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Association between *Shigella* Infection and Diarrhea Varies Based on Location and Age of Children

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Abstract. Molecular identification of the invasion plasmid antigen-H (*ipaH*) gene has been established as a useful detection mechanism for *Shigella* spp. The Global Enteric Multicenter Study (GEMS) identified the etiology and burden of moderate-to-severe diarrhea (MSD) in sub-Saharan Africa and south Asia using a case–control study and traditional culture techniques. Here, we used quantitative polymerase chain reaction (qPCR) to identify *Shigella* spp. in 2,611 stool specimens from GEMS and compared these results to those using culture. Demographic and nutritional characteristics were assessed as possible risk factors. The qPCR identified more cases of shigellosis than culture; however, the distribution of demographic characteristics was similar by both methods. In regression models adjusting for *Shigella* quantity, age, and site, children who were exclusively breast-fed had significantly lower odds of MSD compared with children who were not breast-fed (odds ratio [OR] = 0.47, 95% confidence interval (CI) = 0.28–0.81). The association between *Shigella* quantity and MSD increased with age, with a peak in children of 24–35 months of age (OR = 8.2, 95% CI = 4.3–15.7) and the relationship between *Shigella* quantity and disease was greatest in Bangladesh (OR = 13.2, 95% CI = 7.3–23.8). This study found that qPCR identified more cases of *Shigella* and age, site, and breast-feeding status were significant risk factors for MSD.

INTRODUCTION

The application of molecular diagnostic methods, particularly those which use quantitation of *Shigella* spp. in fecal samples, can contribute to more detailed understanding of aspects of the epidemiology of *Shigella* disease globally, including estimations of disease burden. The recent Global Enterics Multicenter Study (GEMS) reported significant positive associations of *Shigella* with moderate-to-severe diarrhea (MSD) and a high, weighted, adjusted attributable fraction at all seven sites in Africa and south Asia. Mirzapur, Bangladesh proved to be highly endemic for *Shigella*, with attributable fractions that increased with age, reaching 67.6% in the oldest children. Excluding Bangladesh, *Shigella*-attributable fraction ranged from 2% to 7.6% in children aged 0–11 months, 2% to 12.8% in 12–23 months, and 2% to 14.9% in 24–59 months.[1]

Multiple studies have evaluated molecular detection methods to estimate the prevalence of *Shigella*-positive stools, but many of these studies examined only diarrheal cases and/or were not community based.[2–8] Molecular methods range from qualitative polymerase chain reaction (PCR) to multiplex PCR to an absolute quantitative measure using quantitative (q)PCR; most use the invasion plasmid antigen-H (*ipaH*) gene as a molecular marker. These studies consistently report an increased prevalence of *Shigella*-positive stools and a lower limit of detection compared with conventional culture techniques. Vu (2004) suggested that as many as 46% of culture-negative diarrheal stools were positive for *Shigella* by qPCR.[8] We have previously developed a qPCR assay to measure *ipaH* in stools from The Gambia, Mali, Kenya, and Bangladesh and found at least one copy of *ipaH* in 84% of stool samples.[9] Multiple molecular methods increased the rate of detection in diarrheal stools as well as in children reporting no diarrheal disease, presumably because these methods consistently detect smaller quantities of *Shigella*.[10] To overcome this limitation and identify individuals that indicate a probable "true positive" for *Shigella* colonization with expansion to the point of likely disease association, we selected a threshold of approximately 14,000 gene copies of *ipaH* to indicate positivity.[9] Emerging evidence also suggests that in low-income countries carriage of multiple pathogens is common.[5,11–13] The ability to quantitatively evaluate the presence of a pathogen may be useful in assigning or prioritizing specific etiologies of disease.

Although recent studies have shown a greater prevalence of *Shigella* when employing molecular detection methods, none have used these methods to gain insight into the risk factors and potential protective effects of covariates related to disease. Measuring *Shigella* using quantitative molecular methods allows us to further examine the relationship between abundance and disease related to age. In addition, many prior studies have focused on one geographic area; comparing a number of geographic areas with presumably differing levels of disease prevalence is of importance.

The purpose of this study was to perform an in-depth molecular analysis of shigellosis in four of the seven GEMS sites by measuring the abundance of *Shigella* target found in stools across age groups using qPCR and compare qPCR to traditional culture. We characterized the association between quantity of *Shigella* in the stool and MSD by age and site, and examined potentially important covariates such as breast-feeding and malnutrition. As we learn more about how risk factors are associated with the quantity of *Shigella* that is shed in the stool, we can apply this knowledge to examine how these factors, such as breast-feeding and malnutrition, might affect the relationship between *Shigella* and MSD. An examination of a range of ages and site-specific *Shigella* quantification using new molecular techniques may help gain

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insight into disease patterns that could be a key to designing specific interventions that reduce mortality and morbidity due to shigellosis.

METHODS

Study design and participants. We examined stool specimens collected from GEMS, a prospective matched case–control study with the objective of determining the burden, etiology, and adverse outcomes of MSD in infants and young children of 0–59 months of age residing in a censused population at seven sites in sub-Saharan Africa and south Asia.[14] Cases with MSD were enrolled upon presentation to any one of the health clinics or hospitals serving the catchment population at each site. MSD eligibility criteria included one of the signs of dehydration (sunken eyes, loss of normal skin turgor), or a decision to initiate intravenous hydration, presence of blood in the stool (dysentery), or decision to hospitalize the child. Controls were enrolled from the same catchment area within 14 days after case enrollment and included if they reported no diarrhea within the previous 7 days. Controls were matched to cases on gender, age, and location. For this molecular sub-study, we selected a subset of matched cases and controls from four sites: The Gambia, Mali, Kenya, and Bangladesh. This was largely a convenience sample as all GEMS study sites were invited to participate in this study; however, only four chose to accept.

Nutritional status of children was assessed, including measurements of weight and length/height of each case and control at enrollment. Length/height was measured three times and the median of the three measurements were calculated and expressed as a *z* score according to World Health Organization standard curves.[13] Breast-feeding status of the children was ascertained at the time of enrollment and one stool specimen was collected for each child at this time. All specimens for this study were collected between December 2007 and December 2009. The Institutional Review Boards at all participating institutions (University of Maryland Baltimore, icddr, b, Medical Research Council, Center pour le Developpement des Vaccins du Mali, Bamako, Mali; CDC/Kenya Medical Research Institute) reviewed and approved the protocol.

Specimen collection, culture and quantitative PCR. Stool specimens were handled according to the GEMS protocol.[14] The specimens were collected in sterile containers, kept at 2–8°C while in transit, and examined within 24 hours (< 6 hours between evacuation and placement into transport media, made weekly, and < 18 hours before transport swab inoculated the culture plates). Each fresh stool specimen was aliquoted into multiple tubes, some of which were frozen at −80°C. Identification of *Shigella* spp. was conducted at each site by traditional culture methods.[15] DNA was isolated from frozen stools by using a Bead Beater apparatus with 3-mm diameter solid glass beads (Sigma-Aldrich, St. Louis, MO), and subsequently 0.1 mm zirconium beads (BIO-SPEC Inc., Bartlesville, OK) to disrupt cells. The cell slurry was then centrifuged at 16,000 *g* for 1 minute, the supernatant processed using the Qiagen QIAamp® DNA stool extraction kit (Qiagen, Hilden, Germany). Extracted DNA was ethanol precipitated and shipped to the University of Maryland laboratories, Baltimore, MD, where quantitative qPCR was conducted.

Fecal DNA was tested by qPCR using the Applied Biosystems 7500/700 Fast Real-Time PCR System with software V2.0.5 and SYBR Green-Based fluorescent dye.[9] Reactions included 10–1,717 ng of fecal DNA, 8.5 μL of water, 1.5 μL each of 5 μM forward and reverse *ipaH* primers, and 12.5 μL of SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA).[8] Two negative controls per 96 well plate did not contain DNA and were uniformly negative for amplification after PCR. The PCR was carried out for 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.[8,9,16] Gene copies in specimens were determined by absolute quantification using a standard curve fitted for each plate as described previously.[9] We used a threshold of 14,000 *ipaH* gene copies to categorize individuals as shedding either low levels or high levels of *Shigella* within their stool. The method for establishing this threshold is also outlined in Lindsay (2013).[9] In brief, we used receiver operating characteristic curves to determine the sensitivity and specificity of incremental increases of *ipaH* quantity compared with disease status and set the threshold based on the point that maximized both sensitivity and specificity.

Statistical analysis. All statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC). Odds ratios (ORs) and 95% confidence intervals (CIs) for estimates stratified by sample characteristics were estimated with logistic regression. Differences between categorical variables were assessed using χ^2 tests. Log-likelihood ratio tests were used to test for statistical interaction comparing statistical models with and without interaction terms including site or age.

RESULTS

A total of 2,611 stool samples were included in our analysis, composed of 1,181 matched sets including at least one case and one or more matched controls. The qPCR targeting the *ipaH* gene of *Shigella* was carried out in all of these samples. Details regarding the distribution of sample characteristics are in Table 1. Approximately 56% of the participants were male and the majority from Kenya (50%). In succession, The Gambia, Bangladesh, and Mali sites followed with decreasing numbers of participants. The majority of children (62%) were either in the 6–11-month or 12–23-month age strata. While breast-feeding information was recorded in children older than 36 months, based on the low prevalence of any breast-feeding, we limited breast-feeding analyses to only those less than 36 months.

The proportion of samples with *Shigella ipaH* counts greater than 14,000 was compared between cases and controls in different strata of demographics, breast-feeding, and nutritional status (Table 2). The proportion of cases with high *ipaH* counts was relatively uniform between gender and nutritional status categories; statistical testing of relationship between *Shigella* quantity and MSD stratified by nutritional status (measured by height-for-age z-score [HAZ]) suggested no heterogeneity by nutritional status ($P = 0.67$). This analysis suggested, however, there was some heterogeneity in the association between *Shigella* quantity and MSD by age and site. To test this further, logistic regression models with the interaction terms for site and age were compared via log-likelihood ratio tests. Both age category and site were discovered to be statistically significant effect modifiers of the relationship between *Shigella* quantity and MSD (log-likelihood ratio $P = 0.003$ and $P = 0.0002$, respectively), meaning that the relationship varied across strata of age and site. Given this observation,

TABLE 1

Overall sample nutritional, breast-feeding, *Shigella* status, and demographics (N = 2,611)

	Total
Sex	*N* (%)
Male	1,477 (56)
Age (mean, SD in months)	18.19 (12.5)
Age group	
0–5 months	271 (10)
6–11 months	727 (28)
12–23 months	883 (34)
24–35 months	442 (17)
36–59 months	288 (11)
Site	
The Gambia	668 (25)
Mali	200 (8)
Kenya	1,305 (50)
Bangladesh	438 (17)
Breast-feeding status < 36 months	
None	602 (23)
Partial	1,625 (62)
Exclusive	96 (4)
≥ 36 months	288 (11)
Height-for-age *z* score	
> −2	1,903 (73)
< −2 to > −3	489 (19)
< −3	218 (8)
Weight-for-height *z* score	
> −2	2,218 (85)
< −2 to > −3	265 (10)
< −3	123 (5)
ipaH copies ≥ 14,000	343 (13)
Culture *Shigella* spp. positive	163 (6)
Bloody stools	283 (11)

SD = standard deviation.

we created separate logistic regression models to investigate the relationship between *ipaH* greater than 14,000 and MSD; adjusting for breast-feeding status. Figure 1A presents results of the model including an interaction term for age, and Figure 1B presents results including an interaction term for site. The OR of *ipaH* counts ≥ 14,000 in cases compared with controls was not statistically associated with MSD in children less than 6 months of age, however, it is significantly associated with disease at 6–11 months and increases thereafter at each age increment until 36 months, when it decreases slightly. The proportion of cases with *ipaH* counts ≥ 14,000 is highest in Bangladesh (44%) and is lower in The Gambia (25%), Kenya (14%), and Mali (13%); a high association with diarrhea is seen in Bangladesh (adjusted model, OR = 13.21, 95% CI = 7.34–10.56) compared with the African sites. In models using culture (instead of qPCR results) as an indicator of *Shigella* status showed similar patterns across ages and sites, generally with wider confidence intervals (Figure 1A and B). There was not a statistically significant difference between the proportion of the various serotypes of *Shigella* at the four sites (Supplemental Table 1).

Eighty-six children had levels of *ipaH* ≥ 14,000 but did not report clinical symptoms of diarrhea in the previous 7 days. To investigate potential protective factors, we compared these controls to cases that also had high levels of *Shigella* with regard to age distribution, site, and breast-feeding status (Table 2). No characteristic was significantly different between *ipaH* ≥ 14,000 controls and cases; the distribution nearly differed by site (P = 0.056), with only 19% of high-level *Shigella* controls being from Bangladesh compared with 32% of high-level *Shigella* cases.

Breast-feeding status varied significantly by age (P < 0.0001) and the proportion of exclusively breast-fed children

TABLE 2

Number and proportion with *ipaH* counts ≥ 14,000 by case status and association with MSD stratified by subject characteristics (total N = 2,611)

	Cases (N = 1,181) *ipaH* ≥ 14,000 *N* (%)	Controls (N = 1,430) *ipaH* ≥ 14,000 *N* (%)	OR (95% CI) Association between *ipaH* ≥ 14,000 and MSD	*P* value
Sex				
Male	154 (23)	46 (6)	4.98 (3.52–7.06)	< 0.0001
Female	103 (20)	40 (6)	3.63 (2.46–5.34)	< 0.0001
Age group				
0–5 months	6 (5)	4 (3)	1.65 (0.46–6.01)	0.4378
6–11 months	53 (15)	20 (5)	3.13 (1.82–5.35)	< 0.0001
12–23 months	113 (28)	37 (8)	4.82 (3.24–7.20)	< 0.0001
24–35 months	51 (28)	13 (5)	7.40 (3.88–14.09)	< 0.0001
36–59 months	34 (29)	12 (7)	5.23 (2.57–10.63)	< 0.0001
Site				
Gambia	75 (25)	28 (8)	4.14 (2.59–6.59)	< 0.0001
Mali	13 (13)	9 (9)	1.51 (0.61–3.71)	0.366
Kenya	87 (15)	33 (5)	3.45 (2.27–5.24)	< 0.0001
Bangladesh	82 (44)	16 (6)	11.95 (6.67–21.44)	< 0.0001
Breast-feeding status < 36 months				
None	64 (24)	23 (7)	4.42 (2.66–7.35)	< 0.0001
Partial	157 (20)	48 (6)	4.34 (3.09–6.10)	< 0.0001
Exclusive	2 (6)	3 (5)	1.35 (0.21–8.55)	1
> 36 months	34 (29)	12 (7)	5.23 (2.57–10.63)	< 0.0001
Height-for-age *z* score				
> −2	171 (20)	61 (6)	4.18 (3.07–5.68)	< 0.0001
< −2 to > −3	63 (27)	16 (6)	5.34 (2.98–9.56)	< 0.0001
< −3	23 (23)	9 (8)	3.53 (1.55–8.06)	0.0017
Weight-for-height *z* score				
> −2	196 (21)	75 (6)	4.36 (3.29–5.78)	< 0.0001
< −2 to > −3	41 (26)	9 (8)	3.81 (1.76–8.24)	0.0003
< −3	20 (21)	1 (4)	6.84 (0.87–53.52)	0.0423

CI = confidence interval; MSD = moderate-to-severe diarrhea; OR = odds ratio.

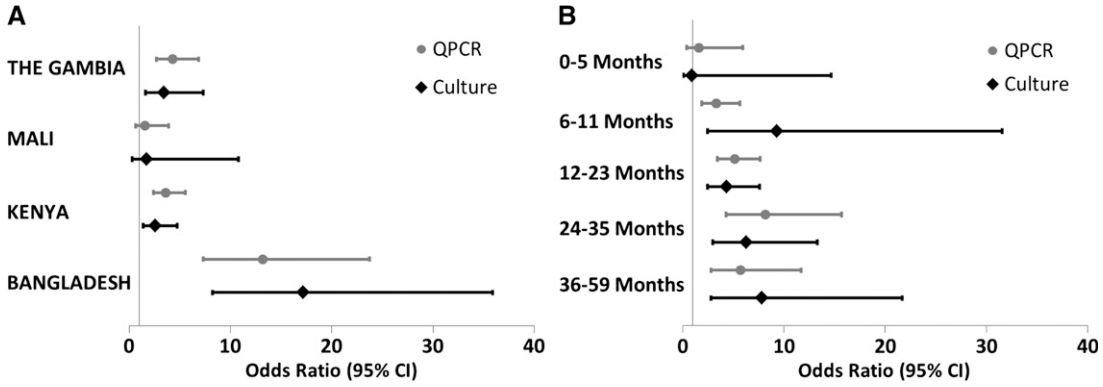

FIGURE 1. Association between *ipaH* copies ≥ 14,000, *Shigella* identification by culture and moderate-to-severe diarrhea (MSD) stratified by site (**A**) and age (**B**) adjusted for breast-feeding status.

fell to virtually zero (~1%) by 1 year of age. Breast-feeding practices also varied significantly by site ($P < 0.0001$). Practices in Bangladesh differed significantly ($P < 0.0001$) on the whole from the African countries; exclusive breast-feeding past the age of 6 months was reported only in African countries (Table 3). In Bangladesh, 78% of children aged 24–35 months were partially breast-fed at enrollment, while partial breast-feeding only accounted for 3–15% in this age group in African sites. In regression models adjusting for *ipaH* quantity, age, and site, children who were exclusively breast-fed had significantly lower odds of MSD compared with children who were not breast-fed (OR = 0.47, 95% CI = 0.28–0.81).

Children who reported no disease and had low levels of *ipaH* quantified by qPCR were exclusively breast-fed at a higher proportion and exhibited the lowest degree of malnutrition (Figure 2). Of control children, 4.5% with low *ipaH* levels were exclusively breast-fed compared with less than 1% of case children with high *ipaH* counts (Figure 2A). Of note, of the 96 children that were exclusively breast-fed, 91 (95%), had *ipaH* counts less than 14,000. The control group with the lowest levels of *ipaH* also exhibited the highest proportion of *z* scores without moderate-to-severe stunting or wasting (≥ −2 SD) (Figure 2B and C). Approximately 67% of high *ipaH* cases had a HAZ > −2 compared with approximately 70% of high *ipaH* controls.

Finally, to test whether the ≥ 14,000 *ipaH* MSD cases identified by qPCR but not by culture differed significantly from the group identified by culture, we compared these groups with regard to gender, age, site, breast-feeding, and nutritional status (Table 4). Here, we see that although *ipaH* ≥ 14,000 cases appeared to be slightly younger ($P = 0.03$) and greater proportions from The Gambia, Mali, and Kenya (83% versus 47%, $P < 0.0001$); breast-feeding and nutritional characteristics were similar.

DISCUSSION

This study found that qPCR identified more cases of *Shigella* and age, site, and breast-feeding status were significant risk factors for MSD. Conversely, nutritional status did

TABLE 3
Distribution of breast-feeding status by age and site in children less than 36 months ($N = 2,323$)

	Age groups				
	0–5 months ($N = 271$)	6–11 months ($N = 727$)	12–23 months ($N = 883$)	23–35 months ($N = 442$)	Total ($N = 2,323$)
	N (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)
All sites					
None	3 (1)	20 (3)	243 (27)	336 (76)	602 (26)
Partial	217 (80)	674 (93)	629 (71)	105 (23)	1,625 (70)
Exclusive	51 (19)	33 (4)	11 (1)	1 (1)	96 (4)
The Gambia					
None	0	0	117 (40)	115 (96)	232 (37)
Partial	25 (69)	158 (89)	170 (57)	4 (3)	357 (57)
Exclusive	11 (31)	19 (11)	9 (3)	1 (1)	40 (6)
Mali					
None	1 (6)	0	13 (24)	21 (91)	35 (19)
Partial	4 (23)	84 (89)	40 (75)	2 (9)	130 (69)
Exclusive	12 (71)	10 (11)	0	0	22 (12)
Kenya					
None	2 (1)	13 (4)	104 (26)	181 (85)	300 (27)
Partial	157 (87)	307 (95)	287 (73)	33 (15)	784 (71)
Exclusive	22 (12)	4 (1)	1 (1)	0	27 (2)
Bangladesh					
None	0	7 (5)	9 (6)	19 (22)	35 (9)
Partial	31 (84)	125 (95)	132 (93)	66 (78)	354 (89)
Exclusive	6 (16)	0	1 (1)	0	7 (2)

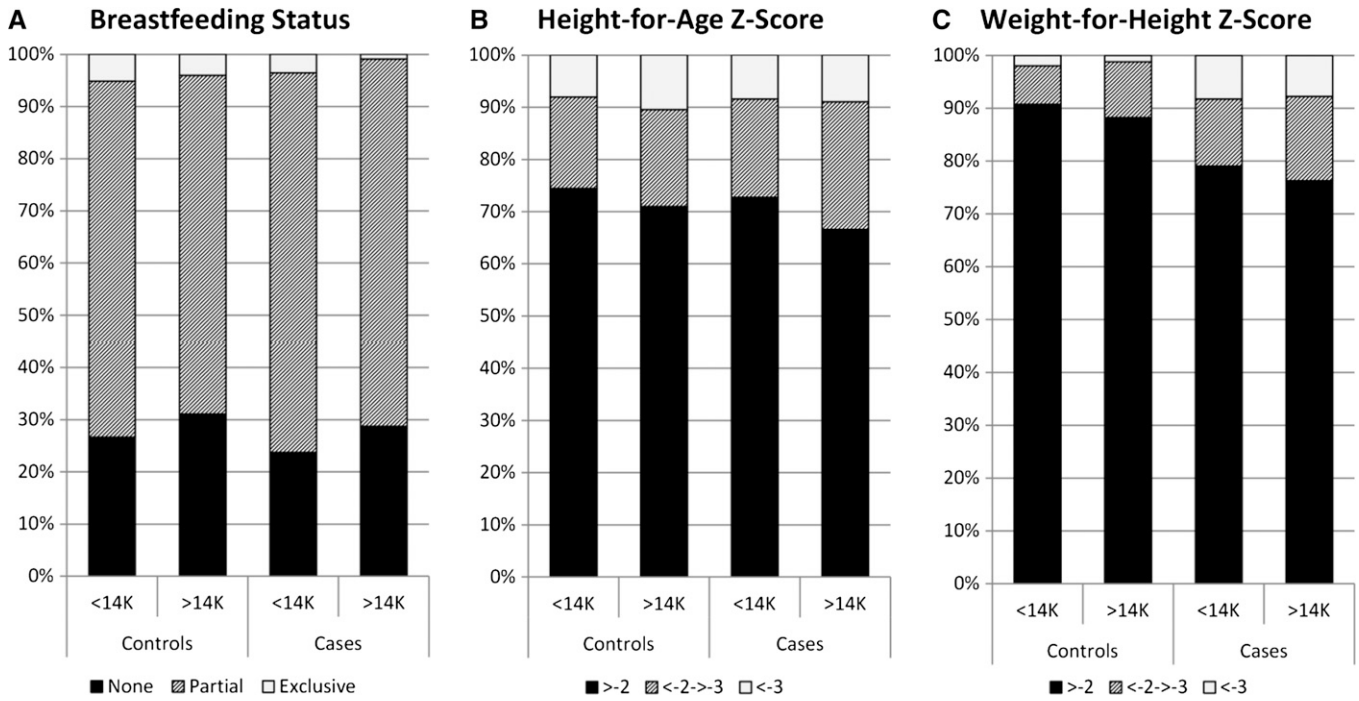

FIGURE 2. Proportions of (**A**) breast-feeding, (**B**) height-for-age, and (**C**) weight-for-height *z* score groups across controls and moderate-to-severe diarrhea (MSD) cases in high (≥ 14K) and low (< 14K) levels of *Shigella* measured by quantitative polymerase chain reaction (qPCR). These graphs demonstrate the breast-feeding and nutritional status of children with *ipaH* < 14,000 in controls vs. *ipaH* ≥ 14,000 in controls in addition to *ipaH* < 14,000 in cases vs. *ipaH* ≥ 14,000 in cases.

not appear to effect the association. Similar age and site trends were observed using culture detection, which identified a much smaller proportion of *Shigella*-positive cases (11% versus 22%). Not surprisingly, a small proportion (5%) of cases less than 6 months of age at all sites shed ≥ 14,000 copies of *ipaH* compared with 15–29% of older children with MSD. Moreover, those infants < 6 months of age with high copy numbers were not significantly more likely to have MSD

TABLE 4

Comparison of *ipaH* > 14,000/*Shigella* culture-negative MSD stools to *Shigella* culture-positive MSD stools

Subject characteristics	*ipaH* ≥ 14K/ culture-negative MSD	Culture-positive MSD	*P* value
	N = 157	*N* = 126	
Male	96 (61)	73 (58)	0.5843
Age mean, SD in months	20 (12.3)	23 (12.3)	0.0396
Age group			
0–5 months	6 (4)	1 (1)	0.1052
6–11 months	36 (23)	22 (17)	–
12–23 months	71 (45)	51 (40)	–
24–35 months	26 (16)	32 (25)	–
36–59 months	18 (11)	20 (16)	–
Site			
The Gambia	56 (36)	25 (20)	< 0.0001
Mali	10 (6)	3 (2)	–
Kenya	65 (41)	32 (25)	–
Bangladesh	26 (16)	66 (52)	–
Breast-feeding status < 36 months			
None	39 (25)	30 (24)	0.4407
Partial	98 (62)	76 (60)	–
Exclusive	2 (1)	0 (0)	–
> 36 months	18 (11)	20 (16)	–
Height-for-age *z* score			
> −2	101 (64)	91 (72)	0.3446
< −2 to > −3	42 (14)	25 (20)	–
<−3	14 (9)	10 (8)	–
Weight-for-height *z* score			
> −2	121 (77)	98 (78)	0.2720
< −2 to > −3	27 (17)	16 (13)	–
< −3	9 (6)	12 (9)	–
Bloody stools	61 (39)	83 (66)	< 0.0001

MSD = moderate-to-severe diarrhea; SD = standard deviation.

than those with < 14,000 copies. This trend is similar to the pattern observed with culture results in the GEMS study: the lowest attributable fraction of disease due to *Shigella* was in the 0–11-month age group. One likely explanation is that exclusive breast-feeding, which was present in 19% of infants < 6 months of age, neutralized the infectious inoculum and prevented disease, as has been shown by others.[17] The association between those with ≥ 14,000 copies and MSD strengthened as children aged, reaching a peak at 24–35 months of age, consistent with the peak age group for shigellosis reported by others through culture identification and has been attributed to a decreased exposure to breast milk and an increased exposure to sources *Shigella* infection on the hands of contacts and in the environment as children become more ambulatory.[17–19] In addition, as children wean, they are exposed to an increased array of food and water that could serve as sources of *Shigella* infection. Von Seidlein suggests a link between quantitation and age in their 2006 population-based surveillance of shigellosis.[6] They report that *ipaH* detection was lowest in children less than 6 months and bacterial load peaked in the second year of life; however, this was with a limited sample size and did not consider disease-free controls.

After the identification of individuals with high levels of *Shigella* using molecular methods, we are now able to reexamine the distribution of disease in different ages and sites. As children are weaned and introduced to solid foods, they may not only lose the protection conferred by maternal antibodies but also be introduced to a greater number of food-borne pathogens leading to the increase in association after 6 months. Results of the standard microbiological analysis of the GEMS study with larger age strata found that the attributable fraction for *Shigella* consistently increased from 0 to 11 months to 12 to 23 months and the highest attributable fraction was seen in 12–23 months for The Gambia and Mali and in 24–59 months in Kenya and Bangladesh.[1] Our analysis supports these observations, however, with a narrower age strata, we see a decrease in the association in children aged 36–59 months compared with those aged 24–35 months for qPCR-identified *Shigella*. We do not see this decrease in culture-identified *Shigella* though with wide confidence intervals perhaps due to limited power. An exploratory analysis of the full GEMS data suggests that this decrease may occur with a larger sample; further analysis may be warranted. The decrease in association in the oldest age group could be a signal of established immunity from previous infection or a more robust immune system. In a comparison of the MSD cases with *ipaH* copies ≥ 14,000 but not detected by *Shigella* culture with those detected by culture; sample characteristics were largely indistinguishable between these groups, with the exception of site, possibly attributed to differing conditions or culture expertise.

The study is limited by the absence of measurement of other potential pathogens within the stool sample and a multiplex approach might be better suited for establishing specific etiology and estimating adjusted attributable risk. Although our sample was large, the study was not designed to evaluate the differences between cases and controls with *ipaH* values ≥ 14,000 and not powered to detect differences between these groups. Further comparisons could perhaps uncover a protective feature found more frequently in controls. Finally, the protective association we found between exclusive breast-feeding and MSD is statistically significant; however, we did not conduct the analysis stratified by age group and measures were collected by self-report, subject to unmeasured cultural and reporting biases that could possibly make these estimates unreliable. Differences by site could be due to some unmeasured confounders; human immunodeficiency virus (HIV) prevalence in Kenya is slightly higher than that in The Gambia and Mali and could result in bias of some estimates; however, data on individual HIV status was not collected.[20]

Strengths of this study are exhibited by the significant results of age and site. The sensitivity of the molecular test and the ample sample size provided critical power to detect differences among the groups. Although many studies have established the use of *ipaH* as a marker for shigellosis, few studies have investigated the quantifiable amounts shed among different sites and age groups in children, making this study of unique importance. As more studies identify more than one pathogen in stools of children with diarrhea in low-income settings, it will be important to use molecular methods that are efficient, quick, and quantitative, providing a better picture of the etiology of a particular episode. Given that shigellosis can be a severe invasive bacterial infection, proper antibiotic therapy is recommended, highlighting the importance of accurate diagnosis. Molecular methods can identify high quantities of *ipaH* in a matter of hours, compared with at least 24 hours required for growth in culture, giving a unique opportunity to initiate the appropriate course of treatment. Statistical modeling suggests that new diagnostics could have a dramatic effect on health outcomes related to acute malnourishment due to enteric pathogens.[21]

This study shows a remarkable variability in the association between *Shigella* and MSD across multiple low-income sites and age groups, exhibited by both qPCR and culture detection. Although many studies have established the increased rate of detection attributable to new diagnostics, we must evaluate the impact of these measurements in community-based settings with a multivariable approach. This study supports the hypothesis that breast-feeding is protective against MSD and age and site are independent risk factors; further research is needed to examine how we may leverage this evidence to support a reduction in overall disease risk.

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