngure FM, Humphrey JH, Mbuya MN *et al.* Formative research on hygiene behaviors and geophagy among infants and young children and implications of exposure to fecal bacteria. *Am J Trop Med Hyg* 2013: **89**: 709–716.
29. Ngure FM, Reid BM, Humphrey JH, Mbuya MN, Pelto G, Stoltzfus RJ. Water, sanitation, and hygiene (WASH), environmental enteropathy, nutrition, and early child development: making the links. *Ann N Y Acad Sci* 2014: **1308**: 118–128.
30. Mattioli MC, Davis J, Boehm AB. Hand-to-mouth contacts result in greater ingestion of feces than dietary water consumption in Tanzania: a quantitative fecal exposure assessment model. *Environ Sci Technol* 2015: **49**: 1912–1920.
31. Liu L, Oza S, Hogan D *et al.* Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 2015: **385**: 430–440.
32. Yeager BA, Lanata CF, Lazo F, Verastegui H, Black RE. Transmission factors and socioeconomic status as determinants of diarrhoeal incidence in Lima, Peru. *J Diarrhoeal Dis Res* 1991: **9**: 186–193.
33. Keusch GT, Denno DM, Black RE *et al.* Environmental enteric dysfunction: pathogenesis, diagnosis, and clinical consequences. *Clin Infect Dis* 2014: **59**(Suppl 4): S207–12.
34. George CM, Oldja L, Biswas S *et al.* Geophagy is associated with environmental enteropathy and stunting in children in rural Bangladesh. *Am J Trop Med Hyg* 2015a: **92**: 1117–1124.
35. Perin J, Thomas A, Oldja L *et al.* Geophagy is associated with growth faltering in children in rural Bangladesh. *J Pediatr* 2016: **178**: 34–39 e1.
36. Campbell DI, Elia M, Lunn PG. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *J Nutr* 2003: **133**: 1332–1338.
37. Campbell DI, McPhail G, Lunn PG, Elia M, Jeffries DJ. Intestinal inflammation measured by fecal neopterin in Gambian children with enteropathy: association with growth failure, *Giardia lamblia*, and intestinal permeability. *J Pediatr Gastroenterol Nutr* 2004: **39**: 153–157.
38. Goto R, Mascie-Taylor CG, Lunn PG. Impact of intestinal permeability, inflammation status and parasitic infections on infant growth faltering in rural Bangladesh. *Br J Nutr* 2009: **101**: 1509–1516.
39. Kosek M, Haque R, Lima A, MAL-ED network *et al.* Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of linear growth deficits in infants. *Am J Trop Med Hyg* 2013: **88**: 390–396.
40. Liu JR, Sheng XY, Hu YQ *et al.* Fecal calprotectin levels are higher in rural than in urban Chinese infants and negatively associated with growth. *BMC Pediatr* 2012: **12**: 129-2431-12-129.
41. Lunn PG, Northrop-Clewes CA, Downes RM. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. *Lancet* 1991: **338**: 907–910.
42. Mondal D, Minak J, Alam M *et al.* Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012: **54**: 185–192.
43. Panter-Brick C, Lunn PG, Langford RM, Maharjan M, Manandhar DS. Pathways leading to early growth faltering: an investigation into the importance of mucosal damage and immunostimulation in different socio-economic groups in Nepal. *Br J Nutr* 2009: **101**: 558–567.
44. Prendergast AJ, Rukobo S, Chasekwa B *et al.* Stunting is characterized by chronic inflammation in Zimbabwean infants. *PLoS One* 2014: **9**: e86928.
45. Weisz AJ, Manary MJ, Stephenson K *et al.* Abnormal gut integrity is associated with reduced linear growth in rural

Malawian children. *J Pediatr Gastroenterol Nutr* 2012: **55**: 747–750.
46. Lunn PG. The impact of infection and nutrition on gut function and growth in childhood. *Proc Nutr Soc* 2000: **59**: 147–154.
47. Kotloff KL, Nataro JP, Blackwelder WC *et al.* Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013: **382**: 209–222.
48. Victora CG, de Onis M, Hallal PC, Blossner M, Shrimpton R. Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics* 2010: **125**: e473–80.
49. Houpt E, Gratz J, Kosek M, MAL-ED Network Investigators *et al.* Microbiologic methods utilized in the MAL-ED cohort study. *Clin Infect Dis* 2014: **59**(Suppl 4): S225–32.
50. Pathela P, Zahid Hasan K, Roy E, Huq F, Kasem Siddique A, Bradley Sack R. Diarrheal illness in a cohort of children 0-2 years of age in rural Bangladesh: I. Incidence and risk factors. *Acta Paediatr* 2006: **95**: 430–437.
51. Quick RE, Venczel LV, Mintz ED *et al.* Diarrhoea prevention in Bolivia through point-of-use water treatment and safe storage: a promising new strategy. *Epidemiol Infect* 1999: **122**: 83–90.
52. George CM, Oldja L, Biswas SK *et al.* Fecal markers of environmental enteropathy are associated with animal exposure and caregiver hygiene in Bangladesh. *Am J Trop Med Hyg* 2015b: **93**: 269–275.
53. Lin A, Arnold BF, Afreen S *et al.* Household environmental conditions are associated with enteropathy and impaired growth in rural Bangladesh. *Am J Trop Med Hyg* 2013: **89**: 130–137.
54. Goto K, Chew F, Torun B, Peerson JM, Brown KH. Epidemiology of altered intestinal permeability to lactulose and mannitol in Guatemalan infants. *J Pediatr Gastroenterol Nutr* 1999: **28**: 282–290.
55. Julian TR, MacDonald LH, Guo Y *et al.* Fecal indicator bacteria contamination of fomites and household demand for surface disinfection products: a case study from Peru. *Am J Trop Med Hyg* 2013: **89**: 869–872.
56. Pickering AJ, Julian TR, Marks SJ *et al.* Fecal contamination and diarrheal pathogens on surfaces and in soils among Tanzanian households with and without improved sanitation. *Environ Sci Technol* 2012: **46**: 5736–5743.
57. Vujcic J, Ram PK, Hussain F *et al.* Toys and toilets: cross-sectional study using children's toys to evaluate environmental faecal contamination in rural Bangladeshi households with different sanitation facilities and practices. *Trop Med Int Health* 2014: **19**: 528–536.
58. Tsou MC, Ozkaynak H, Beamer P *et al.* Mouthing activity data for children aged 7 to 35 months in Taiwan. *J Expo Sci Environ Epidemiol* 2015: **25**: 388–398.
59. Black K, Shalat SL, Freeman NC, Jimenez M, Donnelly KC, Calvin JA. Children's mouthing and food-handling behavior in an agricultural community on the US/Mexico border. *J Expo Anal Environ Epidemiol* 2005: **15**: 244–251.
60. Kwong LH, Ercumen A, Pickering AJ, Unicomb L, Davis J, Luby SP. Hand- and object-mouthing of rural Bangladeshi children 3-18 months old. *Int J Environ Res Public Health* 2016: **13**: 563 doi:10.3390/ijerph13060563.
61. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet* 2009: **374**: 1032–1035.
62. Checkley W, Gilman RH, Epstein LD *et al.* Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am J Epidemiol* 1997: **145**: 156–163.
63. Donowitz JR, Haque R, Kirkpatrick BD *et al.* Small intestine bacterial overgrowth and environmental enteropathy in Bangladeshi children. *MBio* 2016: **7**: e02102–15.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Association between household soil measurements and diarrhea prevalence.

Table S2. Association between fomite- or food-to-mouth contacts and diarrhoea prevalence.

Table S3. Association between fomite- or food-to-mouth contacts and fecal environmental enteropathy markers.

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