

# *Giardia* Detection and Codetection With Other Enteric Pathogens in Young Children in the Vaccine Impact on Diarrhea in Africa (VIDA) Case-Control Study: 2015–2018

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**Background.** *Giardia* has been associated with reduced risk of diarrhea in children in low-resource settings, but the mechanism underlying this association is unknown. To assess whether *Giardia* may shape colonization or infection with other enteric pathogens and impact associations with diarrhea, we examined *Giardia* and enteric pathogen codetection among children <5 years old in Kenya, The Gambia, and Mali as part of the Vaccine Impact on Diarrhea in Africa study.

**Methods.** We tested for *Giardia* and other enteric pathogens using enzyme-linked immunosorbent assays and real-time polymerase chain reaction (PCR) on stool, respectively. We evaluated associations between *Giardia* and enteric pathogen detection using multivariable logistic regression models separately for children with moderate-to-severe diarrhea (MSD, cases) and free of diarrhea (controls).

**Results.** Among 11 039 enrolled children, *Giardia* detection was more common among controls (35%) than cases (28%,  $P < .001$ ). *Campylobacter coli/jejuni* detection was associated with *Giardia* in controls in The Gambia (adjusted odds ratio [aOR] [95% confidence interval {CI}]: 1.51 [1.22–1.86]) and cases across all sites (1.16 [1.00–1.33]). Among controls, the odds of astrovirus (1.43 [1.05–1.93]) and *Cryptosporidium* spp. (1.24 [1.06–1.46]) detection were higher among children with *Giardia*. Among cases, the odds of rotavirus detection were lower in children with *Giardia* in Mali (.45 [.30–.66]) and Kenya (.31 [.17–.56]).

**Conclusions.** *Giardia* was prevalent in children <5 years old and was associated with detection of other enteric pathogens, with differing associations in cases versus controls and by site. *Giardia* may affect colonization or infection by certain enteric pathogens associated with MSD, suggesting an indirect mechanism of clinical impact.

**Keywords.** *Giardia*; pediatric diarrhea; rotavirus; enteric pathogens.

The protozoan parasite *Giardia duodenalis*, also referred to as *Giardia lamblia* and *Giardia intestinalis* (hereafter *Giardia*), is one of the most common enteric pathogens among children and adults worldwide, with >200 million persons infected annually [1]. This high prevalence is particularly pronounced among children in low-resource settings; several studies have found overall prevalence rates in

children of 14–53% [2–4]. A multisite birth-cohort study (the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project [MAL-ED]) found that for two-thirds of children, *Giardia* was detected at least once in their first 2 years of life [2].

Acute *Giardia* infection is treatable but can cause diarrhea and other gastrointestinal symptoms, particularly among adults and children in high-income countries [5]. However, where *Giardia* prevalence is high, as among children in low-resource settings, the relationship between *Giardia* and diarrhea is more complex. The Global Enteric Multicenter Study (GEMS)—a 2007–2011 prospective case-control study of the incidence, etiology, and adverse consequences of medically attended acute moderate-to-severe diarrhea (MSD,  $\geq 3$  loose stools within a 24-hour period and dehydration, dysentery, or hospitalization) among children <5 years old—found that *Giardia* was more frequently detected in the stool of children

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free of diarrhea compared to children with MSD [6], with similar results among children with milder diarrhea enrolled in MAL-ED [7]. A meta-analysis of 12 studies conducted in low-resource settings found children with *Giardia* had lower odds of developing acute diarrhea ( $\geq 3$  loose stools within a 24-hour period lasting  $< 14$  days), although paradoxically, *Giardia* detection was positively associated with persistent diarrhea (uninterrupted diarrhea for  $\geq 14$  days) [8].

The mechanisms underlying the different clinical impacts of pediatric *Giardia* in low-resource settings are still an area of active investigation and are likely due to a combination of differences in the host, parasite, and host-parasite interactions [8]. *Giardia* is known to alter intestinal villi, epithelial cells, mucous barrier, and permeability [9–12], and it can promote the production of host antimicrobial peptides [13] and reduce inflammation [14, 15]. In GEMS, *Giardia* was found to be associated with changes to the microbiome of children with MSD and free of diarrhea [16]. *Giardia*-induced changes in children could impact the ability of other enteric pathogens to invade the intestinal mucosa and epithelium, in turn promoting or preventing their colonization/infection [17]. *Giardia* could then have no association with MSD or be indirectly protective against MSD in children in low-resource settings by preventing colonization/infection with other enteric pathogens associated with MSD.

From 2015 to 2018, the Vaccine Impact on Diarrhea in Africa (VIDA) study collected data to evaluate the etiology and burden of MSD among children  $< 5$  years old [Kotloff et al VIDA]. This prospective case-control study was designed as a follow-on to GEMS in 3 countries—The Gambia, Kenya, and Mali—to investigate the pathogen-specific burden of pediatric diarrhea following rotavirus vaccine introduction. We described *Giardia* prevalence in the VIDA study and assessed whether *Giardia* is associated with detection of other enteric pathogens among children with MSD and children free of diarrhea. Our goal was to investigate whether pathogens known to cause MSD were less prevalent in children with *Giardia* compared to those without *Giardia* in order to understand whether this parasite may be associated with lower risk of MSD via indirect routes, such as inhibition of pathogen colonization/infection.

## METHODS

### VIDA Study Design and Enrollment

The VIDA study design has been described elsewhere [Kotloff et al VIDA; Powell et al VIDA]. Briefly, the study was conducted from May 2015–July 2018 in 5 censused populations with ongoing demographic surveillance systems in 3 countries (study sites): a rural population in Basse and Bansang, The Gambia; 2 urban neighborhoods in Bamako, Mali; and a rural location in Siaya County, Kenya. Children  $< 5$  years old were

enrolled as cases if they presented to healthcare facilities with MSD, defined as:  $\geq 3$  loose stools within a 24-hour period with  $\geq 1$  clinical indication of moderate-to-severe dehydration (the need for intravenous fluids, loss of skin turgor, and/or sunken eyes), dysentery, or admission to the healthcare facility. MSD onset had to be within the past 7 days after  $\geq 7$  days without diarrhea. Also, 1 to 3 controls per case, matched by age group and sex, were randomly selected within 14 days of the index MSD case from near the case-home using site-specific demographic surveillance system records. Control children had to have been diarrhea-free for  $\geq 7$  days before enrollment.

### Data Collection

*Giardia* was detected in stool collected at enrollment using an enzyme-linked immunosorbent assay (ELISA; TechLab, Inc., Blacksburg, Virginia, USA). All other enteric pathogens were detected using TaqMan Array Card (TAC) assays on extracted nucleic acids from stool specimens [18], applying a cycle threshold (Cq) of 35 to determine specimens positive (Cq  $< 35$ ) or negative (Cq  $\geq 35$ ) for a pathogen. We used ELISA as the *Giardia* detection method because its detection probe was excluded from a subset of array cards due to experimental and logistical constraints, which left 34% of specimens untested by TAC assay.

Sociodemographic information was collected at enrollment at healthcare facilities for cases and households for controls. History of breastfeeding among cases was assessed for whether they had exclusively, partially, or not breastfed during the week before the onset of their diarrhea, whereas controls were assessed by the same criteria as part of their normal diet. Water, sanitation, and animal-associated risk factors for exposure to *Giardia* in each household have been described elsewhere [Berendes et al. VIDA] and were not assessed in these analyses.

### Statistical Analysis

All analyses were performed in R (version 4.0.3) [19]. Cases and controls were analyzed separately because associations between *Giardia* and detection of other pathogens may differ between children with and without diarrhea. Unadjusted associations between *Giardia* detection and sociodemographic factors were examined using  $\chi^2$  tests. Cut points for fuel source, caregiver's education, household assets, flooring type, and crowding were selected in order to be consistent with other published analyses [20, 21] [Berendes et al VIDA, Nasrin et al VIDA].

To understand how *Giardia* might impact colonization/infection with pathogens associated with MSD, the 9 enteric pathogens with the largest attributable fractions for MSD in the VIDA study were included in these analyses after excluding pathogens whose codetection with *Giardia* was rare

(<5%: *Aeromonas*, *Salmonella*): *Campylobacter coli* or *Campylobacter jejuni*, *Shigella* spp., *Helicobacter pylori*, enterotoxigenic *Escherichia coli* bearing genes for heat-stable toxin (ST-EPEC), astrovirus, norovirus genotype II (GII), rotavirus, sapovirus, and *Cryptosporidium* spp. [Kotloff et al VIDA]. The association between *Giardia* and enteric pathogen detection was estimated using multivariable logistic regression, treating *Giardia* as the primary exposure and detection of an enteric pathogen (positive/negative) as the outcome variable. Models were adjusted for site, child's age group, sex, caregiver's education, household assets, and flooring type; breastfeeding was not included in these models due to its high collinearity with age group (Supplementary Table 1). We did not adjust by environmental risk factors as we wanted to include possible co-detections due to shared exposure pathways. To consider whether study site or age group acted as potential effect modifiers, regression models were run for each enteric pathogen to include an interaction term between *Giardia* and these variables. Results were presented by strata that showed a significant interaction.

### Ethical Considerations

This study was approved by the ethical review committees at the University of Maryland, Baltimore (HP-00062472), the US Centers for Disease Control and Prevention (CDC) (reliance agreement 6729), The Gambia Government/Medical Research Council/Gambia at the London School of Hygiene & Tropical Medicine (1409), the Comité d'Éthique de la Faculté de Médecine, de Pharmacie, et d'Odonto-Stomatologie, Bamako, Mali (no number), and the Kenya Medical Research Institute Scientific & Ethics Review Unit in Siaya County, Kenya (SSE 2996). Informed, written consent was obtained from all participants prior to initiation of study procedures.

## RESULTS

### *Giardia* Detection and Sociodemographic Factors

A total of 11 039 children were enrolled: 4840 children with MSD (cases) and 6213 matched children free of diarrhea (controls). *Giardia* was detected among 3556 (32%) of the enrolled children and was lower among cases (28%) than controls (35%) ( $P < .001$ ) (Table 1). Among controls, prevalence was highest in The Gambia (43%) and Mali (41%), with lower detection rates in Kenya (21%,  $P < .001$ ), and it was higher among older children, with 50% prevalence in 24- to 59-month-old children as compared to 18% in children 0–11 months old ( $P < .001$ ). Detection was more common in female controls compared to male controls (37% vs 34%,  $P = .007$ ) and was lower among controls whose caregivers completed at least primary school (25%) as compared to controls whose caregivers did not (40%,  $P < .001$ ). Detection was higher among controls in households with >3 assets (television, telephone, radio, bicycle/rickshaw, car/truck, motorcycle/

scooter, refrigerator, motorized boat, animal-drawn cart) than controls living in households with  $\leq 3$  assets (40% vs 29%,  $P < .001$ ). Controls living in households with finished floors had higher rates of *Giardia* detection (38%) as compared to children living in households with rudimentary or natural floors (28%,  $P < .001$ ). Finally, *Giardia* detection was highest among controls who were not breastfed: 47% of controls who were not breastfed, 28% who were partially breastfed, and 8% who were exclusively breastfed had *Giardia* detected in their stool ( $P < .001$ ).

*Giardia* detection by sociodemographic indicators among cases followed similar trends to controls, with the highest prevalence among cases in The Gambia (35%) and Mali (32%) followed by Kenya (18%,  $P < .001$ ). Detection was more common among 24- to 59-month-old cases (42%) compared to younger cases aged 0–11 months old (13%,  $P < .001$ ) and among female (30%) compared to male cases (27%,  $P = .042$ ). *Giardia* prevalence was lower among cases whose caregivers completed at least primary school compared to those who did not (22% vs 31%,  $P < .001$ ), but it was higher among cases living in households with >3 assets compared to households with  $\leq 3$  assets (33% vs 23%,  $P < .001$ ) and among cases living in households with finished floors compared to households with rudimentary or natural floors (30% vs 23%,  $P < .001$ ). As with controls, detection was most common among children who were not breastfed, with 40% of non-breastfed cases having *Giardia* detected in their stool, as compared to 22% of partially breastfed and 10% of exclusively breastfed cases ( $P < .001$ ). For both controls and cases, breastfeeding data were highly co-linear with age, whereby most older children did not breastfeed (Supplementary Table 1), and both groups had the highest *Giardia* prevalence (Table 1). *Giardia* prevalence was not significantly different among children living in households using clean versus other fuel sources or experiencing crowding for both controls and cases.

### Adjusted Associations Between *Giardia* and Enteric Pathogen Detection

We tested whether enteric pathogen detection differed between children with versus without *Giardia*, considering whether differences were unique to either cases or controls. Of the 9 enteric pathogens considered in the study (Table 2), detection of *C. coli/jejuni*, *Shigella* spp., *H. pylori*, and *Cryptosporidium* spp. was more common among children with *Giardia* compared to children without *Giardia* for both controls (*C. coli/jejuni*: 32% vs 26%,  $P < .001$ ; *Shigella* spp.: 28% vs 20%,  $P < .001$ ; *H. pylori*: 14% vs 11%,  $P = .001$ ; *Cryptosporidium* spp.: 21% vs 17%,  $P < .001$ ) and cases (*C. coli/jejuni*: 35% vs 31%,  $P = .014$ ; *Shigella* spp.: 41% vs 32%,  $P < .001$ ; *H. pylori*: 23% vs 17%,  $P = .014$ ; *Cryptosporidium* spp.: 26% vs 22%,  $P = .011$ ). Among controls only, the rate of astrovirus and sapovirus detection was higher among children with *Giardia* relative to children without *Giardia* (astrovirus: 5% vs 4%,  $P = .028$ ; sapovirus: 12% vs 10%,  $P = .042$ ). Among cases only, detection of norovirus GII and

**Table 1. Sociodemographic Indicators Among Children <5 Years With and Without *Giardia* Detection Among Controls and Cases at Enrollment in the VIDA Study, 2015–2018**

	Controls			Cases		
	No <i>Giardia</i>	<i>Giardia</i> n (%)	<i>P</i> value <sup>a</sup>	No <i>Giardia</i> n (%)	<i>Giardia</i> n (%)	<i>P</i> value <sup>a</sup>
All	4021 (65)	2183 (35)	...	3462 (72)	1373 (28)	...
Site			<b>&lt;.001</b>			<b>&lt;.001</b>
The Gambia	1212 (57)	926 (43)		1087 (65)	590 (35)	
Mali	1164 (59)	816 (41)		1100 (68)	508 (32)	
Kenya	1645 (79)	441 (21)		1275 (82)	275 (18)	
Age group			<b>&lt;.001</b>			<b>&lt;.001</b>
0–11 mo	1727 (82)	380 (18)		1487 (87)	232 (13)	
12–23 mo	1299 (61)	827 (39)		1158 (68)	538 (32)	
24–59 mo	995 (50)	976 (50)		817 (58)	603 (42)	
Sex			<b>.007</b>			<b>.042</b>
Male	2210 (66)	1122 (34)		1887 (73)	704 (27)	
Female	1811 (63)	1061 (37)		1575 (70)	669 (30)	
Caregiver education			<b>&lt;.001</b>			<b>&lt;.001</b>
<Primary school	2390 (60)	1626 (40)		2198 (69)	1008 (31)	
≥Primary school	1631 (75)	557(25)		1261 (78)	365 (22)	
Household assets <sup>b</sup>			<b>&lt;.001</b>			<b>&lt;.001</b>
>3	1965 (60)	1332 (40)		1757 (67)	852 (33)	
≤3	2054 (71)	848 (29)		1703 (77)	520 (23)	
Fuel source			.804			.678
Clean fuel <sup>c</sup>	227 (65)	120 (35)		197 (73)	74 (27)	
Other	3784 (65)	2059 (35)		3260 (72)	1298 (28)	
Crowding			.359			.722
≤2 persons sleeping/room	1795 (64)	1001 (36)		1571 (71)	631 (29)	
>2 persons sleeping/room	2226 (65)	1182 (35)		1890 (72)	742 (28)	
Flooring			<b>&lt;.001</b>			<b>&lt;.001</b>
Finished floor <sup>d</sup>	2807 (62)	1722 (38)		2446 (70)	1068 (30)	
Natural or rudimentary floor	1203 (72)	457 (28)		1012 (77)	305 (23)	
Breastfeeding			<b>&lt;.001</b>			<b>&lt;.001</b>
Exclusive	133 (92)	12 (8)		137 (90)	15 (10)	
Partial	2558 (72)	994 (28)		2177 (78)	604 (22)	
None	1330 (53)	1177 (47)		1148 (60)	754 (40)	

The bold values are *P* values < 0.05.

Abbreviation: VIDA, Vaccine Impact on Diarrhea in Africa.

<sup>a</sup>Tests the difference in *Giardia* detection rate across all subgroups.

<sup>b</sup>Includes television, telephone, radio, bicycle/trickshaw, car/truck, motorcycle/scooter, refrigerator, motorized boat, animal-drawn cart.

<sup>c</sup>Includes electric, propane, butane, natural gas.

<sup>d</sup>Includes parquet/polished wood, vinyl/asphalt strips, ceramic tile, cement, or carpet.

rotavirus was lower among children with *Giardia* relative to children without *Giardia* (norovirus GII: 9% vs 12%, *P* = .002; rotavirus: 9% vs 15%, *P* < .001).

When examining the association between *Giardia* and detection of other enteric pathogens, no significant associations were found in both cases and controls across all sites. There was also no significant effect of age on *Giardia*-enteric pathogen codetection in our models despite the high prevalence of *Giardia* among older children. Only *C. coli/jejuni* detection was found to be positively associated with *Giardia* detection in both controls and cases; however, although in controls this association was site-specific to The Gambia (adjusted odds ratio [aOR] [95% confidence interval {CI}]: 1.51 [1.22–1.86]), in cases it was across all 3 sites (1.16

[1.00–1.33]) (Figure 1, Supplementary Table 2). There were positive associations between *Giardia* and astrovirus (1.43 [1.05–1.93]) and *Cryptosporidium* spp. detection (1.24 [1.06–1.46]) among controls across all 3 sites, but not among cases (Figure 1, Supplementary Tables 3 and 4). There was a site-specific significant association between *Giardia* and rotavirus detection unique to cases; the odds of rotavirus detection were lower among cases with *Giardia* in Mali (0.45 [.30–.66]) and Kenya (0.31 [.17–.56]) but not in The Gambia (0.87 [.65–1.17]) (Figure 1, Supplementary Table 5). No other enteric pathogens associated with MSD in the VIDA study were associated with *Giardia* detection in our analyses (Figure 1, Supplementary Tables 6–10).

**Table 2. Detection of Enteric Pathogens Overall and Codetection With *Giardia* Among Controls and Cases in the VIDA Study**

	Controls				Cases			
	Detection n (%) <sup>a</sup>	Codetection With <i>Giardia</i> n (%) <sup>b</sup>	Detection Without <i>Giardia</i> n (%) <sup>c</sup>	<i>P</i> value <sup>d</sup>	Detection n (%) <sup>a</sup>	Codetection With <i>Giardia</i> n (%) <sup>b</sup>	Detection Without <i>Giardia</i> n (%) <sup>c</sup>	<i>P</i> value <sup>d</sup>
<b>Bacteria</b>								
<i>Campylobacter coli/jejuni</i>	1359 (28)	530 (32)	829 (26)	<b>&lt;.001</b>	1545 (32)	475 (35)	1070 (31)	<b>.014</b>
<i>Shigella</i> spp.	1084 (23)	468 (28)	616 (20)	<b>&lt;.001</b>	1641 (34)	553 (41)	1088 (32)	<b>&lt;.001</b>
<i>Helicobacter pylori</i>	558 (12)	226 (14)	332 (11)	<b>.001</b>	879 (18)	311 (23)	568 (17)	<b>.014</b>
ST-EPEC	944 (20)	340 (21)	604 (19)	.225	1084 (23)	321 (24)	763 (22)	.336
<b>Viruses</b>								
Astrovirus	210 (4)	87 (5)	123 (4)	<b>.028</b>	304 (6)	91 (7)	213 (6)	.509
Norovirus GII	513 (11)	167 (10)	346 (11)	.343	552 (11)	125 (9)	427 (12)	<b>.002</b>
Rotavirus	208 (4)	74 (5)	134 (4)	.717	642 (13)	122 (9)	520 (15)	<b>&lt;.001</b>
Sapovirus	529 (11)	203 (12)	326 (10)	<b>.042</b>	668 (14)	194 (14)	474 (14)	.649
<b>Parasites</b>								
<i>Cryptosporidium</i> spp.	873 (18)	349 (21)	524 (17)	<b>&lt;.001</b>	1106 (23)	348 (26)	758 (22)	<b>.011</b>

The bold values are *P* values < 0.05.

Abbreviations: ST-EPEC, heat-stable enterotoxigenic *E. coli*; VIDA, Vaccine Impact on Diarrhea in Africa.

<sup>a</sup>Percent is representative of detection among controls and cases in the study overall.

<sup>b</sup>Percent is representative of detection among controls and cases with *Giardia*.

<sup>c</sup>Percent is representative of detection among controls and cases without *Giardia*.

<sup>d</sup>Tests the difference between enteric pathogen detection in children with versus without *Giardia*.

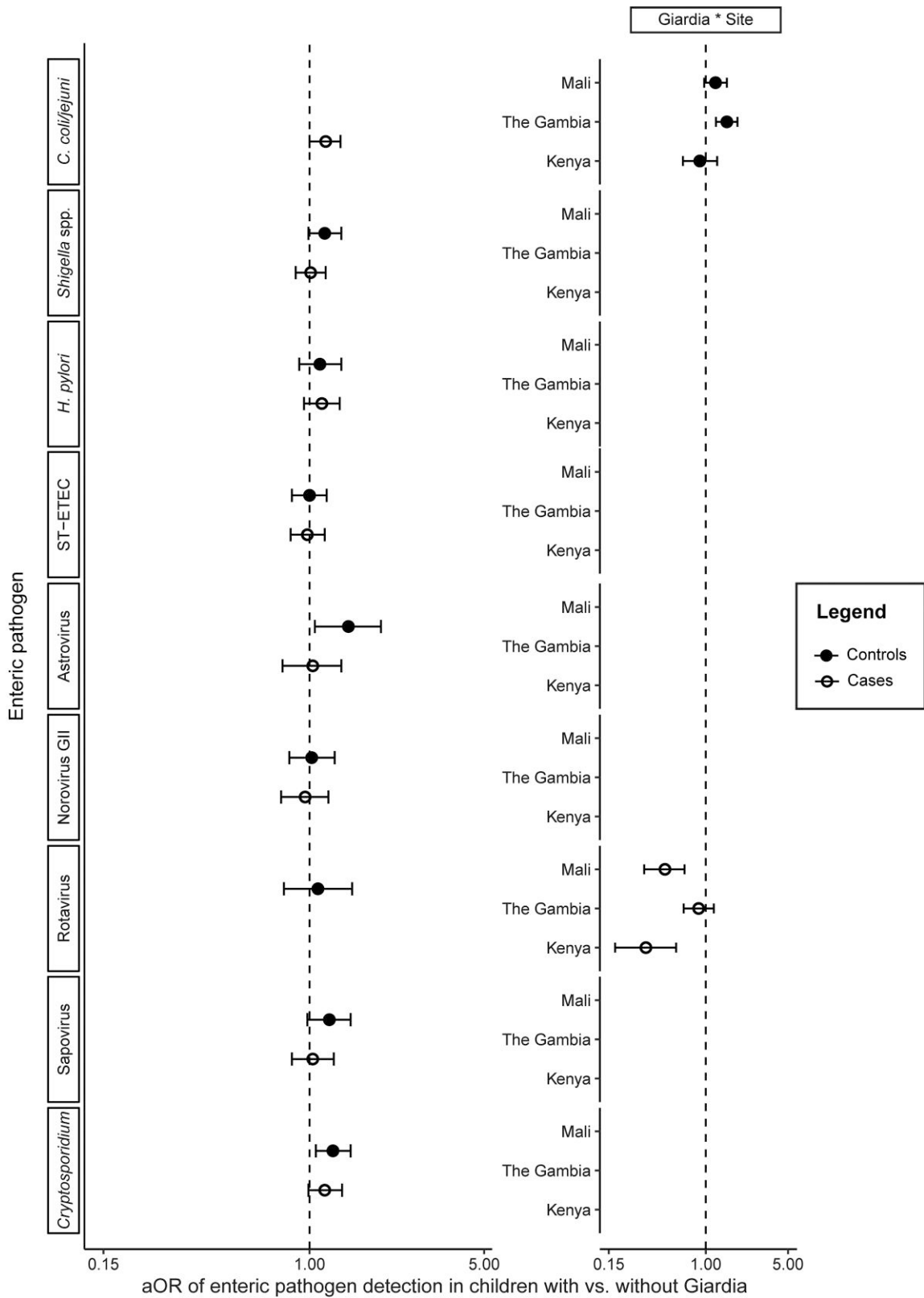
## DISCUSSION

We investigated whether detection of *Giardia* in children free of diarrhea and children with MSD was associated with detection of other enteric pathogens to better understand the relationship between *Giardia* and diarrhea among children in low-resource settings. In a population of children where *Giardia* detection was more common in children free of diarrhea compared to children with MSD, we found significant positive associations between detection of *Giardia* and *C. coli/jejuni*, *Cryptosporidium* spp., and astrovirus and a significant negative association between *Giardia* and rotavirus, but none of these associations were found among both clinical groups across all 3 sites. These findings suggest that interactions between *Giardia* and other pathogens may impact clinical outcomes normally associated with either pathogen, but that the outcome of this interaction could vary by context or other risk factors.

We observed a negative association between *Giardia* and rotavirus detection among children with MSD in Mali and Kenya. This finding that a pathogen known to cause MSD [6] [Kotloff et al VIDA] was less prevalent in children with *Giardia* compared to those without *Giardia* suggests that one possible mechanism behind *Giardia*'s protective effect against diarrhea could be that it decreases the pathogenicity of specific co-infecting organisms. A study in Bedouin infants found that infants coinfecting with *Giardia* and rotavirus experienced reduced diarrheal disease severity compared to children with rotavirus alone [22]. Although another case-control study

contradicts these results, showing greater risk of diarrhea in individuals with rotavirus and *Giardia* as compared to single-pathogen infections, the study population included children and adults, and controls were not matched to cases on age [23]. Alternatively, colonization/infection with one of these enteric pathogens could promote a gut environment not conducive to colonization/infection with the other. Indeed, *Giardia* has been shown to promote secretion of antimicrobial peptides, nitric oxide, reactive oxygen species, mucins, and other immune factors [24] which could in turn inhibit the pathogenesis of diarrhea due to other pathogens, including rotavirus. Understanding the possible molecular interactions between *Giardia* and rotavirus might be relevant to understanding vaccine performance given high *Giardia* prevalence and routine infant rotavirus immunization over the last decade in Mali and Kenya, where studies have found lower efficacy of these vaccines [25].

We found a positive association between *Giardia* and *C. coli/jejuni* detection in both children with MSD and free of diarrhea; only in the case of children free of diarrhea was this effect site-specific in The Gambia. This positive link is compatible with results from the MAL-ED study showing a positive correlation between *Giardia* and *Campylobacter* carriage [2]. Codetection could be common because *Giardia* and *Campylobacter* have shared pathways of exposure through untreated water and contact with dogs and cats [26, 27]; however, no shared pathways were found when examining environmental risk factors in the VIDA study [Berendes et al VIDA]. Alternatively, one pathogen could foster an environment



**Figure 1.** The aORs (*circles*) and 95% confidence intervals (*whiskers*) of enteric pathogen detection for children with versus without *Giardia*. Each pathogen row represents 2 models: 1 model for the association between *Giardia* and codetection of that pathogen in controls (*filled circles*), and 1 model for that same association in cases (*open circles*). The vertical dotted line at aOR = 1.0 represents no association between *Giardia* detection and enteric pathogen codetection; aOR < 1.0 represent a negative association, while aOR > 1.0 represent a positive association. If significant country-specific interactions with *Giardia* were detected (*Giardia \* Site*), country-level estimates are presented in lieu of single pan-site estimates. Abbreviation: aOR, adjusted odds ratio.

conducive to colonization/infection with the other. *Giardia* attaches to the intestinal epithelium, a site critical for production of pro-inflammatory chemokines in response to microbial infection. In *in vitro* and *ex vivo* experiments, *Giardia* attenuated secretion and directly degraded a pro-inflammatory chemokine, CXCL8, produced by human intestinal epithelial cells following exposure to *Salmonella* Typhimurium [14]. By dampening the immune response to other microbes, *Giardia* could create an environment conducive to *Campylobacter* colonization.

Similarly, *Giardia* was found to be positively associated with both *Cryptosporidium* spp. and astrovirus detection; however, this relationship was only present across all sites in children free of diarrhea at enrollment, despite both these pathogens being associated with MSD [6, 7] [Kotloff et al VIDA]. As with *C. coli/jejuni*, one enteric pathogen could promote a gut environment conducive to the other, but because the *Cryptosporidium* spp. and astrovirus link with *Giardia* was unique to children free of diarrhea, co-carriage could reduce inflammation that would otherwise cause MSD. Indeed, previous work found that children in Tanzania with *Giardia* had reduced levels of C-reactive protein in their serum, a marker for inflammation, whereas *Giardia* detection was associated with lower rates of subsequent diarrheal episodes in those children [28].

This study is subject to several limitations. First, although cases and controls are analyzed independently in our analyses, the selection of controls was based on matching to cases, so data from controls may not be generalizable to all children without diarrhea in these sites. Second, these analyses only consider single instances of codetection between *Giardia* and one enteric pathogen; as a result, multiple combinations of enteric pathogens in association with *Giardia* not captured in our analyses may have biased estimated effects. Third, some codetections may have been missed because we used assays with differing sensitivities to detect *Giardia* (ELISA) versus other enteric pathogens (TAC) in stool. However, the use of TAC for *Giardia* would have excluded more than 2000 specimens from these analyses. Finally, our analyses are cross-sectional and therefore cannot determine the causal relationship between *Giardia* and other enteric pathogens.

Our analyses demonstrate that *Giardia* is prevalent in young children living in low-resource settings and is associated with detection of other enteric pathogens associated with MSD, although there were no consistent associations across all sites and children with MSD or free of diarrhea. Given our findings, although *Giardia* on its own may not cause diarrhea in high prevalence settings, assessment of the risk of MSD from other enteric pathogens should include this pathogen in future models. Possible associations and underlying mechanisms between *Giardia* and enteric pathogen codetection and altered clinical outcomes, including diarrhea or malnutrition, should be analyzed in future studies.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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