

# Effect of a Homestead Food Production and Food Hygiene Intervention on Biomarkers of Environmental Enteric Dysfunction in Children Younger Than 24 Months in Rural Bangladesh: A Cluster-Randomized Controlled Trial

Anna A. Müller-Hauser,<sup>1,2\*</sup> Tarique Md. Nurul Huda,<sup>3,4</sup> Shafinaz Sobhan,<sup>1,2</sup> Nathalie J. Lambrecht,<sup>1,2</sup> Jillian L. Waid,<sup>2,5</sup> Amanda S. Wendt,<sup>2</sup> Shahjahan Ali,<sup>6,7</sup> Mahbubur Rahman,<sup>4</sup> and Sabine Gabrysch<sup>1,2,5</sup>

<sup>1</sup>Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Public Health, Berlin, Germany; <sup>2</sup>Research Department 2, Potsdam Institute for Climate Impact Research, (PIK), Member of the Leibniz Association, Potsdam, Germany; <sup>3</sup>Department of Public Health, College of Public Health and Health Informatics, Qassim University, Al Bukairiyah, Saudi Arabia; <sup>4</sup>Environmental Interventions Unit, Infectious Diseases Division, icddr,b, Dhaka, Bangladesh; <sup>5</sup>Heidelberg Institute of Global Health, Heidelberg University, Heidelberg, Germany; <sup>6</sup>Infectious Diseases Division, icddr,b, Dhaka, Bangladesh; <sup>7</sup>Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, Colorado

**Abstract.** Poor sanitation and hygiene practices and inadequate diets can contribute to environmental enteric dysfunction (EED). We evaluated the impact of a combined homestead food production and food hygiene intervention on EED biomarkers in young children in rural Bangladesh. The analysis was conducted within the Food and Agricultural Approaches to Reducing Malnutrition (FAARM) cluster-randomized trial in Sylhet, Bangladesh. The FAARM trial enrolled 2,705 married women and their children younger than 3 years of age in 96 settlements (geographic clusters): 48 intervention and 48 control. The 3-year intervention (2015–2018) included training on gardening, poultry rearing, and improved nutrition practices and was supplemented by an 8-month food hygiene behavior change component, implemented from mid-2017. We analyzed data on 574 children age 0 to 24 months with multilevel linear regression. We assessed fecal myeloperoxidase (MPO), neopterin (NEO), and alpha-1-antitrypsin (AAT) as biomarkers of EED, and serum C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) as biomarkers of systemic inflammation, using ELISA. There was no intervention effect on NEO, AAT, CRP, and AGP concentrations, but, surprisingly, MPO levels were increased in children of the intervention group (0.11 log ng/mL; 95% CI, 0.001–0.22). This increase was greater with increasing child age and among intervention households with poultry that were not kept in a shed. A combined homestead food production and food hygiene intervention did not decrease EED in children in our study setting. Small-scale poultry rearing promoted by the intervention might be a risk factor for EED.

## INTRODUCTION

Worldwide, nearly one quarter of children younger than 5 years are too short for their age, which indicates widespread undernutrition. The developmental consequences of undernutrition include compromised immune function and impaired cognitive development.<sup>1,2</sup> Alongside insufficient intake of nutritious food and recurring diarrheal diseases, environmental enteric dysfunction (EED) is hypothesized to be a major cause of undernutrition.<sup>3–5</sup>

EED is a subclinical disorder of the small intestine characterized by villous atrophy, crypt hyperplasia, and lymphocyte infiltration, which result in intestinal permeability, nutrient malabsorption, and intestinal and systemic inflammation.<sup>3,6–9</sup> EED is thought to contribute to undernutrition through decreased nutrient absorption, as well as increased energy requirements resulting from ongoing intestinal and systemic inflammation and immune activation.<sup>4,6,10–14</sup> There is no agreed-upon case definition for EED. As the manifestation of EED is highly variable among individuals, studies usually measure a combination of biomarkers that reflect the different domains of EED: intestinal damage and repair (e.g., measured by Regenerating [REG] family proteins), permeability and absorption (e.g., fecal alpha-1-antitrypsin [AAT] or zonulin), microbial translocation (e.g., serum lipopolysaccharides [LPS] or anti-LPS IgG), intestinal inflammation (e.g., fecal myeloperoxidase [MPO] and neopterin [NEO]),

and systemic inflammation (e.g., C-reactive protein [CRP] or alpha-1-acid glycoprotein [AGP]).<sup>15</sup> Several of these biomarkers have been associated with poorer child growth outcomes.<sup>16–25</sup>

Environmental enteric dysfunction often begins in early infancy. Repeated exposure to enteric pathogens from contaminated food, water, or the environment resulting, for example, from poor water, sanitation, and hygiene (WASH) conditions or exposure to livestock in the household setting, is thought to contribute to EED.<sup>26–30</sup> In addition, poor infant feeding practices and intake of food with low nutrient densities have been associated with an increased risk of developing EED<sup>14,31</sup>—for example, by weakening intestinal barrier function and mucosal repair.<sup>32</sup>

Therefore, a combined nutrition and hygiene intervention may have the potential to reduce or prevent EED in children. However, recent trials investigating combined WASH and nutrition interventions could not report a clear and consistent effect on EED biomarkers.<sup>33,34</sup> Although the WASH Benefits trial<sup>33</sup> in Bangladesh observed lower concentrations for some biomarkers of intestinal permeability and inflammation among the intervention arms during the course of the study (3- and 14-month assessments), by the age of 28 months, children in the intervention arms showed higher biomarkers of EED compared to control subjects. Also, the SHINE trial<sup>34</sup> in Zimbabwe observed only minor, inconsistent effects of its nutrition and WASH interventions on EED biomarkers. A study in India<sup>25</sup> assessing the effect of a household-level water and sanitation infrastructure intervention observed a decrease in intestinal permeability but not intestinal inflammation in children from intervention households compared with control subjects. Findings from these studies suggest

\*Address correspondence to Anna A. Müller-Hauser, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Public Health, Berlin, Germany. E-mail: anna.mueller-hauser@charite.de

that the interventions did not interrupt contamination pathways sufficiently, or that other unaddressed pathways, such as livestock exposure and food contamination, exposed children to pathogens.

Microbial contamination of food is highly prevalent in resource-poor settings and is a known contributor to intestinal infection and diarrheal disease.<sup>35–38</sup> There is evidence that suboptimal household hygiene around food preparation and feeding leads to contamination of complementary foods.<sup>39–44</sup> Therefore, good caregiver food hygiene practices could contribute to a reduction in complementary food contamination and intestinal disease. Recent studies<sup>45–47</sup> showed that interventions using social and behavior change techniques were successful in improving caregiver food hygiene practices in low-income settings in sub-Saharan Africa and South Asia. However, there is currently no evidence on whether food hygiene behavior change interventions can improve child health outcomes, or if a combined approach to improve food hygiene behavior and dietary intake through nutrition diversification can affect EED in children.

The Food Hygiene to reduce Environmental Enteric Dysfunction (FHEED) study was designed to evaluate a behavior change intervention within the Food and Agricultural Approaches to Reducing Malnutrition (FAARM) cluster-randomized controlled trial in Sylhet, Bangladesh. In this study, we assess the effect of an intervention that trained women's groups in gardening, poultry rearing, and food hygiene on EED in children younger than 24 months.

## MATERIALS AND METHODS

**Study design and participants.** Data analyzed for this study are from the FAARM cluster-randomized controlled trial (ClinicalTrials.gov, ID: NCT02505711) conducted from 2015 to 2020, and the FHEED substudy. Detailed information about the FAARM trial design, study setting, and study population can be found in the study protocol.<sup>48</sup> In brief, the FAARM trial evaluated the impact of a homestead food production (HFP) program, implemented through Helen Keller International from mid 2015 to late 2018, on child undernutrition in the rural Habiganj District, Sylhet Division, northeastern Bangladesh. The trial enrolled 2,705 married women, with a self-reported age of 30 years or younger at enrollment, in 96 settlements (geographic clusters). Covariate-constrained randomization was used to assign 48 settlements to the intervention and 48 to the control group.

The HFP program included training on year-round home gardening, small-scale poultry rearing, and improved nutrition and hygiene practices. The project staff conducted training sessions with woman farmer groups at least every 2 months, supplemented by individual household visits every 2 months.<sup>48</sup> The HFP program had a strong focus on diversified food production and improved nutrition practices; improving hygiene was limited to messages around handwashing and instructions to build handwashing stations. Therefore, an additional food hygiene module was added (implemented from June 2017 to February 2018) to strengthen household food hygiene practices, especially around food preparation and child feeding.

The food hygiene module was based on a Nepali intervention,<sup>45</sup> adapted to the FAARM population, and integrated to fit the ongoing intervention activities and study setting. Using

a behavior-centered design and emotional drivers, the module promoted four specific food hygiene behaviors: washing hands with soap and water, washing feeding utensils with soap and water, cooking fresh food or reheating leftover food before feeding, and storing food and water safely. This was done under the hypothesis that improved food hygiene practices would prevent or decrease complementary food contamination and thereby reduce the risk of undernutrition from intestinal infections and disease. A detailed description of the design, implementation, and uptake of the food hygiene module has been published.<sup>49</sup>

The FHEED substudy was designed to analyze the impact of the combined HFP and food hygiene intervention on household food hygiene practices, contamination of complementary foods, and intestinal infection and inflammation among children 0 to 18 months old at the trial's endline assessment in 2019. All children enrolled in the FHEED substudy were thus born during or after delivery of the food hygiene module, ensuring they could benefit from that intervention throughout infancy. Supplemental Figure 1 depicts the timeline of the FAARM trial and FHEED substudy.

**Sample size.** The main outcome variables were fecal biomarker concentrations of MPO, AAT, and NEO at endline. To detect intervention-related differences with a minimal standardized effect size of 0.3 for these biomarkers, a sample size of ~300 children per study group (on average, six children per cluster) was deemed necessary ( $\alpha = 0.05$ ,  $\beta = 0.20$ ). To achieve the required sample size, we included all children born between November 2017 and October 2019 in the endline assessment, which increased the age range of the final sample from 0 to 18 to ~0 to 24 months.

**Randomization and masking.** The randomization procedure is explained in detail in the FAARM study protocol.<sup>48</sup> The nature of the intervention did not allow masking of the implementation team and participants in intervention settlements. A distance of at least 400 m between settlements was chosen to avoid spillover of intervention activities to control settlements. Data collectors and laboratory staff of the FHEED substudy were not engaged in any intervention activities and were not informed about settlement allocation. However, data collectors could have observed intervention materials during data and sample collection in the intervention settlements.

**Data collection.** For this analysis, we used data from the following FAARM surveys: 1) demographic characteristics at baseline (March to May 2015), 2) data on poultry ownership from the routine assessment conducted as part of the FAARM surveillance system (May to September 2019), 3) characteristics and child health status from the FAARM endline survey (September 2019 to April 2020), 4) child health status during collection of stool samples at the FAARM endline survey (September to December 2019).

The FAARM baseline and endline surveys collected data on women and household characteristics such as education, age, religion, and household wealth, as well as on sanitation facilities of all households. Education was measured as the number of school years completed and was grouped into none, some or completed primary education, and some or completed secondary education. At baseline and endline, we calculated wealth quintiles using principal component analysis of household assets, in line with the Bangladesh Demographic and Health Survey.<sup>50</sup> A handwashing station

was considered functional when it was equipped with water, a cleaning agent, and a pouring device. Access to basic sanitation facilities was defined as the presence of a flush/pour flush toilet connected to a piped sewer system, septic tank, or pit latrine; a pit latrine with a slab (including a ventilated pit latrine); and a composting toilet, not shared with other households.<sup>51</sup>

During the surveillance system's routine assessment in May to September 2019, data on household poultry ownership, including the reported number of poultry in a household, reported ownership of a poultry shed (traditional or improved), and observed use of the poultry shed (for keeping poultry or other) were collected as prespecified pathway indicators of the FAARM trial.

At the time of serum and fecal sample collection during the FAARM endline survey, data on child health were collected. Caregivers were asked whether their child was sick with loose or mushy stools within the past 7 days prior to the survey. If caregivers reported loose or mushy stools, they were asked for the number of stools passed on the worst day. A child was considered sick with diarrhea when a caregiver reported three or more loose, mushy stools on at least 1 day within the 7 days prior to the survey. We also collected information on disease severity, including caregivers' report of blood in the stool or a fever. Questionnaires were administered by trained data collection officers (holding at least a bachelor's degree) who conducted face-to-face interviews with the respondents. All survey data were collected using the tablet-based application Open Data Kit.<sup>52</sup>

**Biological sample collection.** Fecal samples of participating children were collected by their caregivers at endline. Caregivers were provided with a sterile stool collection tube, gloves, and instructions on how to collect the stool sample from their children (infographic as well as verbal explanation and practical demonstration by the sample collector). If possible, mothers collected the first stool sample in the morning. The field team collected the stool samples from the households and stored them in a cool box with icepacks, monitoring the temperature in the cool box (8–10°C) at all times. If a child did not pass stool in the morning, the sample collector returned to the household later that day to collect the sample. Samples were brought to the field laboratory by noon, when they were aliquoted and stored without additives at –20°C until shipment to Germany, where they were stored at –80°C until further analysis.

Serum samples of participating children older than 6 months were collected by trained and experienced phlebotomists at endline. Approximately 3 mL of venous blood was drawn from the arm vein and collected into a serum tube (BD Vacutainer trace element serum tubes, BD, Franklin Lakes, NJ). Samples were stored in a cool box with icepacks and brought to the field laboratory within 5 hours after sample collection. For serum separation, samples were centrifuged for 15 minutes at 1,500 × *g*. Serum was aliquoted and stored at –20°C until further analysis.

**Biomarker analyses.** Biomarkers of EED (MPO and NEO measuring intestinal inflammation, AAT measuring intestinal permeability) were measured in stool samples by ELISA. Commercially available ELISA kits for MPO (R&D Systems, Minneapolis, MN), AAT (R&D Systems, Minneapolis, MN), and NEO (IBL International, Hamburg, Germany) were used according to the manufacturer's instructions. A detailed description of the assay procedures can be found in Supplemental Appendix 1. In brief,

samples were homogenized in assay buffer (Ref. KENO751, IBL International) for 2 × 1 minute using a bead beater. Samples were centrifuged at 2,500 × *g* at 4°C to clear the samples. Supernatants were diluted 1:5,000 (MPO), 1:20,000 (AAT), and 1:100 (NEO) with the respective assay buffer. ELISAs were run in technical duplicates. Samples with out-of-range values were retested at an appropriate dilution.

Biomarkers of systemic inflammation (CRP as a marker for acute inflammation and AGP as a marker for chronic inflammation) were measured in serum samples using a combined sandwich ELISA technique by the VitMin Laboratory (Willstaett, Germany).<sup>53</sup>

**Outcomes.** Prespecified outcomes were fecal concentrations of MPO, AAT, and NEO and serum concentrations of CRP and AGP in children younger than 2 years of age. For their use in regression analyses, biomarker concentrations were log-transformed to achieve a normal distribution. Additional outcomes of interest in this sample were 7-day period prevalence of caregiver-reported diarrhea in children and diarrhea severity.

**Statistical analysis.** All data analyses were performed in Stata SE version 14.2 (StataCorp, College Station, TX). Biomarker concentrations were summarized as median and interquartile range or were log-transformed and summarized as mean and SE. In addition, we calculated Pearson correlations between concentrations of each biomarker. Other continuous variables were summarized using mean and SD, and categorical variables using proportions.

We used mixed-effects linear regression models to estimate the intervention effect on each log-transformed biomarker, with settlement-level random effects and adjustments for age and sex of the child to increase precision. Intervention effects by child age were calculated using an interaction term between intervention allocation and grouped child age (0–5 months, 6–11 months, 12–17 months, and 18–24 months). Predicted biomarker concentrations were calculated using the *margins* command, and the *lincom* command was used to calculate point estimates and 95% CIs. Mixed-effects linear regression models also adjusting for baseline household wealth were run as sensitivity analyses, as household wealth was slightly imbalanced between the intervention and control groups. All analyses estimating the intervention effect on EED biomarkers were intention-to-treat.

We also conducted post hoc analyses to explore whether the intervention's promotion of poultry may have increased EED biomarkers. To assess associations between poultry ownership and log-transformed biomarkers, we used mixed-effects linear regression models, with settlement-level random effects and adjusting for age and sex of the child 1) including only poultry variables as the exposure, 2) including intervention allocation as a covariate, and 3) including an interaction term between intervention allocation and poultry variables to calculate effects stratified by poultry ownership and poultry location, or including the settlement-level average number of poultry. The Stata command *margins dydx* was used to calculate marginal effects. A likelihood ratio test was used to compare models with and without the interaction term.

## RESULTS

Our analysis includes data from 574 children 0 to 24 months old and their households, collected during the FAARM

endline survey in 2019 (90% of the 635 children born between November 1, 2017 and October 31, 2019). Analyses of the serum biomarkers CRP and AGP were performed on a smaller subset of 428 children, as blood was collected only from children 6 months or older. Figure 1 provides details on how many children were included in the analysis of different biomarkers, as well as reasons for exclusion.

Background characteristics of the intervention and control households were largely similar regarding household size, religion, and wealth, with a slight imbalance in that there were slightly fewer intervention households in the least-wealthy quintiles (Table 1). At endline in 2019, ~60% of households had access to a functional handwashing facility, equipped with soap and water, and more than 50% had access to a basic sanitation facility that was not shared with another household. Approximately 85% of women had at least some primary education, and the index child was 13 months old on average on the day of stool collection (Table 1).

The median values for biomarkers of enteric dysfunction were 23,233 ng/mL MPO, 422 µg/mL AAT, and 1,534 nmol/L NEO. Median values for biomarkers of systemic inflammation were 0.62 mg/L CRP and 0.77 g/L AGP (Table 2). MPO, NEO, and CRP showed a decreasing trend in concentration with increasing child age, whereas median AAT concentrations were slightly higher in girls (Table 2). Pairwise correlations between log MPO, log AAT, and log NEO were rather weak, with the greatest correlation between MPO and AAT (correlation coefficient  $r = 0.4$ ). C-reactive protein and AGP showed a high correlation ( $r = 0.66$ ). No correlation was observed between biomarkers of enteric dysfunction and systemic inflammation (Supplemental Table 1).

Approximately 6% of mothers reported that their children had diarrhea within the past 7 days, and the prevalence did not differ between children in the intervention and control groups (Supplemental Table 2). Biomarker levels of EED

were not elevated in children with reported diarrhea in the past 7 days; children with diarrhea even showed lower levels of MPO and AAT compared to children without diarrhea (Supplemental Table 3).

There was no effect of the intervention on AAT and NEO concentrations nor on CRP and AGP concentrations among children younger than 24 months of age using linear mixed-effect models adjusting for clustering, child age, and sex (Table 3). There was some evidence that the intervention led to an increase in MPO levels (0.11 log ng/mL; 95% CI, 0.001–0.22;  $P = 0.047$ ; Table 3), with MPO concentrations in intervention children ~11% greater than in control children. The increase in MPO concentration was dependent on child age. Although MPO concentrations were similar in children 0 to 5 months old, they diverged with increasing child age and showed the greatest difference in children 18 to 24 months of age (Figure 2 and Supplemental Table 4).

As the FHEED study was originally designed to investigate the effect of the food hygiene and HFP intervention on children 0 to 18 months old, additional regression models were run, including only children younger than 18 months. For this subgroup, the intervention had no effect on any of the inflammatory biomarkers, including MPO (Supplemental Table 5).

Given the slight imbalance in household wealth at baseline between study groups, we also assessed the intervention effects on each EED biomarker while adjusting not only for age and sex of the child, but also for baseline wealth as a sensitivity analysis. We found no major differences in the effect estimates compared with the models not adjusting for baseline wealth (Supplemental Table 5).

Among other activities, the HFP intervention promoted small-scale poultry rearing in the intervention households. Accordingly, in 2019, in the intervention arm, more children were in households that owned poultry (62% versus 56%, intervention versus control), and among poultry owners,

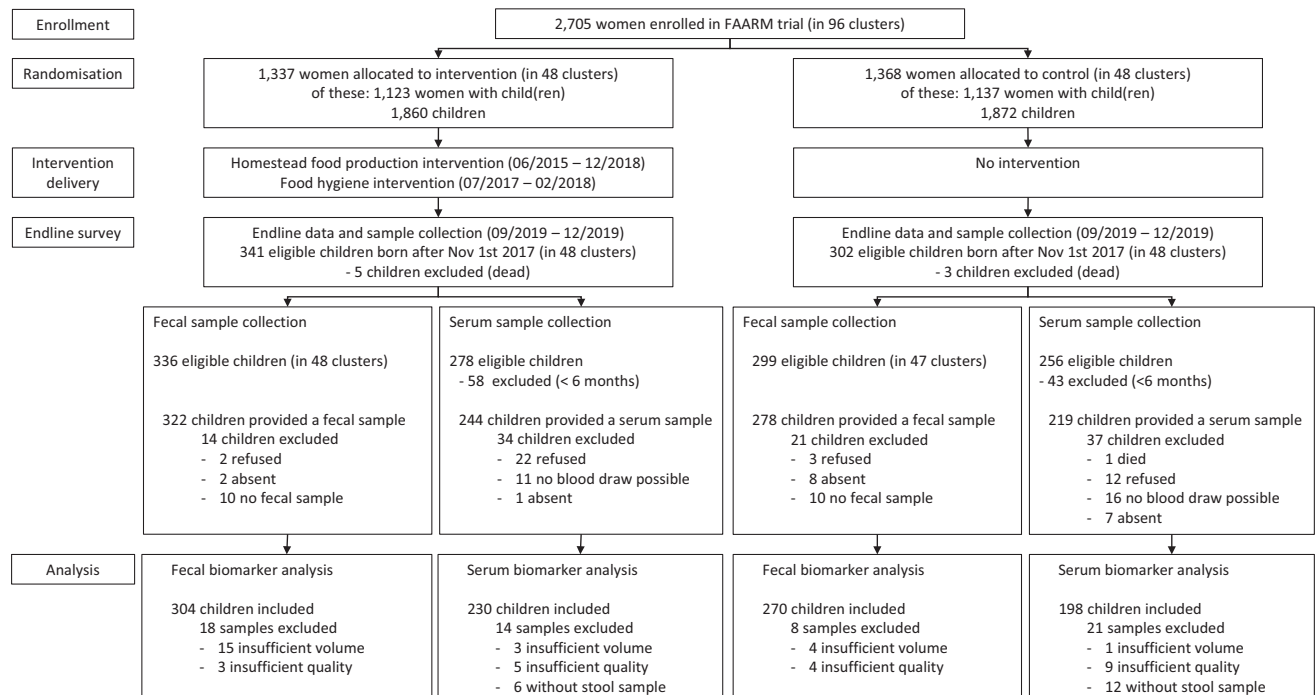


FIGURE 1. Study flow diagram. FAARM = Food and Agricultural Approaches to Reducing Malnutrition.

TABLE 1  
Characteristics of households, women, and children of the study population in rural Sylhet, Bangladesh

Characteristic*	Overall, % or mean (SD)	Control, % or mean (SD)	Intervention, % or mean (SD)
<b>Household characteristics</b>			
Intervention allocation			
Control	47	–	–
Intervention	53	–	–
Religion			
Muslim	75	73	77
Hindu	25	27	23
No. of household members†	7.2 (3.4)	7.3 (3.3)	7.0 (3.4)
Wealth at baseline‡			
Poorest	25	26	23
Lower	23	26	21
Middle	19	18	20
Upper	19	17	22
Wealthiest	14	13	14
Wealth at endline§			
Poorest	29	32	26
Lower	19	21	18
Middle	19	16	22
Upper	19	17	20
Wealthiest	14	14	14
Access to a functional handwashing station at endline	58	56	60
Access to basic sanitation facilities at endline¶	57	56	57
<b>Women characteristics</b>			
Age at baseline, years	22.8 (3.9)	22.7 (3.8)	23.0 (3.9)
Education#			
None	14	14	15
Partial/complete primary	47	48	46
Partial secondary or more	39	38	39
<b>Children characteristics</b>			
Age at endline stool collection survey, months	12.9 (6.5)	12.5 (6.3)	13.2 (6.5)
Sex			
Male	54	55	54
Female	46	45	46

N = 574; n = 270 (control), n = 304 (intervention).

\* If not indicated otherwise, characteristics were collected during the baseline survey in 2015.

† For number of household members, n = 546; missing data, n = 28.

‡ For wealth at baseline, n = 562; missing data, n = 12.

§ For wealth at endline, functional handwashing station, and access to latrines, n = 570; missing data, n = 4.

|| A functional handwashing station is equipped with water, detergent, and a pouring device.

¶ Access to basic sanitation facilities includes flush/pour flush toilets connected to a piped sewer system, septic tanks or pit latrines, pit latrines with slabs (including ventilated pit latrines), and composting toilets not shared with other households.

# Partial/complete primary education consists of up to 5 years of schooling. Partial secondary or more education consists of up to 10 years of schooling or higher education.

intervention households had a slightly greater number of poultry (3.9 versus 3.3). The average number of poultry per settlement was also slightly greater in the intervention settlements compared with control settlements (2.9 versus 2.0). More intervention households owned a modern poultry shed (56% versus 0.4%) and kept poultry in a shed for at least

some time during the day or night (34% versus 4%, Supplemental Table 6).

As previous studies showed an association between poultry ownership and increased EED biomarkers, enteric infections, and diarrheal disease,<sup>28,54,55</sup> we investigated the association of MPO and poultry ownership in a post hoc

TABLE 2  
Summary measures of EED and systemic inflammation biomarker concentrations overall by child age and sex

Variable	n	Biomarker in stool			Biomarker in serum		
		MPO, ng/mL; median (IQR)	AAT, µg/mL; median (IQR)	NEO, nmol/L; median (IQR)	n	CRP, mg/L; median (IQR)	AGP, g/L; median (IQR)
Overall	574	23,233 (9,481–55,227)	422 (233–720)	1,534 (753–3,089)	428	0.62 (0.14–2.42)	0.77 (0.58–1.11)
<b>Child age, months</b>							
0–5	79	39,686 (21,105–92,967)	455 (228–731)	1,920 (1,265–3,412)	*	*	*
6–11	179	28,691 (12,746–56,629)	425 (232–707)	2,064 (1,175–3,441)	148	0.84 (0.17–2.86)	0.84 (0.59–1.18)
12–17	134	21,740 (8,194–44,635)	398 (225–654)	1,367 (701–2,718)	119	0.61 (0.10–2.0)	0.73 (0.58–1.09)
18–24	182	17,170 (6,701–39,899)	412 (246–747)	922 (395–1,872)	161	0.51 (0.09–2.02)	0.75 (0.57–1.01)
<b>Child sex</b>							
Male	312	22,935 (8,841–53,546)	399 (216–669)	1,557 (795–3,126)	230	0.69 (0.17–2.25)	0.78 (0.59–1.08)
Female	262	23,323 (10,472–57,474)	454 (262–788)	1,455 (717–2,956)	198	0.55 (0.12–2.56)	0.76 (0.57–1.14)

AAT = alpha-1-antitrypsin; AGP = alpha-1-acid glycoprotein; CRP = C-reactive protein; EED = environmental enteric dysfunction; IQR = interquartile range; MPO = myeloperoxidase; NEO = neopterin.

\* Serum was not collected from children younger than 6 months of age.

TABLE 3  
Effect of the intervention on of EED and systemic inflammation biomarker concentrations

Inflammatory marker	<i>n</i>	Mean	Robust SE	Coefficient (95% CI)*	<i>P</i> value*
log MPO ng/mL					
Control	270	4.28	0.044	0.12 (0.001 to 0.22)	0.047
Intervention	304	4.38	0.038		
log AAT $\mu$ g/mL					
Control	270	2.58	0.033	0.05 (−0.04 to 0.14)	0.29
Intervention	304	2.63	0.033		
log NEO nmol/L					
Control	270	3.13	0.030	0.06 (−0.02 to 0.15)	0.13
Intervention	304	3.18	0.032		
log CRP mg/L <sup>#</sup>					
Control	198	−0.31	0.069	0.13 (−0.04 to 0.29)	0.13
Intervention	230	−0.20	0.052		
log AGP g/L <sup>#</sup>					
Control	198	−0.09	0.017	−0.01 (−0.05 to 0.03)	0.55
Intervention	230	−0.11	0.013		

AAT = alpha-1-antitrypsin; AGP = alpha-1-acid glycoprotein; CRP = C-reactive protein; EED = environmental enteric dysfunction; MPO = myeloperoxidase; NEO = neopterin.

<sup>#</sup>All children younger than 6 months on the day of blood collection were excluded from sampling because they did not provide a serum sample.

\* From multilevel regression models, adjusted for child age and sex, with settlement random effects.

analysis as a potential explanation for the increase in MPO levels in children's stool samples. Overall, there was rather weak evidence that MPO levels were increased by poultry ownership (0.07 log ng/mL; 95% CI, −0.02 to 0.16; *P* = 0.11) or not keeping poultry in a shed (versus no poultry: 0.09 log ng/mL; 95% CI, −0.009 to 0.19; *P* = 0.07). Poultry tend to roam; therefore, we examined not only household poultry ownership, but also the average number of poultry at the settlement level.<sup>56</sup> There was also weak evidence for increased MPO concentrations with a greater average number of poultry at the settlement level (0.04 log ng/mL; 95% CI, −0.003 to 0.08; *P* = 0.07; Supplemental Table 7).

Evidence for an association between poultry location and MPO levels was slightly stronger when accounting for intervention allocation as a covariate (poultry not in shed versus no poultry: 0.1 log ng/mL MPO; 95% CI, 0.002–0.20, *P* = 0.05). When stratifying the intervention effect on MPO by poultry ownership or poultry location, using an interaction term, we found no effect on MPO concentrations among intervention households without poultry, weak evidence for an increase in MPO among intervention households owning poultry (0.13 log ng/mL; 95% CI, −0.006 to 0.26; *P* = 0.06), and some evidence for an increase among intervention households not keeping poultry in a shed (versus no poultry: 0.2 log ng/mL; 95% CI, 0.05–0.36; *P* = 0.01); however, there was no evidence of an interaction of these subgroups with the intervention (Supplemental Table 8).

## DISCUSSION

Our results suggest that a combined nutrition and food hygiene intervention integrated in an HFP program in rural Bangladesh had no beneficial impact on biomarkers of EED and systemic inflammation in children younger than 24 months of age. There was no evidence of an intervention effect on concentrations of AAT, NEO, CRP, and AGP. For MPO, we found weak evidence for an adverse effect of the intervention, possibly on older children of the study population, and in households that did not keep poultry in a shed.

Approximately 15% of study children had increased levels of CRP and 30% increased levels of AGP (> 5 mg/L and > 1 g/L, respectively; the defined cutoff values indicative of

inflammation<sup>57</sup>), indicating ongoing systemic immune activation in a substantial proportion of children in our study population. There are no agreed-upon reference values for MPO, AAT, and NEO concentrations in children of this age group. The median concentrations for AAT and NEO measured in our study were comparable to concentrations reported in the MAL-ED study<sup>17</sup> and WASH Benefits trial<sup>33</sup> in Bangladesh. However, our MPO concentrations were greater and more comparable to concentrations reported from MAL-ED sites in South Africa or Tanzania.<sup>17</sup> As described in previous studies,<sup>33,34,58</sup> we also observed a decrease in NEO and MPO concentrations with increasing child age.

Similar to our study, two previous large-scale cluster-randomized trials that evaluated combined nutrition and WASH interventions on EED biomarkers in children failed to show clear beneficial effects.<sup>33,34</sup> The SHINE trial<sup>34</sup> in Zimbabwe, assessing the effect of a combined infant and young child feeding (IYCF) and WASH intervention on biomarkers of EED in children 1, 3, 6, 12, and 18 months old observed a slight decrease in NEO concentrations in children of 18 months in the IYCF group compared with the non-IYCF group. There was also a small decrease in kynurenine in children 12 months old and an increase in insulin-like growth factor 1 in children 18 months of age in the WASH group compared with the non-WASH group, but no effects on other EED biomarkers.<sup>34</sup> The WASH Benefits trial in Bangladesh,<sup>33</sup> which evaluated a WASH intervention with or without nutrition supplementation, observed lower concentrations for some biomarkers of intestinal permeability (lactulose and mannitol) and inflammation (NEO) in children 3 and 14 months in the intervention arms; however, by the age of 28 months, they showed higher biomarker levels of intestinal inflammation (MPO) than controls.<sup>33</sup> A matched cohort study<sup>25</sup> in India that assessed the effect of a household-level water and sanitation infrastructure intervention observed a decrease in intestinal permeability (AAT) but not intestinal inflammation in children from intervention households compared with control households. Similarly, a substudy<sup>59</sup> of the WASH for WORMS cluster-randomized trial, evaluating a community-wide WASH intervention in Timor-Leste, reported slightly lower MPO concentrations in children 1 to 5 years of age from intervention households, but no effect on other biomarkers that were measured.

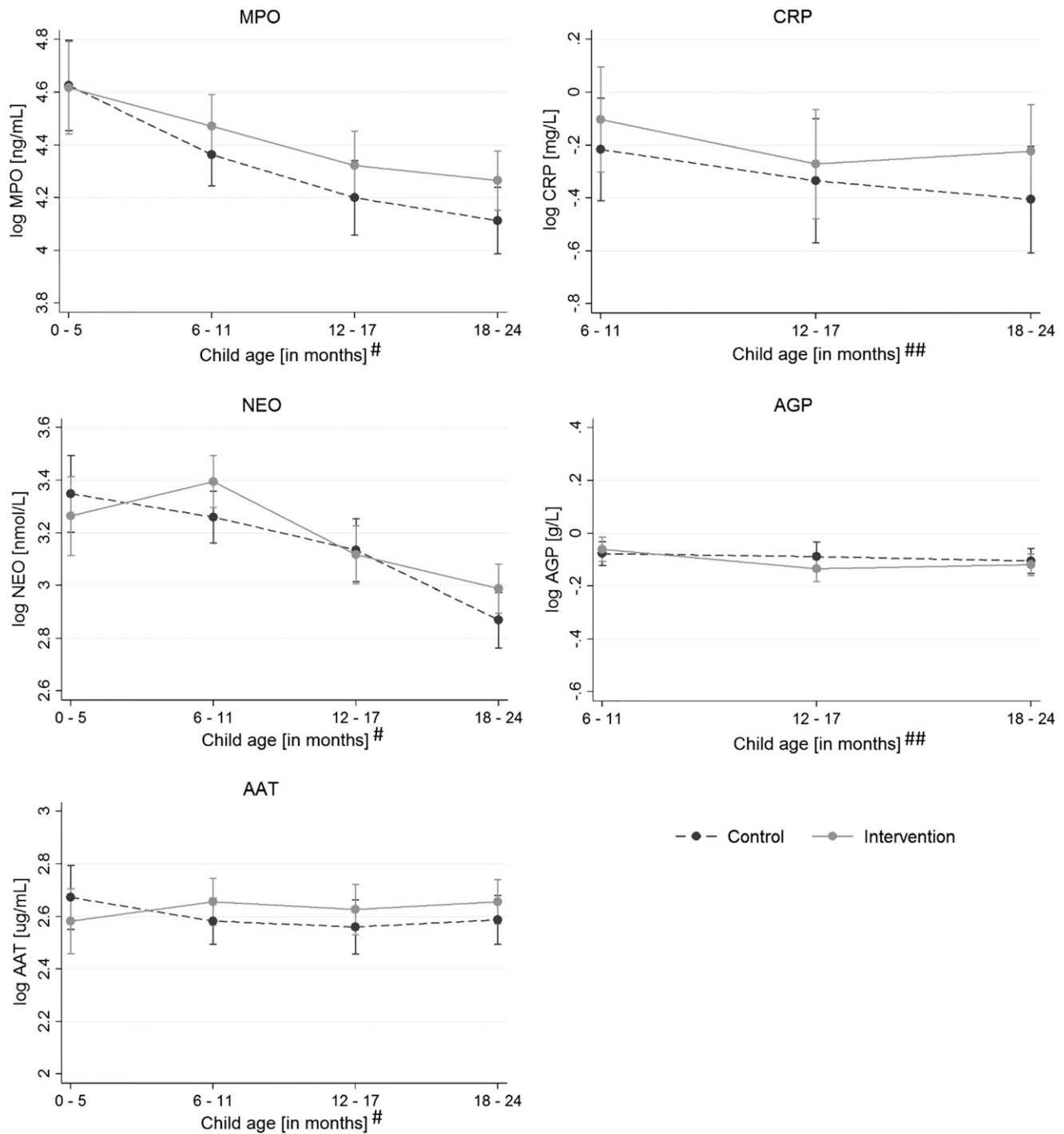


FIGURE 2. Concentration of biomarkers for environmental enteric dysfunction (EED) and systemic inflammation by intervention group and child age. The graphs show predicted concentrations and 95% CIs using marginal standardization from multilevel regression models, adjusted for child sex, with settlement random effects ( $n = 574$  for log myeloperoxidase [MPO], log neopterin [NEO], log alpha-1-antitrypsin [AAT];  $n = 428$  for log C-reactive protein [CRP] and log alpha-1-acid glycoprotein [AGP]). #Child age at the time of stool sample collection. ##Child age at the time of blood sample collection. Light-gray solid lines indicate intervention; dark-gray dashed lines indicate control.

In contrast to the previously mentioned studies, the FAARM trial focused on skill building and only supplied assets for HFP. The additional food hygiene intervention did not provide any infrastructure, but rather concentrated on changing caregivers' household food hygiene practices through social and behavior change techniques. Therefore, the success of the intervention relied mainly on faithful

implementation of the intervention curriculum, high uptake of intervention messages, and consistent practice of the promoted behaviors by the women and households. According to our theory of change, the two possible pathways through which the intervention could have affected EED positively are through its food hygiene component and through improved consumption of nutrient-rich foods.

An effect through nutrient-rich food consumption could be expected, given that the HFP intervention had a rich training curriculum on diversified food production and improved nutrition practices. Attendance in the HFP training sessions was 80% on average, and pathway indicators collected through the program monitoring system show improvement for intervention households. Women from intervention households breastfed their children exclusively for approximately half a month longer, and children's dietary diversity increased by 0.3 food groups (Waid et al., under review). Despite the improvement in diets, we did not see a decrease in EED biomarkers, which is in contrast to two previous studies<sup>14,31</sup> that found some evidence for a decrease in EED biomarkers with increased nutrient intake from complementary foods.

Regarding the impact pathway through food hygiene, the rather light hygiene component from the HFP program had been enhanced by an additional dedicated food hygiene module. Attendance and participation in the food hygiene training sessions was high (87% on average),<sup>49</sup> and knowledge of promoted food hygiene behaviors was widespread (Huda et al., manuscript in preparation). We also saw improvements of several food hygiene behaviors, including using clean feeding utensils, preparing food fresh or reheating stored food before feeding, and washing hands before food preparation and child feeding. However, handwashing was rare overall, and most behaviors were practiced inconsistently (Sobhan et al., under review). This indicates an attenuation of the intervention effect along the causal pathway from participation via knowledge to practice in a "funnel of attrition."<sup>60</sup> The observed improvements in food hygiene practice were not sufficient to reduce complementary food contamination (Huda et al., manuscript in preparation), which makes an impact on EED through the food hygiene pathway rather unlikely. This is in line with the lack of impact we found in our study, and with the observed null effect of the intervention on diarrhea prevalence.<sup>61</sup>

Young children in resource-poor settings are likely to come into contact with fecal pathogens in their home environment from a range of sources. Besides pathogen exposure through poor sanitation infrastructure or contaminated foods and water, frequent contact with animals, or unknowingly touching and ingesting feces while crawling or playing might present an equally important source of pathogen contact.<sup>27,28,30,62</sup> Interventions such as WASH (or other hygiene interventions), which target only one or a few pathways of exposure to fecal contamination, might thus not be able to reduce the level of exposure sufficiently to translate into a beneficial health outcome (such as reduction of EED or diarrhea). Therefore, comprehensive WASH interventions, including profound, long-lasting changes in the physical household environment that allow a drastic reduction of fecal contamination, might be needed to achieve health impacts.<sup>63–65</sup>

Although the intervention did not affect most EED biomarkers, we found increased levels of MPO. The difference in MPO increased with child age and was most pronounced in children 18 to 24 months of age. Interestingly, the WASH Benefits trial<sup>33</sup> in Bangladesh observed a similar effect in 28-month-old children. When thinking about possible reasons for this potentially harmful effect, greater mobility of children with increasing age could play a role in that it leads

to greater pathogen exposure from the environment—if the intervention increases pathogen loads. As our intervention increased poultry numbers somewhat, both at the household and settlement levels (household: 3.9 birds versus 3.3 in controls (among poultry-owning households); settlement-level average: 2.9 birds versus 2.0 in controls), and handwashing practices are very poor in this population, this may be a possible mechanism for the increase in MPO levels. Although our exploratory analysis found only weak evidence linking higher average poultry numbers at the settlement level to higher MPO levels, we found that exposure to poultry in the household, if not kept in a shed, was associated with higher MPO levels, and that any harmful intervention effect on MPO was in this subgroup, but without any evidence for a statistical interaction.

The HFP intervention promoted small-scale poultry rearing in combination with good poultry management techniques, including improved poultry sheds. In 2019, approximately two thirds of intervention households that kept poultry owned an improved poultry shed, and more than half of the intervention households with poultry used a shed for keeping them (compared with less than 10% in controls). A study in Peru<sup>66</sup> found that an intervention to promote poultry corraling, surprisingly, increased the risk of *Campylobacter* infection—a pathogen that has been associated with EED.<sup>67</sup> However, in our study households, greater shed use in the intervention group seemed to have had a protective effect, possibly counteracting any negative effects from the moderate increase in poultry numbers. Poultry in our study were kept in the shed primarily overnight and roamed freely during the day in almost all households. In households without a shed, poultry were mostly kept inside the house overnight, in the kitchen or sleeping area. Intervention households that did not opt to build a shed, or did not keep their poultry in the shed, may thus have had increased exposure to poultry inside the house overnight; however, this exposure was also true for controls and to an even greater extent. We are thus not sure which feature of our poultry intervention, if any, may have contributed to potentially increased intestinal inflammation in the intervention children. Although the evidence is not very strong, we feel it is important to highlight this potential harm that might originate from poultry. Previous research in Ethiopia<sup>68</sup> found that keeping poultry inside the house overnight decreased children's height-for-age z-score, likely because of an increased risk of infection. Also, other studies<sup>28,55,69,70</sup> provide evidence that exposure to poultry or other livestock in the household setting is associated with enteric infections, diarrheal disease, and EED in children. Unfortunately, the FHEED study was not designed to look at these associations in more detail. Future studies are needed to understand more fully the risk of poultry interventions for intestinal health as well as safety measures to reduce potential risk factors.

Our study has several strengths and limitations. Its cluster-randomized design, the use of covariate-constrained randomization, and its relatively large number of clusters helped to balance observed and unobserved characteristics that could otherwise confound the results. Moreover, the high completion of ~90% of the attempted sample minimizes the risk of a selection bias. In addition, the rich data collected during the course of the FAARM trial as part of the surveillance system enabled us to explore poultry as a



potential cause of increased MPO levels, thereby aiding the interpretation of study findings, despite not being the focus of this study. However, the study also has limitations. Because of financial constraints, we did not analyze EED biomarkers over time for all children included in the FHEED study, but only once at endline, which did not allow us to investigate changes in EED over time. In addition, we did not analyze enteropathogens for all FHEED children, which could have potentially provided an explanation for the increased MPO levels found. However, longitudinal data on EED biomarkers and enteropathogens has been analyzed in a smaller subset of children, and will be reported in a forthcoming article. The chosen age cutoff of 24 months for the FHEED study excludes the older FAARM children, who may have also shown an increase in MPO levels. This limited our sample size for examining the association between poultry and intestinal inflammation, or other potential contributors to increased MPO levels, in more detail. Finally, our analyses did not address intervention fidelity and adoption of intervention messages, and how these influenced the overall results, leaving these aspects to be addressed quantitatively in future work.

In conclusion, our results suggest that a food hygiene intervention integrated in an HFP program did not succeed in reducing biomarkers of EED and systemic inflammation among young children in rural Bangladesh. Surprisingly, children of intervention households showed increased levels of MPO compared with control households, which may be because of greater poultry ownership in the intervention group, particularly when poultry were not kept in a shed. Although data were collected only from two rural subdistricts in the Sylhet Division, the household environment resembles typical rural areas in Bangladesh. It is thus likely that our results are relevant for regions with similar demographic characteristics across the country, and other countries in South Asia. Future nutrition and hygiene interventions might need to incorporate more comprehensive WASH components, with profound, long-lasting changes in the household and community environment to achieve an impact on health outcomes. A better understanding of the potential benefits and risks of complex interventions is also needed to increase knowledge of potential risk factors associated with adopted intervention practices (such as increased poultry production), to identify potential mitigation strategies, and to tailor intervention components to individual contexts.

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**Authors' addresses:** Anna A. Müller-Hauser, Shafinaz Sobhan, and Nathalie J. Lambrecht, Charité–Universitätsmedizin Berlin, Institute of Public Health, Berlin, Germany, and Research Department 2, Potsdam Institute for Climate Impact Research, Potsdam, Germany, E-mails: [anna.mueller-hauser@charite.de](mailto:anna.mueller-hauser@charite.de), [shafinaz.sobhan@charite.de](mailto:shafinaz.sobhan@charite.de), and [nathalie.lambrecht@charite.de](mailto:nathalie.lambrecht@charite.de). Tarique Md. Nurul Huda, Department of Public Health, College of Public Health and Health Informatics, Qassim University, Al Bukairiyah, Saudi Arabia, and Environmental Interventions Unit, Infectious Diseases Division, icddr,b, Dhaka, Bangladesh, E-mail: [t.huda@qu.edu.sa](mailto:t.huda@qu.edu.sa). Jillian L. Waid, Research Department 2, Potsdam Institute for Climate Impact Research, Potsdam, Germany, and Heidelberg Institute of Global Health, Heidelberg University, Heidelberg, Germany, E-mail: [waid@pik-potsdam.de](mailto:waid@pik-potsdam.de). Amanda S. Wendt, Research Department 2, Potsdam Institute for Climate Impact Research, Potsdam, Germany, E-mail: [wendt@pik-potsdam.de](mailto:wendt@pik-potsdam.de). Shahjahan Ali, Infectious Diseases Division, icddr,b, Dhaka, Bangladesh, and Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO, E-mail: [shahjahanjohny@gmail.com](mailto:shahjahanjohny@gmail.com). Mahbubur Rahman, Environmental Interventions Unit, Infectious Diseases Division, icddr,b, Dhaka, Bangladesh, and Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO, E-mail: [mahbubur@icddr.org](mailto:mahbubur@icddr.org). Sabine Gabrysch, Charité–Universitätsmedizin Berlin, Institute of Public Health, Berlin, Germany, Research Department 2, Potsdam Institute for Climate Impact Research, Potsdam, Germany, and Heidelberg Institute of Global Health, Heidelberg University, Heidelberg, Germany, E-mail: [sabine.gabrysch@pik-potsdam.de](mailto:sabine.gabrysch@pik-potsdam.de).

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