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Growth Status, Inflammation, and Enteropathy in Young Children in Northern Tanzania

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Abstract. Recent evidence suggests that enteropathy of the gut due to environmental conditions (i.e., environmental enteropathy [EE]) in young children is negatively associated with linear growth. Using a case–control study design, we examined the potential determinants of stunting in stunted and non-stunted children 22–28 months of age. Potential determinants included inflammation biomarkers C-reactive protein, alpha-1-acid glycoprotein (AGP), and endotoxin-core antibody (EndoCAb) measured in serum samples; enteropathy markers alpha-1-antitrypsin, neopterin, myeloperoxidase (MPO) measured in stools samples; and demographic, health, feeding, and household characteristics. We also explored the determinants of EE by testing associations of composite EE scores and individual biomarkers with potential risk factors. Fifty-two percent of children (n = 310) were found to be stunted, and mean height-for-age Z scores (HAZ) were −1.22 (standard deviation [SD] ± 0.56) among non-stunted (control) children and −2.82 (SD ± 0.61) among stunted (case) children. Child HAZ was significantly ($P < 0.05$) and inversely associated with AGP, and child stunting was significantly positively associated ($P < 0.05$) with low dietary diversity, severe household hunger, and absence of soap in the household. Alpha-1-acid glycoprotein and EndoCAb concentrations were also significantly higher ($P < 0.05$) among children in households with no soap. Our study documented a seemingly localized cultural practice of young children (25%) being fed their dirty bathwater, which was associated with significantly higher concentrations of MPO ($P < 0.05$). Alpha-1-acid glycoprotein showed the most consistent associations with child growth and hygiene practices, but fecal EE biomarkers were not associated with child growth. The lack of retrospective data in our study may explain the null findings related to fecal EE biomarkers and child growth.

INTRODUCTION

Stunted growth remains a public health challenge globally, and reducing childhood stunting has been identified as one of the six global nutrition goals adopted by the World Health Assembly.[1,2] Many factors can affect child growth and result in stunting, including suboptimal maternal health and nutrition status, inadequate home environment, inadequate complementary feeding, and clinical and subclinical infection.[3]

The linkages between child growth and household-level water, sanitation, and hygiene (WASH) facilities and practices are mixed. Although observational studies suggest associations between child growth or stunting prevalence and adequacy of household sanitation,[4,5] recent randomized controlled trials in Kenya and Bangladesh found no impact of improved WASH facilities and practices on linear growth in children.[6,7] Despite these recent findings, nascent research suggests that poor sanitation and hygiene practices compromise children's gut function and nutrient absorption.[8] Caregiver hygiene and animal exposure[9] have been associated with intestinal enteropathy in children, and it has been observed that young children were repeatedly exposed to *Escherichia coli* via the consumption of contaminated water and the ingestion of soil and chicken feces.[10]

Environmental enteropathy (EE) refers to malfunctioning of the small intestine that results in inflammation and reduced surface area of intestinal villi. It is posited that repeated exposure to enteropathogens via fecal–oral transmission could result in EE,[11] and that if occurring during infancy and early childhood, EE could be a potential independent predictor of stunting that is not correlated with recent diarrhea,[8] a health outcome commonly seen as a sequelae to poor sanitation and hygiene conditions. Moreover, research from multiple countries[12–14] has found associations between gut function and linear growth in young children.

Examining the association between EE and linear growth is particularly relevant to Tanzania, as child growth has been previously associated with inadequate household sanitation there.[15] The prevalence of child stunting in children aged < 5 years in Tanzania has consistently decreased in the past decade, from 44.3% in 2004, 42.0% in 2010, to 34.4% in 2015.[16–18] Despite this reduction, the prevalence of stunting remains high according to the World Health Organization (WHO) classifications.[19] According to the 2015–2016 Demographic and Health Survey (DHS), the national prevalence of stunting was the highest (44.4%) among children 24–35 months of age.[18]

The primary objective of this case–control study is to identify the associations between stunting and child growth and indicators of inflammation, EE, and reported demographic, health, feeding, and hygiene practices. A secondary objective of the study is to examine the risk factors of EE.

METHODS

Study design. We designed a case–control study to compare inflammation and EE biomarkers against sociodemographic, health and hygiene characteristics between stunted children (cases) and non-stunted children (controls) living in the same communities. The case–control study was nested into the endpoint assessment of the *Creating Homestead Agriculture for Nutrition and Gender Equity* (CHANGE) impact evaluation—a trial examining the impact of an integrated nutrition intervention (including micronutrient powders [MNPs], homestead food production, and WASH and nutrition education) on child anemia and growth.[20] The CHANGE project

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aimed to improve child growth by supporting caregivers to adopt optimal child-feeding practices and improved WASH practices through a behavior change approach and to adopt improved homestead food production (e.g., vegetable gardens and chicken rearing) through training and service provision. As part of the research design, MNPs were given to all children participating in the program as well as to a control group, with the original goal of reducing anemia levels to understand the ability of the integrated CHANGE package to maintain these reduced anemia levels.

For the case–control study, participants were enrolled from January to February 2016 in the control areas of the CHANGE evaluation in which only a limited set of activities were implemented. Specifically, children in the control arm received anemia and malaria diagnosis and treatment during baseline and follow-up surveys of the CHANGE evaluation, which happened 18, 15, and 6 months before recruitment into the case–control study. In addition, they received a 2-month supply of MNPs at the CHANGE baseline and 12-month follow-up surveys (i.e., when children were 6–12 months of age and then 18–24 months of age). No other activities (e.g., support for agriculture and livestock production or behavior change communication on WASH, nutrition, or malaria prevention) were organized as part of the CHANGE program in the control communities.

Data collection procedures. The case–control study recruited all children living in the 10 control wards (i.e., smallest administrative units) of the Sengerema district in Tanzania's Lake Zone who provided blood samples during the CHANGE end line assessment. These children were also included in previous rounds of the CHANGE study, from whom questionnaire data and blood samples were collected during previous survey rounds. The enrolled children had an age range of 22–28 months during the end line assessment. This design was expected to identify 128 cases and 192 controls (320 children in total), considering an expected stunting rate of 40% and an 80% response rate for stool samples, with a statistical significance level of 0.05 and power of 0.8. Based on the aforementioned assumptions, the target sample size would presumably allow the study to detect an odds ratio of 2 between stunted and non-stunted children with differing endotoxin exposures and other stool biomarkers that were considered clinically relevant a priori.

A comprehensive questionnaire was administered as part of the larger study to caregivers of enrolled children. The child's age was collected as part of the household questionnaire and further validated by confirming the birth date listed on the child's health card and records from previous rounds of the CHANGE evaluation. The questionnaire also included information on household and individual demographic variables (e.g., age and gender), household food security, household WASH facilities and practices, maternal health and education characteristics, and infant and young child feeding practices.

Following the completion of the questionnaire, height and weight were measured from all children using portable wooden stadiometers (Infant/Child ShorrBoard®, Olney, MD) and standing scales (Seca, 874 U, Hamburg, Germany). In preparation for the fieldwork, a height measurement standardization exercise was conducted, and only the best-performing nurses were hired.

Capillary blood samples were collected from each child's middle or ring finger by trained nurses. After the lancet puncture, the first two drops of blood were swiped with dry gauze, and the third and fourth blood droplets were used to measure hemoglobin concentration and malaria status. Following this, approximately 300–400 μL of blood was collected into capillary blood collection tubes with coagulation activator (Microvette® 300 Z, 20.1308; Sarstedt, Nümbrecht, Germany). The blood samples were kept cold (+1 to +4°C) and were centrifuged on the day of blood collection. After centrifugation and separation, the serum was frozen at –20°C.

Following blood collection, nurses provided pre-labeled stool sample containers, a clean plastic spoon, and paper to place below a child when defecating, should the child not be wearing a diaper. Each child's caretaker was instructed to scoop approximately 100 mL of stool directly from the child's diaper or from clean paper into the pre-labeled stool container directly after a child defecated, securely close the lid, and place the sample in a cool dark spot until it was collected. Three research assistants on motorbikes accompanied the nurses to the field and collected the stool samples the same day, such that the cold chain began 1–4 hours after the stool was passed. After collection, the stool samples were kept in cold boxes (+4 to +8°C) until the samples were delivered to the the National Institute for Medical Research (NIMR) field laboratory in Sengerema, where a slide for microscopy was prepared within 12–24 hours after the stool was passed and a 10–15 mL aliquot of stool was frozen at –20°C.

Laboratory analyses—Blood. Hemoglobin concentration was measured onsite using a portable hemoglobinometer (Hb201+; HemoCue AB, Ängelholm, Sweden). Current and recent malaria status was assessed using a rapid diagnostic test (Malaria Ag P.f/Pan; Standard Diagnostics, Gyeonggi-do, Republic of Korea) with the ability to detect antigens from *Plasmodium faliciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*.

Three markers of systemic inflammation were measured in serum. C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were measured in duplicate at VitMin Lab laboratory (Willstaett, Germany) using the sandwich enzyme-linked immunosorbent assay (ELISA) technique described by Erhardt et al.[21] Single IgG endotoxin-core antibody (EndoCAb) concentrations were assessed using commercially available kits (Hycult Biotech, Inc., Uden, The Netherlands) using a 1:400 dilution factor. Approximately, 15% of samples were measured in duplicate, and the results showed high correlation ($R^2 = 0.91$).

Laboratory analyses—stool. Microscopy to detect *Ascaris lumbricoides* (large roundworm) and *Strongyloides stercoralis* (threadworm) was performed separately by two microscopists with extensive experience detecting soil transmitted helminths burden in stool samples.

Analysis of stool biomarkers was conducted at NIMR Mwanza Laboratory (Mwanza, Tanzania). Commercial ELISA kits were used to measure concentrations of three biomarkers in stool samples: neopterin (NEO; GenWay Biotech, San Diego, CA), alpha-1-antitrypsin (AAT; BioVendor, Karasek, Czech Republic), and myeloperoxidase (MPO; Immundiagnostik, Bensheim, Germany). During the extraction process, NEO was diluted with 0.9% saline solution at a dilution factor of 50, and AAT and MPO were diluted with the manufacturer's dilution buffer at a dilution factor of 63.5. All aliquots were vigorously vortexed for 1 minute, centrifuged according to the manufacture's specifications, and 1 mL of the resulting supernatant was then removed and frozen at –20°C. Following

an initial screening for each biomarker, a second dilution step was conducted to yield concentrations within the standard curves. The final dilutions for NEO, AAT, and MPO were 1:500, 1:12,500, and 1:200, respectively. Of note, the NEO ELISA manufactured by Genway Biotech is only marketed for analysis of serum, plasma, and urine but has been repeatedly used by other researchers to measure NEO in stool.[14,22]

Positive controls were included as part of each kit for AAT, MPO, and NEO, and controls for all plates were consistently within the ranges provided by the manufacturer. For NEO, the interassay coefficients of variation (CVs) were 21.2% and 14.3% for the low (target concentration: 5.7 nmol/L) and high (target concentration: 18.8 nmol/L) controls, respectively. The interassay CVs for AAT were 4.1% for the low (target concentration: 8.3 ng/mL) and 6.4% high (target concentration: 26.2 ng/mL) controls. For MPO, the interassay CVs were 14.9% for the low (target concentration: 6.9 ng/mL) and 5.9% high (target concentration: 26.4 ng/mL) controls.

Indicator definitions and cut-offs. C-reactive protein and AGP were used in tandem to capture the various stages of inflammatory response as defined by Thurnham et al.[23] Incubation was defined as elevated CRP (> 5 mg/L) alone, early convalescence as elevated CRP and AGP (> 1.0 g/L), and late convalescence defined as elevated AGP alone. Incubation, early convalescence, or late convalescence was defined as *any* inflammation, and no inflammation was defined as CRP ≤ 5 mg/L and AGP ≤ 1.0 g/L.

The weight and height measurements were used to calculate gender-specific height-for-age *Z* scores (HAZ) and weight-for-height *Z* scores (WHZ) according to the WHO Child Growth Standards.[24] Children with HAZ and WHZ scores < –2.0 were classified as stunted and wasted, respectively.[25] Hemoglobin concentration < 110 g/L was used to define anemia[26] following a subtraction of 2 g/L hemoglobin to adjust concentrations for altitudes 1,000–2,000 m above sea level.[26]

Using questionnaire data collected within the CHANGE study, minimum dietary diversity for each child was calculated using WHO guidelines,[27] although most of the children in our study are outside the age range (i.e., > 24 months of age) recommended by WHO. Despite the age of most children in our study, dietary diversity is still likely a relevant indicator of dietary adequacy as it is often used in other population groups, such as women of reproductive age.[28] In addition, caretakers reported recent illnesses of the children in the past 2 weeks according to their own understanding of descriptions used by interviewers. Illnesses examined were watery diarrhea (i.e., "stool like water") and fever ("hot body"). Caretakers were also asked about when they washed their child's hands and were able to respond without prompting about all instances where the child's hands are washed. The responses "after defecation" and "before eating" were the only two responses mentioned of the five critical moments for handwashing.[29] With the inclusion of the response that the child's hands were washed "frequently throughout the day," a handwashing score was calculated, to identify if the child had zero, one, two, or three times when hands were washed routinely, and then dichotomized to determine if at least one of these key practices was used. Caretakers also reported if the child was fed his/her own bath water 1) anytime when the child was < 1 year of age and 2) anytime in the past month. The practice of feeding children their own bath water had been observed in the region during previous rounds of the CHANGE evaluation.

The interviewers observed the presence and type of sanitation facility at the household, using WHO/UNICEF criteria[30] and determined if the sanitation facilities were considered "improved" (i.e., flush to piped sewer system/septic tank/pit, ventilated improved pit latrine, pit latrine with a slab, composting toilet) or "un-improved" (i.e., pit latrine without slab, bucket toilet, hanging toilet, no facilities). Household food security in the past month was measured using the standard household food insecurity access scale (HFIAS) classification into food secure, or mildly, moderately, or severely food insecure.[31] In addition to the HFIAS, the standard household hunger scale (HHS), which captures more severe hunger-related behaviors,[32] was calculated and separated into three categories (little to no hunger, moderate hunger, and severe hunger).[33]

We constructed a composite "EE score" modifying the approach developed by Kosek et al.[14] Using this approach, each child was assigned to one of three categories based on their percentile for NEO, AAT, and MPO concentrations, "where AAT, MPO, and NEO categories were defined as 0 (≤ 25th percentile), 1 (25–75th percentile), or 2 (≥ 75th percentile)."[14] In addition, we augmented the "EE score" developed by Kosek et al.[14] by incorporating the systemic inflammation markers EndoCAb and AGP into the equation because these markers have been associated with growth or stunting in previous studies.[34,35] In this "expanded EE score," EndoCAb and AGP are categorized using the same approach as developed by Kosek et al., and weighted similar to NEO in the index model (see equation below).

$$2 \times (\text{AAT category}) + 2 \times (\text{MPO category}) + 1 \times (\text{NEO category}) + 1 \times (\text{EndoCAb category}) + 1 \times (\text{AGP category}) = \text{Expanded EE score}$$

Statistical analysis. To identify factors associated with stunting, inflammation, EE, demographic, health, feeding, and household characteristics were compared independently in stunted and non-stunted children. For these associations, measures of central tendency and variance were calculated for stunted and non-stunted children separately and compared. For normally-distributed variables, means and standard deviations were calculated, and the *P*-values expressing the difference between non-stunted and stunted children were calculated using the analysis of variance (ANOVA) test. *P* values less than 0.05 were considered statistically significant. We did not adjust further for multiple comparisons because the goal of the paper was to draw hypotheses on the pathway from inflammation to stunting, rather than prove predefined key hypothesis of these pathways for final decision making.[36] For non-normally distributed variables, the medians of each subgroup were compared using the Wilcoxon rank sum test. For dichotomous and categorical variables, odds ratios and their respective confidence intervals (95%) were calculated.[37] In addition, adjusted odds ratios were calculated using a logistic regression model to account for child's gender, child's age in months, child's anemia status, mother's anemia status, household ownership of soap, and household hunger. To identify continuous associations between growth, inflammation, and EE, pairwise correlations between HAZ and all markers of inflammation and EE were also conducted.

Lastly, to identify risk factors of EE, the mean EE and expanded EE scores were compared for various health, feeding, and hygiene factors. Data analysis was conducted using Stata 14 (StataCorp, College Station, TX).

Ethics and consent. Ethical approval for the case–control study was received from the Lake Zone Ethics Committee (REF: MR/53/100/3) housed at the NIMR Mwanza Center and the International Food Policy Research Institute's (IFPRI) Institutional Review Board (REF: 2016-7-PHND-M). Ethical clearance for the CHANGE study was obtained from Medical Research Coordinating Committee housed at NIMR headquarters in Dar es Salaam (REF: NIMR/HQ/R.8a/Vol.IX/1721) and IFPRI's Institutional Review Board (REF: 2014-PHND-12-M). A separate written informed consent statement was used to request consent to collect stool samples for the measurement of EE markers and to measure EndoCAb from serum samples collected for micronutrient biomarkers. This consent statement was read aloud in the local language to the child's caretaker, who gave consent on the child's behalf and agreed to collect the stool sample according to the procedures described by the nurse. To meet the requirements of NIMR's National Ethics Committee, a single dose of albendazole was given to all children who provided stool samples according to procedures described in Tanzania's public health policy.[38] The consent forms were stored in a locked cabinet categorized per NIMR's standardized filing and storage procedure. Stool sample data contained no identifying information.

RESULTS

Inflammation, enteropathy, and other potential risk factors of stunting. Complete questionnaire, anthropometry, blood biomarker, and stool biomarker data were available for 310 children. Fifty-two percent of children were found to be stunted, and mean HAZ was –1.22 (SD ± 0.56) among non-stunted (control) children and –2.82 (SD ± 0.61) among stunted (case) children. Mean WHZ was significantly lower ($P = 0.016$) in stunted children compared with non-stunted children.

As shown in Table 1, no significant differences were found between median concentrations of CRP and AGP in non-stunted and stunted children. Similarly, there was no significant difference in the median EndoCAb, NEO, AAT, and MPO concentrations between non-stunted and stunted children. Although there were no significant differences in hemoglobin concentration, the prevalence of anemia was significantly ($P = 0.016$) higher in stunted children. We found no difference in malaria prevalence or recent diarrhea and fever between non-stunted and stunted children.

Nearly all children (96.7%) received MNPs in the 6 months preceding the survey. Nearly half of all children had a minimum diverse diet in the 24 hours before the survey, but the proportion of non-stunted children (54.4%) receiving a minimum diverse diet was significantly higher than that of stunted children (42.5%; $P = 0.037$). Regarding handwashing, nearly all caretakers reported washing their children's hands at least once throughout the day, with no significant difference between stunted and non-stunted children. Washing a child's hands before eating (78.5% non-stunted; 83.9% stunted, NS) and after defecation (47.1% non-stunted; 53.5% stunted, NS) were the most frequently reported handwashing practices; only 5.0% and 7.1% of caretakers of non-stunted and stunted

TABLE 1
Inflammation, enteropathy, and other risk factors for stunting among non-stunted and stunted children 22–28 months of age

Indicator	Non-stunted children ($n = 149$)	Stunted children ($n = 161$)	P-value*
Inflammation and enteropathy biomarkers			
C-reactive protein (mg/L); median (IQR)	1.3 (0.5, 5.8)	1.4 (0.6, 3.5)	0.932
AGP (g/L); median (IQR)	1.1 (0.8, 1.7)	1.3 (0.8, 2.1)	0.119
EndoCAb (GMU/mL); median (IQR)	100.2 (57.0, 150.0)	89.2 (60.8, 152.8)	0.799
Neopterin (nmol/L); median (IQR)	609.0 (338.5, 1,015.5)	585.0 (334.5, 1,048.0)	0.701
Alpha-1-antitrypsin (mg/g); median (IQR)	0.15 (0.03, 0.33)	0.18 (0.7, 0.39)	0.147
MPO (ng/mL); median (IQR)	1,340.8 (346.0, 4,756.8)	1,614.0 (368.0, 4,483.8)	0.656
Child demographic characteristics			
Female child, %	49.7	46.6	0.588
Age in months; mean (SD)	25.2 (±1.4)	25.4 (±1.4)	0.263
Caregiver age in years; mean (SD)	30.9 (±8.6)	29.6 (±9.7)	0.189
Child health characteristics			
Hemoglobin (g/L); mean (SD)	107 (±13)	105 (±15)	0.131
Anemia, %	53.0	66.5	**0.016**
Malaria parasitemia, %	17.3	21.6	0.356
Watery diarrhea in past 2 weeks, %	2.0	4.9	0.161
Fever in past 2 weeks, %	47.8	44.9	0.639
Weight-for-height Z scores; mean (SD)	0.37 (±1.00)	0.10 (±0.95)	**0.017**
Child-feeding characteristics			
Micronutrient powders in past 6 months, %	95.9	97.5	0.443
Minimum dietary diversity, %†	54.4	42.5	**0.037**
At least 1 handwashing practice, %	90.0	94.8	0.115
Child fed own bathwater in past month, %	27.5	23.6	0.431
Child fed own bathwater when child was < 1 year old, %	39.6	40.4	0.889
Household characteristics			
Number of members; mean (SD)	7.0 (±2.6)	6.8 (±2.07)	0.459
Household food insecurity and access scale—severely food insecure, %	42.9	45.0	0.706
Household Hunger Scale—severe hunger, %	1.4	5.6	**0.045**
Improved sanitation facility, %	8.1	6.8	0.682
Any sanitation facility, %	72.1	71.9	0.964
Soap present, %	67.1	55.3	**0.033**

AGP = alpha-1-acid glycoprotein; EndoCAb = IgG endotoxin-core antibody; MPO = myeloperoxidase. Bolded P-values illustrate a statistically significant association (<0.05).
* P-value represents Mann–Whitney test for comparisons of medians, Pearson's chi square test for dichotomous variables, and ANOVA for continuous variables.
† Measured in children outside the age range recommended by the World Health Organization.[27]

children reported washing a child's hands frequently throughout the day. No individual handwashing practice was significantly associated with stunting status (data not shown). Approximately 25% and 40% of children were fed their own bathwater in the past month and when less than 1 year old, respectively. There was no statistically significant difference in the proportion of non-stunted and stunted children fed their own bathwater.

The number of household members and a household's classification as experiencing severe food insecurity in the past month were not associated with stunting. Severe household hunger, although only affecting a small proportion of households, was statistically significantly higher ($P = 0.045$) in the households of stunted children. Only 7% of households had an "improved" sanitation facility, and no statistically significant difference in the proportions of any sanitation facility or improved sanitation facility was observed for non-stunted and stunted children. More than half of the households possessed soap, and soap ownership was significantly more frequent in the homes of non-stunted children ($P < 0.05$). Possession of soap was also significantly associated with lower odds of stunting (odds ratio [OR] = 0.61; 95% confidence interval [CI]: 0.38, 0.96; data not shown). Of note though, only 2.3% of households had a fixed station for handwashing (data not shown), with no statistical difference between cases and controls. Ascaris and strongyloides helminthes were very rare and were found in only four and six children, respectively. All children with ascaris were stunted, and four out of six children with strongyloides were stunted (data not shown).

Table 2 presents the pairwise correlations between HAZ and all inflammation and enteropathy biomarkers. Only AGP was statistically significantly and negatively associated with HAZ (i.e., lower AGP concentrations are associated with higher HAZ). Amongst inflammation markers, positive and statistically significant associations were found between CRP and AGP, AGP and EndoCAb, and MPO and AAT.

Elevated CRP and elevated AGP were common, affecting approximately 20% and 58% of children, respectively. About 60% of children had elevated CRP and/or AGP (i.e., *any* inflammation), and there was a nearly complete overlap of elevated CRP and AGP; only about 2% of children had elevated CRP without elevated AGP.

As shown in Table 3, the proportions of non-stunted and stunted children with elevated inflammation and EE biomarkers and higher EE scores were similar. Both unadjusted and adjusted odds ratios illustrated that no statistically significant differences were found between non-stunted and stunted children. However, some but weak associations were found in calculations of adjusted odds ratios for elevated CRP ($P = 0.056$) and elevated EndoCAb ($P = 0.076$), showing a negative association between these markers of systemic inflammation and stunting.

TABLE 2

Spearman pairwise correlation coefficients for HAZ and inflammation and enteropathy markers in children 22–28 months of age, cases, and controls

	HAZ	CRP	AGP	EndoCAb	NEO	AAT	MPO
HAZ	1.00	–	–	–	–	–	–
CRP	0.04	1.00	–	–	–	–	–
AGP	−0.14*	0.50*	1.00	–	–	–	–
EndoCAb	−0.04	0.06	0.23*	1.00	–	–	–
NEO	0.07	−0.07	−0.08	0.02	1.00	–	–
AAT	0.05	−0.05	0.02	−0.03	0.00	1.00	–
MPO	0.03	−0.03	−0.01	0.05	0.11	0.26*	1.00

AAT = Alpha-1-antitrypsin; AGP = alpha-1-acid glycoprotein; CRP = C-Reactive Protein; EndoCAb = IgG endotoxin-core antibody; HAZ = Height-for-age *Z* score, lnEndoCAb = natural log of IgG endotoxin-core antibody; MPO, myeloperoxidase; NEO = Neopterin.
* Indicates significance at $P < 0.05$.

Risk factors of inflammation and enteropathy. Table 4 presents associations between the potential risk factors of inflammation or enteropathy and the *EE score* and *Expanded EE Score*, showing that no statistically significant differences were found for any health, feeding, or hygiene characteristics. We also measured the associations between the same risk factors and individual inflammation and enteropathy indicators (i.e., CRP, AGP, AAT, NEO, MPO) and found significantly higher concentrations of 1) AGP ($P = 0.015$) and EndoCAb ($P = 0.018$) in children from households without soap; 2) AGP ($P = 0.028$) in children with fever in the past 2 weeks; 3) NEO ($P = 0.031$) in children without minimum dietary diversity; and 4) MPO ($P = 0.039$) in children who were fed their bathwater in the past month (data not shown).

DISCUSSION

Child growth and inflammation and enteropathy. Our study found a significant negative association between AGP and HAZ, indicating that chronic inflammation is negatively associated with child growth. Other studies have observed similar associations; a recent publication Merrill et al.[34] found consistent associations between elevated AGP and the odds of stunting in children 6–59 months of age residing in Sub-Saharan African countries. Similarly, a publication of the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) project,[39] which investigates the associations between child growth and EE among children 3–20 months of age in multiple countries, found significant associations between AGP and future growth in early childhood. Moreover, the authors concluded that "evidence for the association with reduced linear growth was stronger for systemic inflammation than for gut inflammation."[39] Of note, the MAL-ED examined the association between systemic inflammation in early childhood and future growth later in childhood. By contrast, our study measured child growth and EE and systemic inflammation markers at the same time point.

Unlike AGP, elevated CRP concentrations were not associated with child growth or stunting in our study. This lack of association is likely explained by the fact that elevated CRP concentrations capture acute inflammation, which relates principally to recent illnesses and likely serves as a poor proxy for repeated illnesses at early ages. Prendergast et al.[40] observed that CRP and AGP concentrations were significantly higher in Zimbabwean stunted children from 6 weeks to 12 months of age; however, by 18 months of age there was no difference in CRP and AGP concentrations between non-stunted and stunted children. This finding suggests that acute and chronic inflammation may have a larger impact on child growth during infancy and young childhood compared with when children are older (e.g., > 2 years). This is supported by Merrill et al.,[34] who observed that the odds of having elevated AGP were significantly lower in children 36–59 months compared with children 12–23 months. Although Merrill et al. found

TABLE 3

Unadjusted and adjusted odds ratios for being stunted, and prevalence of inflammation and enteropathy biomarkers for non-stunted and stunted children 22–28 months of age

Characteristic	Non-stunted, *n* (%)*	Stunted, *n* (%)*	Unadjusted OR (95% CI)	Adjusted OR (95% CI)†
Systemic inflammation				
Elevated CRP	38 (25.7)	33 (20.5)	0.75 (0.44, 1.27)	0.57 (0.32, 1.01)
Elevated AGP	83 (56.1)	96 (59.6)	1.15 (0.74, 1.82)	1.07 (0.66, 1.74)
Any inflammation (elevated CRP and/or AGP)	89 (59.7)	98 (60.9)	1.05 (0.66, 1.65)	0.96 (0.59, 1.56)
Thurnham inflammation categories				
No inflammation	60 (40.3)	63 (39.1)	Reference	Reference
Incubation (elevated CRP only)	5 (3.3)	2 (1.2)	0.38 (0.07, 2.07)	0.34 (0.06, 1.87)
Early convalescence (elevated CRP & AGP)	34 (22.8)	31 (19.3)	0.87 (0.47, 1.59)	0.65 (0.34, 1.26)
Late convalescence (elevated AGP only)	50 (33.6)	65 (40.4)	1.24 (0.74, 2.07)	1.23 (0.72, 2.09)
Elevated endotoxin core antibody; > median 92.4 GMU/mL	77 (53.5)	72 (45.5)	0.73 (0.46, 1.15)	0.65 (0.41, 1.05)
Enteropathy				
Elevated NEO; > median of 587.5 nmol/L	69 (51.5)	64 (48.1)	0.87 (0.54, 1.41)	0.81 (0.49, 1.35)
Elevated AAT; > median of 0.15 mg/g	64 (48.5)	74 (52.2)	1.16 (0.72, 1.87)	1.22 (0.74, 2.01)
Elevated myeloperoxidase; > median of 1,591.7 ng/mL	50 (46.3)	63 (51.6)	1.24 (0.73, 2.08)	1.41 (0.81, 2.45)
Kosek EE score; > median of 5	33 (33.0)	38 (35.8)	1.13 (0.64, 2.02)	1.15 (0.62, 2.14)
Expanded EE score; > median of 7	33 (34.0)	38 (36.9)	1.13 (0.63, 2.02)	1.46 (0.79, 2.70)

AAT = alpha-1-antitrypsin; AGP = alpha-1-acid glycoprotein; CRP = C-reactive protein; EE = environmental enteropathy; NEO = neopterin.

* Note: The *n*'s are numbers of those exposed to the characteristic in each subgroup.

† OR adjusted for child's gender, child's age in months, child's anemia status, mother's anemia status, household ownership of soap, and household hunger.

few associations between child age and stunting when age was measured as a continuous variable, this categorical analysis suggests that age likely has a modifying effect on the association between AGP and stunting.

Our study found no significant association between child growth and EndoCAb. Endotoxin-core antibody is an antibody produced in response to exposure to lipopolysaccharide—a component of the cell wall of Gram-negative bacteria—following its translocation of the mucosal lining of the intestinal wall.[41] As such, EndoCAb has been suggested as a potential indicator of EE.[11] However, the current evidence examining the association between EndoCAb and stunting or child growth is mixed: Campbell et al. observed significant associations between EndoCAb concentrations and child growth and intestinal permeability (measured using lactulose:mannitol ratio) in Gambian children 1–16 months of age.[12] Other researchers[13,35] found an association of EndoCAb and growth in Gambian children below the age of 2 years, whereas no associations between EndoCAb concentrations and child growth were found in Malawian children aged 24–59 months[35] or Bangladeshi children 10–48 months of age.[13] Significant associations in studies, including only children less than 2 years, compared with the older age of children in this study may suggest that EndoCAb, similar to AGP, is more closely associated with growth in young children. The mediating effect of age on the association between inflammation and growth may be due to the fact that the period of rapid growth from 6 to 23 months of age is accompanied by repeated and increased exposure to environmental pathogens, as children consume complementary foods that are potentially prepared in unhygienic conditions and become more mobile and come into greater contact with their environment.[34,42] During this period of increased exposure to environmental pathogens, a child's adaptive immune system gradually matures, which influences a child's inflammatory response to infections.[43,44]

Contrary to other evidence, our study found no significant association between stunting or child growth status and fecal EE biomarkers or composite EE scores. To illustrate, Kosek et al. measured NEO, AAT, and MPO in children at either 3, 6, or 9 months of age in eight countries and found that children with the highest EE scores grew about 1 cm less than children with the lowest EE scores in the 6 months following the last

TABLE 4

Mean enteropathy scores by various risk factors of inflammation and enteropathy in children 22–28 months of age

Indicator	Mean Kosek EE score			Mean expanded EE score		
Characteristic (no/yes)	No	Yes	*P*-value*	No	Yes	*P*-value*
Child health characteristics						
Fever in past 2 weeks	4.8	4.6	0.601	7.8	7.6	0.642
Watery diarrhea in past 2 weeks	4.8	4.8	0.945	7.8	7.6	0.944
Child feeding characteristics						
Minimum dietary diversity†	4.7	4.8	0.837	7.9	7.7	0.633
Child fed own bathwater in past month	4.8	4.6	0.624	7.7	7.8	0.927
Child fed own bathwater when child was < 1 year old	4.9	4.6	0.363	7.8	7.7	0.794
Hygiene characteristics						
Improved sanitation facility	4.8	5.5	0.145	7.7	8.6	0.157
At least one handwashing practice	4.8	4.8	0.936	7.7	7.8	0.939
Soap present	4.6	4.9	0.497	8.0	7.6	0.298

EE = environmental enteropathy.

* *P*-value represents ANOVA test for continuous variables.

† Measured in children outside the age range recommended by World Health Organization.[27]

measurement (i.e., at either 9, 12, or 15 months of age).[14] The ability of Kosek et al. to clearly detect a relationship may be partly due to the longitudinal study design, the ability to control for incidence of diarrhea, and the fact that all subjects were within the typical window of growth faltering (i.e., < 24 months of age).[45]

In addition to differences in study design and subject age, our study observed relatively low concentrations of EE biomarkers compared with other studies. Kosek et al.[14] observed median NEO, AAT, and MPO concentrations of 1,847 nmol/L, 0.44 mg/g, and 11,118 ng/mL, respectively, across eight countries. In Bangladesh, George et al.[22] observed median NEO, AAT, and MPO concentrations of 1,506 nmol/L, 0.26 mg/g, and 3,577 ng/mL, respectively. It is not clear why our study observed lower fecal EE concentrations than these other studies. The implementation of a stringent cold chain during field collection undoubtedly prevented degradation. However, our study provided caretakers with simple plastic stool containers and not containers with a catalyst (e.g., Anaerocult® [Merck, Darmstadt, Germany], AnaeroGen™ [Hardy Diagnostics, Santa Maria, CA]) to produce an anaerobic environment for the sample. Thus, some degradation of proteins between defecation and collection by field staff could have occurred. That said, Kosek et al.[14] and George et al.[22] did not report using anaerobic catalysts as part of their stool collection, so our collection methods appear to closely match theirs.

Low concentrations of fecal EE biomarkers are surprising considering the suboptimal household sanitation conditions, and the fact that nearly all children consumed MNPs containing iron in the 6 months before data collection. Jaeggi et al.[46] found that consumption of MNPs with iron increased levels of enterobacteria and fecal calprotectin concentrations in children 6–10 months of age. Further research in northern Tanzania is required to identify why fecal EE biomarkers are lower than in other locations, as is it clear that the suboptimal sanitation and feeding practices would suggest that fecal EE markers should be high.

Household possession of soap. Presence of soap at the household level was the sole indicator related to hygiene and sanitation that was significantly associated with stunting and inflammation and EE indicators (i.e., AGP and EndoCAb). These findings are likely explained as regular handwashing with soap can prevent contamination of food stuffs and transmission of pathogens.[47] Although there is little data examining the linkages between handwashing and EE, a recent study in Bangladesh found that children 0–30 months of age were more likely to have elevated levels of fecal calprotectin—a marker of gut inflammation—when their caretaker had visibly soiled hands.[9] A recent study from India also found the risk of stunting in children 0–23 months of age was significantly lower for caregiver's self-reporting washing hands with soap before meals or after defecation.[48]

Notably, these studies observed linkages with caretaker hand hygiene rather than the child's hand hygiene; our study only collected information related to when caretakers washed their child's hand. Notably, our study found a higher proportion of caretakers reporting washing their child's hands before eating and after defecation than those possessing soap. This difference could be due to the fact that soap possession was determined based on interviewer observation, whereas the question about washing the child's hands (i.e., "When do you wash your child's hands?") did not specify that soap was used during hand washing. This could suggest that, in some instances, caretakers wash their child's hands without soap. Similar findings were found in by the CHANGE study's 2014 WASH formative research report,[49] in which data were collected through systematized caretaker and child observation of sanitation and hygiene practices in Tanzania's Sengerema and Ukerewe districts before the project being implemented. This report found that among the 60 households interviewed, 53 households had soap. Among soap-possessing households, soap used the day before the interview was primarily for washing dishes (81%), washing clothes (58%), bathing of adults (51%), and bathing children (47%). Contrarily, fewer respondents reported washing the child's hands (11%), washing hands after defecating (9%), and washing hands before giving food to the child (8%) the previous day. The report cited comments from focus group discussions, where participants noted that handwashing is not often practiced before eating but only after eating to clean food and oil from one's hands.

Although household ownership of soap may serve as an accurate proxy for washing dishes and clothes—activities that are routinely practiced on a daily and weekly basis—it may not be a suitable proxy for caretaker or child bathing. Daily bathing of Nepali children 0–59 months of age has been shown to reduce the odds of underweight,[50] but no other studies could be found. As bathing frequency of child and caretaker can be considered a strong proxy of general hygiene practices, future studies examining the determinants of stunting should consider collecting this information and examining associations with child growth.

Dietary diversity and household hunger. Our study found significant associations between dietary diversity and stunting. Of note, the proportion of children in our study with minimum dietary diversity (54% in non-stunted children; 42% in stunted children) was notably higher than that reported in the 2015–2016 DHS for children 6–23 months of age (i.e., 26%).[18] This difference was likely explained by the higher age of our study population. Previous findings from multicountry analyses suggest that dietary diversity is associated with linear growth. A pooled analysis of DHS data from 14 countries (including Tanzania) conducted by Marriot et al.[51] found that children 6–23 months of age with minimum dietary diversity were less likely to be stunted but only among children whose caretakers had completed higher levels of education (i.e., secondary school or greater). In another pooled analysis of 21 countries (not including Tanzania), Onyango et al.[52] found that 12 countries had significant associations between dietary diversity and linear growth in children 6–23 months of age.

Although no data on breastfeeding practices were collected from the children during the CHANGE endpoint assessment (i.e., when children were 22–28 months of age), the CHANGE baseline survey collected information on breastfeeding practices when children were 6–12.9 months of age. Among the children in the control areas of the CHANGE baseline survey, 45% were given breastmilk within 1 hour of birth, and 87% were fed colostrum. Nearly all children (98%) were ever breastfed and 83% received breast milk, as well as solid, semisolid, or soft foods, during the previous day.[53] As children enrolled in the baseline survey were older than 6 months of age, no data were collected on exclusive breastfeeding.

Regarding households hunger and food security, we found that of the two household-level scores, HFIAS and HHS, the stunting prevalence was significantly higher only for children in households reporting a HSS in the "severe" category. Because of the low proportion of children residing in households with severe household hunger, its contribution to the high stunting prevalence observed is minimal. Differing associations between HFIAS and HHS can be explained by the fact that they capture different dimensions of food insecurity. According to Maxwell et al. " ... the HHS measures the most extreme consequences of food insecurity, while the HFIAS captures a greater range of the food security spectrum."[32] Conversely, Psaki et al.[54] observed a significant relationship between HFIAS and HAZ but not HHS and HAZ. Thus, future studies examining the determinants of child growth should consider using both measures of household food insecurity as the influence of each indicator on child growth may vary depending on the local context.

Drinking bathwater and geophagy practices. Our study documents, for the first time, a seemingly infrequent cultural practice, whereby children were fed their bathwater after being bathed. Little is known about the rationale for this practice, the authors could not find any documentation of this practice in scientific literature, and this practice was not considered in the CHANGE study's 2014 formative research.[49] It is possible that feeding bathwater is practiced to protect children against pathogenic bacteria and other toxins and could thus relate to geophagy, a practice of soil eating. A 2011 review of geophagy practices[55] postulates that the practice of eating soil is most commonly a preventive approach to "inhibit parasites" and notes that geophagy is most common among pregnant women.[55] In Tanzania, a study of pregnant women in Dar es Salaam found that 29% of subjects regularly consumed soil,[56] and a study on Pemba Island found that 6% of women consumed soil and that consumption of soil was not associated with increased helminth infection (i.e., Ascaris, Trichuris, or hookworm).[57] The practice of eating earthy substances in humans can also be performed to augment a scanty or mineral-deficient diet or as part of a cultural tradition, but this cannot be said of drinking bathwater.

In contrast to research related to pregnant women, there are few studies that examine this practice in children. Though unclear, the practice of feeding a child his/her dirty bathwater appears to be a practice localized to Tanzania's Lake Zone. Although feeding a child bathwater in the past month or at any time when the child was < 1 year of age was not associated with stunting or growth status in the population, a bathwater feeding in the previous month was linked to higher MPO concentrations, and thus, may result in enteric inflammation in children. Additional research is needed to understand the rationale behind this practice, which could likely pose health risks to infants and young children as dirty bathwater likely contains soil, feces, and potentially toxins. In addition, understanding this practice is important for government and local organizations as feeding children bathwater may undermine interventions in stunting reduction programs.

Limitations. We acknowledge that our study has a few notable limitations. First, our case–control study did not contain retrospective information on EE status, and thus we used current EE status as a proxy indicator of previous exposure. As such, we were unable to measure EE during periods of typical growth faltering when children are between 6 and 24 months of age. Second, the age of our subjects was mostly outside the window of growth faltering. Thus, our study assumed that enteropathy at 22–28 months of age served as an adequate proxy for enteropathy during periods of more intense growth faltering. To address this, future case–control studies should be conducted in younger children (e.g., 6–18 months of age) to determine if enteropathy (measured cross-sectionally) is associated with child growth during the beginning and middle stages of growth faltering. Third, we were unable to measure hookworm infection, which has been shown to be more common than ascaris helminths among school aged children in Tanzania's Lake Zone.[58] However, soil-transmitted helminths such as hookworms affect more school aged children than preschool aged children,[59] and thus may not have been widely present in our study population. Further, examining hookworm infection requires that stool sample smears are prepared within 1 hour following the stool's passing because hookworm eggs deteriorate quickly.[60] Fourth, our study did not assess frequency of diarrhea and diarrheal status of the stool samples collected, and all stool samples were included in the analysis. As diarrheal stool samples have a greater variability of water content than non-diarrheal samples,[61] the concentrations of stool biomarkers could have been lower than non-diarrheal samples.

CONCLUSION

This study used a case–control design to examine the potential determinants of stunting in stunted and non-stunted children 22–28 months of age in Tanzania's Lake Zone, with a focus on inflammation and EE markers. The results showed that chronic inflammation, measured by elevated AGP, was significantly associated with child growth. Household possession of soap was associated with higher HAZ and lower concentrations of inflammatory biomarkers. The ownership of soap likely had protective effects on child growth by reducing exposure to bacteria and pathogens that may have resulted in inflammation and EE. Although no significant association was observed between EE and growth, this may be due to the non-retrospective nature of the study and the fact that most children were outside the age of typical growth faltering.

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