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SUPPLEMENT ARTICLE

Etiology, Presentation, and Risk Factors for Diarrheal Syndromes in 3 Sub-Saharan African Countries After the Introduction of Rotavirus Vaccines From the Vaccine Impact on Diarrhea in Africa (VIDA) Study

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Background. Diarrheal disease is heterogeneous, including watery diarrhea (WD) and dysentery, some cases of which become persistent diarrhea (PD). Changes in risk over time necessitate updated knowledge of these syndromes in sub-Saharan Africa.

Methods. The Vaccine Impact on Diarrhea in Africa (VIDA) study was an age-stratified, case-control study of moderate-to-severe diarrhea among children <5 years old in The Gambia, Mali, and Kenya (2015–2018). We analyzed cases with follow-up of about 60 days after enrollment to detect PD (lasting ≥14 days), examined the features of WD and dysentery, and examined determinants for progression to and sequelae from PD. Data were compared with those from the Global Enteric Multicenter Study (GEMS) to detect temporal changes. Etiology was assessed from stool samples using pathogen attributable fractions (AFs), and predictors were assessed using χ^2 tests or multivariate regression, where appropriate.

Results. Among 4606 children with moderate-to-severe diarrhea, 3895 (84.6%) had WD and 711 (15.4%) had dysentery. PD was more frequent among infants (11.3%) than in children 12–23 months (9.9%) or 24–59 months (7.3%), $P = .001$ and higher in Kenya (15.5%) than in The Gambia (9.3%) or Mali (4.3%), $P < .001$; the frequencies were similar among children with WD (9.7%) and those with dysentery (9.4%). Compared to children not treated with antibiotics, those who received antibiotics had a lower frequency of PD overall (7.4% vs 10.1%, $P = .01$), and particularly among those with WD (6.3% vs 10.0%; $P = .01$) but not among children with dysentery (8.5% vs 11.0%; $P = .27$). For those with watery PD, *Cryptosporidium* and norovirus had the highest AFs among infants (0.16 and 0.12, respectively), while *Shigella* had the highest AF (0.25) in older children. The odds of PD decreased significantly over time in Mali and Kenya while increasing significantly in The Gambia.

Conclusions. The burden of PD endures in sub-Saharan Africa, with nearly 10% of episodes of WD and dysentery becoming persistent.

Keywords. diarrhea; dysentery; persistent; global; infection.

Diarrheal disease is the third leading cause of mortality among children <5 years of age globally, with 1 in 10 deaths attributed to diarrhea in 2019, and the greatest burden among children in South Asia and sub-Saharan Africa [1]. Although commonly described as a single entity, diarrheal disease comprises multiple clinical syndromes, each associated with different risk factors, causes, geographic distribution, pathophysiology, and sequelae. These include acute watery diarrhea (AWD) and bloody diarrhea (ie, dysentery), some cases of which progress to persistent diarrhea (PD). AWD is predominant in young children and characterized by frequent nonbloody loose or watery stools that can result in life-threatening dehydration and electrolyte abnormalities. Less common is bloody diarrhea, which historically has been associated with an increased risk of stunting [2], episodes of longer duration, and, in some settings, an increased risk of death compared with watery diarrhea (WD) [3–5]. Diarrheal episodes lasting ≥14 days, termed PD, are seen disproportionately among children in low- and middle-income countries and have

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been associated with more growth faltering and higher mortality rates [4, 6, 7].

During the past 3 decades, remarkable improvement has been observed in mortality rates associated with diarrheal disease in children <5 years old, attributed to declines in risks, such as unsafe water and sanitation and stunting [8], in association with social and economic development in low- and middle-income countries, coupled with improvements in case management and rotavirus vaccine introduction [9]. It is reasonable to expect that these ongoing shifts will result in changes in the etiology, manifestations, and outcomes of diarrhea in young children. However, progress has not been distributed equitably, in particular leaving areas of sub-Saharan Africa with a high prevalence of risk factors and poor outcomes [10]. Therefore, it is important to update our understanding of diarrheal diseases in sub-Saharan Africa to inform initiatives for preventing disease and death associated with diarrhea.

The Vaccine Impact on Diarrhea in Africa (VIDA) study was an age-stratified, matched case-control study that examined the incidence, etiology, and adverse clinical outcomes of moderate-to-severe diarrhea (MSD) among infants and young children after the introduction of rotavirus vaccine at 3 sites in sub-Saharan Africa. Herein we describe the features of WD and dysentery, and determinants for progression to and sequelae from PD in the VIDA study. We also examine temporal trends in PD, comparing the results with those from the Global Enteric Multicenter Study (GEMS), a similarly designed study conducted at the same sites 1 decade earlier [11, 12].

METHODS

Study Design and Participants

Between 11 May 2015 and 23 July 2018, children 0–59 months of age residing in 3 censused populations in sub-Saharan Africa with ongoing demographic surveillance systems (DSSs) were enrolled in VIDA [13, 14]. These sites (Basse, The Gambia; Bamako, Mali; and Siaya County, Kenya) had previously participated in GEMS between 1 December 2007 and 7 March 2011 before the introduction of the rotavirus vaccine [11, 12, 15–17]. Bansang, a demographically similar DSS area adjacent to Basse, was added in VIDA to increase the number enrolled. To permit comparisons, VIDA's clinical, epidemiological, and microbiological methods closely matched those used in GEMS. VIDA's methods [14] and the main results from GEMS and VIDA are reported elsewhere [11, 13]. Key methods are summarized below.

Enrollment in both GEMS and VIDA occurred over a 36-month period at each site. Eligible case patients were brought for care at sentinel health centers (SHC) serving the DSS population at each site for a new episode of diarrhea (≥3 abnormally loose stools within 24 hours with onset within 7 days after ≥7 diarrhea-free days), with ≥1 of the following features of MSD: sunken eyes (confirmed by the caregiver as more than normal), decreased skin turgor, intravenous hydration administered or prescribed, blood in the stool, or hospital admission recommended. We aimed to enroll the first 8–9 eligible cases per fortnight in each age stratum (infants [age 0–11 months], toddlers [12–23 months[, and children [24–59 months]). For every enrolled case patient, eligible controls were randomly selected from the site's DSS database; 1–3 diarrhea-free controls were enrolled within 2 weeks of the index case enrollment, matched for sex, residential area, and age (±2 months for children aged <12 months and ±4 months for those aged 12–59 months) [11].

Clinical and Epidemiological Procedures and Definitions

At enrollment, the participant's primary caretaker underwent a standardized interview to document demographic, epidemiological, and clinical information. Each child's height/length was measured and converted to a height-for-age *z* score (HAZ) based on World Health Organization (WHO) standards [18], with HAZ <−2 considered to indicate stunting [19]. Each case patient provided a fresh stool sample to be assessed for enteropathogens. Treatment data were collected for the duration of the child's stay at the sentinel health center and for a prescription given for home treatment. A child was considered to have received antibiotics based on intent to treat (ie, administered at the SHC or a prescription was given). To estimate the duration of diarrhea, caretakers recorded daily diarrhea (presence or absence) for the ensuing 14 days onto a memory aid (Supplementary Figure 1) [12]. Degree of dehydration was categorized per WHO guidelines [20]. A modified Vesikari score was calculated based on diarrhea and vomiting duration, the maximum daily frequency of diarrheal stools and emesis episodes, fever, and the degree of dehydration (Supplementary Table 1) [21].

About 60 days after enrollment (range, 50–90 days), fieldworkers visited participants at home to assess the child's vital status and to repeat anthropometric measurements. The memory aids were reviewed with the caretaker and collected.

The diarrheal syndromes are defined as follows: (1) AWD, nonbloody MSD lasting <14 days; (2) persistent WD, WD lasting ≥14 days; and (3) bloody diarrhea (dysentery), MSD with blood in stool observed by the caretaker, clinician, or laboratory staff. Owing to a paucity of persistent bloody diarrhea cases, acute and persistent bloody diarrhea were combined for etiologic analyses.

Laboratory Procedures

Enteropathogens were identified in whole-stool samples using a customized TaqMan Array Card that compartmentalized probe-based quantitative polymerase chain reaction (qPCR) assays [13, 17]. A qPCR cycle cutoff value <35 was considered positive. In VIDA, stool samples from all enrolled cases patients and from the first diarrhea-free control were tested. In GEMS, a random sample of case patients and their first diarrhea-free controls were tested [17].

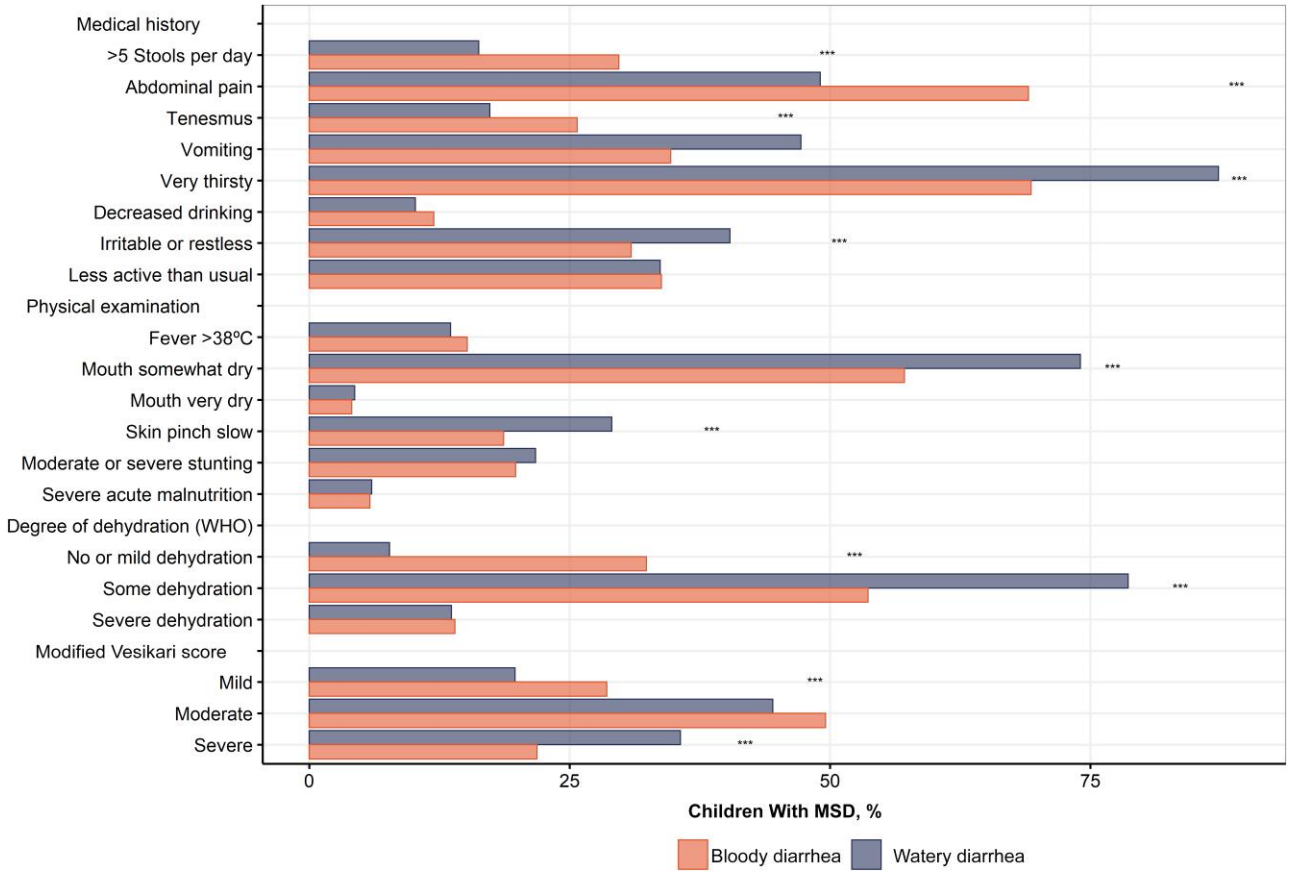

Figure 1. Clinical features among children with bloody or watery syndromes of moderate-to-severe diarrhea (MSD), based on findings at enrollment from medical history and physical examination. The level of severity was assessed for each syndrome at physical examination using 2 severity scores: the World Health Organization (WHO) dehydration assessment and the modified Vesikari score. ***$P < .001$ (χ^2 test).

Statistical Methods

Etiology of MSD by Syndrome and Study

Pathogen attributable fractions (AFs) were calculated for the 3 sites combined, stratified by diarrheal syndrome, study (GEMS or VIDA), and age, as described elsewhere [14]. Briefly, a conditional logistic regression model was used to assign a population AF of cases to a given pathogen, adjusting for other pathogens and allowing for interactions between qPCR cycle values, diarrheal syndrome, and age group. Although GEMS originally used different analytic methods, GEMS data were reanalyzed here using the VIDA methods.

Risk Factors for Progression to PD and Diarrhea Duration in VIDA

Clinical, demographic, and socioeconomic predictors of PD were examined in bivariate analysis using χ^2 tests. Among MSD case patients, clinical and demographic predictors of PD included diarrhea type, clinical findings, age, site, HAZ, and receipt of antibiotics; caregiver completion of at least primary school and electricity in the household were selected a priori as key socioeconomic predictors. The analysis for the association between prescription of antibiotics and PD focuses on antibiotics recommended by WHO for dysentery (ciprofloxacin, third-generation cephalosporins, azithromycin, and pivmecillinam) [22]. There are currently no recommendations for the use of antibiotics in the treatment of WD other than for cholera. Owing to the low number of PD episodes, further exploration of subgroup-specific associations between treatment and duration of diarrhea used the median duration of diarrhea as the outcome instead of PD. Bivariate assessment of differences between median durations by subgroup was done using Wilcoxon rank sum tests. Owing to the high prevalence of *Shigella* detection in WD identified herein and elsewhere [17], we included WD episodes in this analysis despite a lack of WHO recommendations for this indication.

We examined the odds that a diarrheal episode would become PD with increasing levels of pathogen presence (AF). Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for developing PD were calculated using multivariate logistic regression, adjusting for child age, caregiver education, the presence of electricity in the household, enrollment HAZ, and site. All other pathogens, as well as pathogen × site interaction terms, were tested for inclusion and maintained in the model if significant.

Change in PD Over Time

We assessed whether the odds of PD among MSD cases had changed between GEMS versus VIDA, using regression models that tested for interaction between the studies and age category, study site, and diarrhea type (bloody or watery). Odds ratios and 95% CIs were reported for the probability of persistence, comparing VIDA and GEMS by age and study site. Adjusted models were constructed, including interaction terms for bloody diarrhea versus WD and site, and adjusted for stunting, antibiotic prescription, fever, vomiting, stool frequency, lethargy, dehydration, caregiver education, and household electricity.

Clinical Outcomes

We examined the association of each syndrome with growth faltering by comparing the change in HAZ between enrollment and the 60-day follow-up visit among case patients in VIDA and their matched controls using adjusted linear regression, adjusting for the following variables selected a priori: age, study site, enrollment HAZ, caregiver education level, and follow-up time.

Few deaths were reported in the study, so to include the maximum number of participants when examining the association between diarrheal syndromes and deaths, participants without memory-aid data were included if they had a final "child health" variable (where death was recorded) and the death date could be used to infer the duration of diarrhea. Owing to low outcome numbers, adjusted models were not feasible. We used χ^2 tests to assess the differences in frequency of deaths, comparing those who had dysentery versus WD and comparing those with PD versus no PD. Dichotomous variables were compared using χ^2 tests, and continuous variables using Wilcoxon rank sum tests. Differences were considered statistically significant at $P < .05$.

Ethical Review

The current study was approved by the ethical review committees at the University of Maryland, Baltimore (no. HP-00062472), the Centers for Disease Control and Prevention (reliance agreement 6729), The Gambia Government/Medical Research Council/Gambia at the London School of Hygiene & Tropical Medicine (no. 1409), the Comité d'Ethique 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 (no. SSE 2996). Informed, written consent was obtained from caretakers for all participants before initiation of study procedures.

RESULTS

Participants and Clinical Syndromes

A total of 4606 children with MSD from the VIDA study who had pathogens assessed by means of qPCR and follow-up data are included in this analysis, with 1574, 1550, and 1482 children in The Gambia, Mali, and Kenya, respectively (Table 1). In total, 3895 case patients (84.6%) had WD, of which 376 cases (9.7%) became persistent; 711 (15.4%) had bloody diarrhea, of which 67 cases (9.4%) became persistent.

Clinical Presentation of Diarrheal Syndromes in VIDA

Children with bloody diarrhea were significantly more likely to pass >5 stools per day and to experience abdominal pain and tenesmus (Figure 1), while those with WD were significantly more likely to appear dehydrated, with increased thirst, decreased skin turgor, and dry mouth, which corresponded to a higher likelihood of WHO-defined dehydration and a severe modified Vesikari score.

Etiology of MSD by Syndrome and Study

Pathogen AFs varied by MSD syndrome, age group, and study, although CIs overlapped (Figure 2). Among infants with AWD, rotavirus (AF, 0.16) and *Cryptosporidium* (AF, 0.13) were the pathogens most frequently classified as etiologic in VIDA. Results were similar for GEMS.

Shigella had the highest AFs among older children with AWD (0.17 and 0.14 for toddlers and children, respectively) in VIDA, similar to the GEMS findings. The AFs were also high for rotavirus, *Cryptosporidium, Campylobacter,* and *Helicobacter pylori* (Figure 2). *Shigella* had the highest AF among children with bloody diarrhea in all age groups in VIDA, and the AFs increased with age (0.28, 0.71, and 0.61, among children aged 0–11 months, 12-23 months, 24–59 months, respectively). *Campylobacter* had high AFs in the 2 youngest age groups (0.18 and 0.14, 0–11 months and 12–23 months, respectively). Results were similar in GEMS.

For those with persistent WD, *Cryptosporidium* (AF, 0.16) and norovirus (AF, 0.12) had the highest AFs among infants. *Shigella* (AF, 0.25), *Cryptosporidium* (AF, 0.23), and enterotoxigenic *Escherichia coli* (encoding heat-stable toxin (ST-ETEC); AF, 0.20) had the highest AFs among toddlers. ST-ETEC outranked *Shigella* in the oldest stratum (AF, 0.21), in contradistinction to GEMS. *Cryptosporidium* (AF, 0.13) and *Campylobacter* (AF, 0.10) were also prominent (Figure 2).

Risk Factors for Progression to PD and Diarrhea Duration in VIDA

The risk of PD in VIDA was highest among infants (11.3%) and decreased with age ($P = .001$) (Table 1). The risk was significantly higher in Kenya (15.5%) and The Gambia (9.3%) than in Mali (4.3%). Lower caregiver education and lack of household electricity significantly increased the risk of PD. Children with MSD who progressed to PD were significantly more likely to present with lethargy, dehydration, and a modified Vesikari score in the severe range compared to those who did not progress to PD (Table 1).

When examined categorically, stunting at enrollment was not associated with the development of PD (Table 1). When

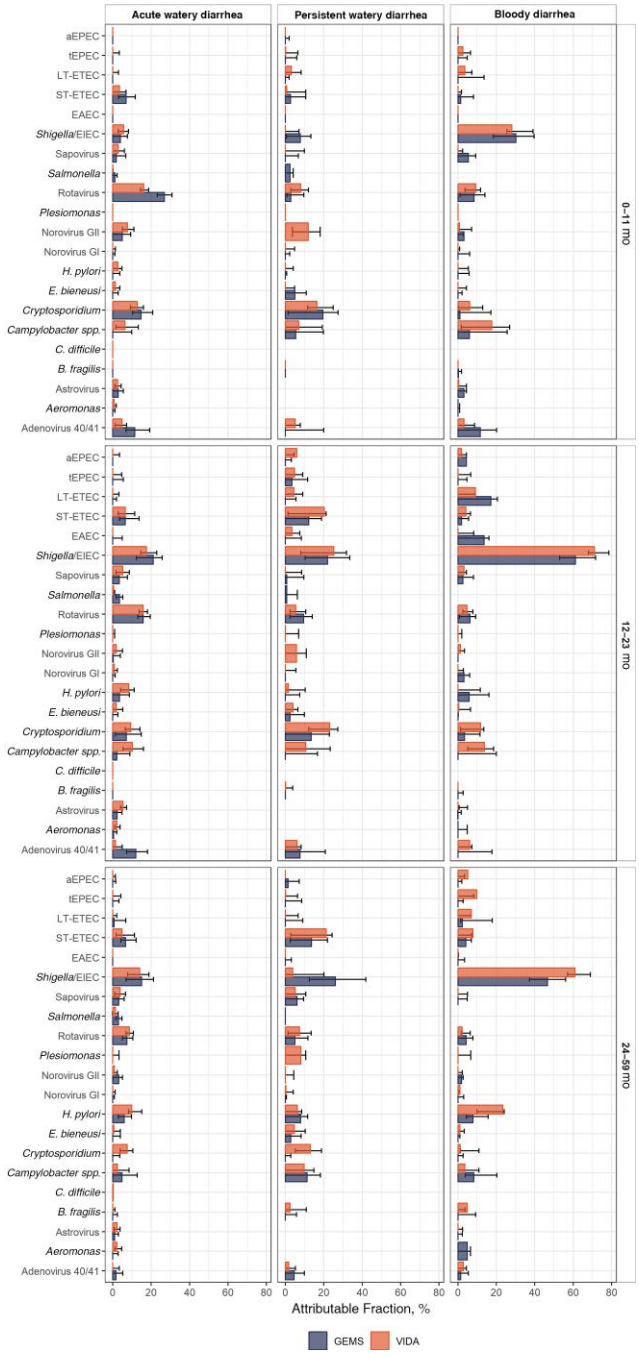

Figure 2. Attributable fractions and 95% confidence intervals among pathogens significantly associated with moderate-to-severe diarrhea among children residing at 3 sites in sub-Saharan Africa, by diarrhea syndrome, in the Vaccine Impact on Diarrhea in Africa (VIDA) study and the Global Enteric Multicenter Study (GEMS). Pathogen detection was performed by means of quantitative polymerase chain reaction using a customized TaqMan Array Card. *A, B,* and *C* display results for the 0–11-, 12–23-, and 24–59-month age strata, respectively. Abbreviations: aEPEC, atypical enteropathogenic *Escherichia coli*; *B. fragilis, Bacteroides fragilis*; *C. difficile, Clostridioides difficile*; *E. bieneusi, Enterocytozoon bieneusi*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; *H. pylori, Helicobacter pylori*; LT-ETEC, heat-labile enterotoxigenic *E. coli*; ST-ETEC, heat-stable enterotoxigenic *E. coli*; tEPEC, typical enteropathogenic *E. coli*.

HAZ at enrollment was examined as a continuum in multivariable regression, an increase in HAZ was not significantly associated with decreased odds of PD (aOR, 0.92 [95% CI, .84–1.01]) (Figure 3).

In multivariable regression for the association between pathogen levels (AFs) and PD, neither inclusion of caretaker education nor the presence of electricity in the household improved model fits, so these factors were not included in the final models. *Cryptosporidium* was the only pathogen significantly associated with an increased aOR for PD after adjustment for age and site. rotavirus, *H. pylori,* and *Shigella* were associated with decreased odds of PD (Figure 3 and Supplementary Figure 2).

Impact of Treatment on PD and Duration of Diarrhea in VIDA by Clinical Syndrome

Among 4560 case patients with available data, 424 of 697 (62.3%) with dysentery and 448 of 3863 (11.6%) with WD were prescribed WHO-recommended antibiotics (Supplemental Table 3). PD developed less often in children who were prescribed antibiotics (7.4%) than in those who were not (10.1%) overall ($P = .01$), and among those with WD (6.3% vs 10.0%; $P = .01$) but not among children with dysentery (8.5% vs 11.0%; $P = .27$) (Table 2). However, when we examined the impact of treatment on the linear duration of bloody diarrhea, a significant shortening was observed ($P = .001$) (Table 3).

In subgroup analyses, treatment was significantly associated with shorter diarrhea duration among toddlers 12–23 months of age ($P = .002$) and among children with *Shigella* detection ($P = .03$). The effect was comparable in children with and those without stunting ($P = .02$ for both). A similar trend was seen for WD ($P = .06$), where a significant decrease in diarrhea duration associated with treatment was also seen in The Gambia ($P = .03$) and in children with shigellosis ($P = .02$).

Overall, zinc was prescribed in VIDA in 96.7% of MSD cases in Kenya, 5.7% in Mali, and 48.5% in The Gambia (Supplementary Table 4). There was no association between zinc and PD in Kenya and Mali, likely owing to the homogeneity of zinc prescribing. In The Gambia, zinc was associated with a decreased risk of PD ($P = .01$).

Change in Odds of PD Between GEMS and VIDA

By comparison, 12.2% of the 4535 GEMS cases of WD became persistent, along with 14.7% of the 565 bloody diarrhea cases (Supplementary Table 2). Compared with GEMS, in the VIDA study the odds of MSD persisting decreased significantly in Mali and Kenya for both WD and bloody diarrhea (Table 4). These trends did not vary significantly by age group and remained after adjustment for other factors. By contrast, in The Gambia, the odds of WD becoming persistent were >2-fold higher in VIDA compared with GEMS; this risk was observed in all age groups and after adjustment for other factors. The

Table 1. Bivariate Association Between Risk Factors for Development of Persistent Diarrhea Among 4606 Children With Moderate-to-Severe Diarrhea in the Vaccine Impact on Diarrhea in Africa Study

Risk Factor	Children With MSD, No. (n = 4606)	Children With PD, No. (%) (n = 443 [9.6%])	*P* Value[a]
Demographic features			
Age group			
0–11 m	1631	184 (11.3)	.001[a]
12–23 m	1618	160 (9.9)	
24–59 m	1357	99 (7.3)	
Study site			
Kenya	1482	229 (15.5)	<.001[a]
The Gambia	1574	147 (9.3)	
Mali	1550	67 (4.3)	
Socioeconomic indicators			
Caretaker's educational level			
Less than primary school	3054	255 (8.4)	<.001[a]
At least primary school	2303	174 (7.6)	
Electricity in home			
No	2303	269 (11.7)	<.001[a]
Yes	2303	174 (7.6)	
Clinical findings			
Diarrhea type			
Watery	3895	376 (9.7)	.85
Bloody	711	67 (9.4)	
Stunting (HAZ >−2)			
No	3583	333 (9.3)	.16
Yes	1023	110 (10.8)	
Fever			
No	3972	381 (9.6)	.89
Yes	634	62 (9.8)	
Vomiting			
No	2463	224 (9.1)	.20
Yes	2143	219 (10.2)	
Diarrhea stools per day			
≤5	3804	345 (9.1)	.006[a]
>5	802	98 (12.2)	
WHO-defined dehydration			
None	438	41 (9.4)	.004[a]
Some	3527	313 (8.9)	
Severe	641	89 (13.9)	
Lethargy			
No	3043	244 (8.0)	<.001[a]
Yes	1562	199 (12.7)	
Modified Vesikari score			
Severe	1219	142 (11.7)	<.001[a]
Moderate	1845	187 (10.1)	
Mild	1538	114 (7.4)	
Skin pinch slow			
No	3291	312 (9.5)	.62
Yes	1315	131 (10.0)	
Malnutrition			
No	4025	386 (9.6)	.87
Yes	581	57 (9.8)	

Abbreviations: HAZ, height-for-age *z* score; MSD, moderate-to-severe diarrhea; WHO, World Health Organization.

[a]Significant at $P < .05$ (*P* values based on χ^2 test).

odds of bloody diarrhea becoming persistent were also higher in The Gambia in VIDA compared with GEMS. However, this increase was not statistically significant overall or in any age group. The adjustment did not change this relationship.

Clinical Outcomes

We examined the association of each syndrome with growth faltering by comparing the change in HAZ between enrollment and the 60-day follow-up visit among case patients in VIDA and their matched controls (Table 5). Compared with controls, all cases had more growth faltering, except those with persistent bloody diarrhea. There was no association between PD and growth faltering when comparing AWD with watery PD. Thirty-seven MSD case patients in VIDA died. There was no significant difference in the frequency of death comparing children with bloody diarrhea and those with WD (5 of 722 [0.69%] vs 32 of 3934 [0.81%], respectively; $P = .74$). Among the those who died, 35 had sufficient duration data, and the proportion who died was higher among those with than among with those without PD (6 of 440 [1.36%] vs 29 of 4214 [0.69%], respectively; $P = .12$), but this difference was not significant.

DISCUSSION

Our findings indicate that PD continues to be a public health problem in sub-Saharan Africa, though the proportion of diarrheal episodes progressing to PD has decreased in the decade between GEMS and VIDA in Mali and Kenya but not in The Gambia [23, 24]. Among episodes of medically attended MSD, we found that PD developed in nearly 1 in 10 children. Those with persistent WD not only had significantly more growth faltering compared with controls, but they also had a higher probability of dying within 2–3 months of enrollment than those with AWD, although this difference was not significant.

Site-to-site differences in the proportion of MSD episodes that proceed to PD were apparent, ranging from 4.3% in Mali to 15.5% in Kenya. In addition, after introduction of rotavirus vaccination, the proportional distribution of PD increased in The Gambia but decreased significantly in both Mali and Kenya. Because we used consistent methods across our sites, we suspect that the patterns reflect inherent differences in exposure to factors that may affect diarrhea duration, such as malnutrition [25]), improved sanitation and hygiene [26], treatment with antibiotics and zinc [27], reduced human immunodeficiency virus exposure [28], and AF of etiologic agents.

We found that the risk of PD was higher among infants 0–11 months of age than in older children, as others have reported [7, 29–33]. This pattern coincides with the peak incidence of acute diarrhea and has been attributed to increased susceptibility to enteric infections among immunologically naive infants,

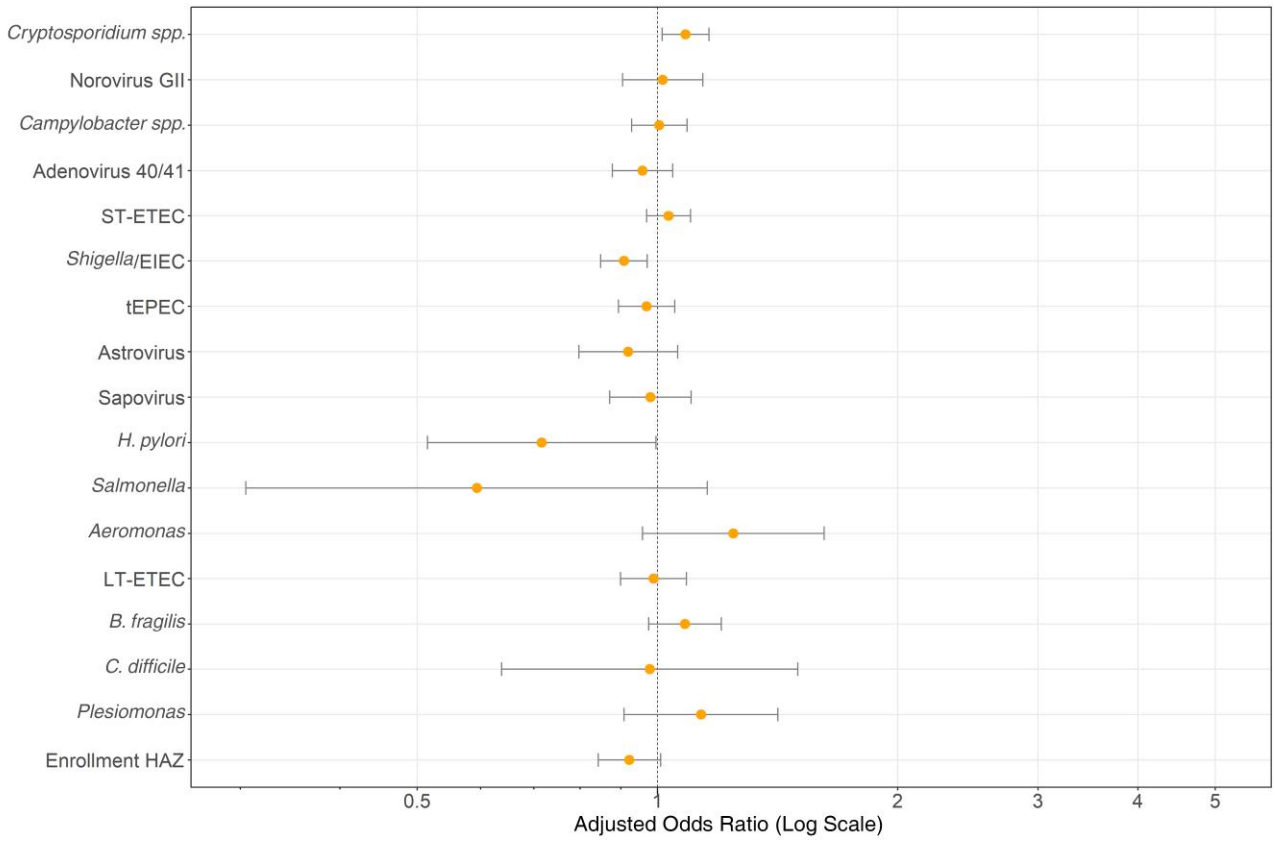

Figure 3. Pathogen-specific odds that an episode of moderate-to-severe diarrhea will become persistent (duration ≥14 days) among children from 3 sites in sub-Saharan Africa participating in the Vaccine Impact on Diarrhea in Africa (VIDA) study. A multivariate logistic regression model was used, combining watery and bloody diarrheal episodes, with adjustment for child age, height-for-age *z* score (HAZ) at enrollment, and study site. Results of pathogen × site interactions are shown in Supplementary Figure 2. Abbreviations: *B. fragilis, Bacteroides fragilis*; *C. difficile, Clostridioides difficile*; EIEC, enteroinvasive *Escherichia coli*; *H. pylori, Helicobacter pylori*; LT-ETEC, heat-labile enterotoxigenic *E. coli*; ST-ETEC, heat-stable enterotoxigenic *E. coli*; tEPEC, typical enteropathogenic *E. coli*.

perhaps in concert with other age-related vulnerabilities [31, 33–35]. In our study, as in GEMS, the clinical severity of the presenting illness increased the likelihood of progression to PD. Similar findings have been reported elsewhere [32, 36, 37], although the impact of dehydration as a predisposing condition has been inconsistent [29]. Notably, PD was associated with severe but not acute dehydration, which raises the possibility that clinical signs we observed, such as lethargy and decreased intake, which meet WHO criteria for severe dehydration, may actually have been attributable to an alternative disease process.

In contrast to other reports [3–5], the likelihood of progressing to PD was not greater for bloody diarrhea than for WD. On the other hand, sociodemographic factors such as lack of maternal primary education and indicators of low household wealth have consistently been found as risk factors in our study and others [31, 34, 38]. The presence of rotavirus was associated with decreased odds of progression to PD, which may have been related to the characteristically short lived rotavirus infection.

Defining whether malnutrition is a risk factor for the development of PD has proved to be challenging [39]. We did not observe an association between stunting as a categorical variable and PD, but we did observe a trend suggesting an association between lower enrollment HAZ and PD.

We estimated the population AF of enteropathogens present early in the illness and significantly associated with each diarrheal syndrome controlling for the presence of other pathogens. Rotavirus remained a significant cause of AWD among children <24 months of age, despite the introduction of rotavirus vaccination between GEMS and VIDA at all sites. *Shigella* was the most important pathogen among cases of bloody diarrhea in all age groups in both GEMS and VIDA. *Campylobacter* spp. was also associated with bloody diarrhea, as reported elsewhere, having increased among children <24 months of age in VIDA compared with GEMS [3, 40–42]. In VIDA, the large AF for *H. pylori* among episodes of both bloody diarrhea and AWD among children 24–59 months of age was unexpected, although similar observations were seen in GEMS and remain of uncertain significance [43].

Table 2. Bivariate Association Between Prescription of World Health Organization–Recommended Antibiotics and Development of Persistent Diarrhea (≥14-Day Duration)

Diarrhea Type by Antibiotic Prescription Status	Children With MSD, No. (%)[a]			*P* Value
	Total	Not Persistent	Persistent	
Any diarrhea	n = 4560	n = 4124	n = 436	
No antibiotics prescribed	3678 (80.4)	3307 (80.2)	371 (85.1)	.01[b]
Antibiotics prescribed	882 (19.6)	817 (19.8)	65 (14.9)	
Acute bloody diarrhea	n = 697	n = 631	n = 66	
No antibiotics prescribed	263 (37.7)	234 (37.1)	29 (43.9)	.27
Antibiotics prescribed	434 (62.3)	397 (62.9)	37 (56.1)	
Acute watery diarrhea	n = 3863	n = 3493	n = 370	
No antibiotics prescribed	3415 (88.4)	3073 (88)	342 (92.4)	.01[b]
Antibiotics prescribed	448 (11.6)	420 (12)	28 (7.6)	

Abbreviation: MSD, moderate-to-severe diarrhea.

[a]Including all children with MSD for whom prescribing information was available.

[b]Significant at $P < .05$ (P values based on χ^2 test).

Table 3. Association Between Treatment With World Health Organization—Recommended Antibiotics and Duration of Diarrhea in Children With Moderate-to-Severe Diarrhea From the Vaccine Impact on Diarrhea in Africa Study, Stratified by Enrollment Characteristics

Characteristic by Diarrhea Type at Enrollment	Duration of Diarrhea, Median (IQR), d		
	No Antibiotic Prescribed (n = 3678)	Antibiotic Prescribed (n = 882)	*P* Value
Bloody diarrhea	6 (4–9)	5 (4–8)	**.001**[a]
Age group			
0–11 m	7 (5–11)	6 (4–10)	.15
12–23 m	7 (5–9)	5 (4–8)	**.002**[a]
24–59 m	5 (3–9)	5 (3–7.5)	.56
Site			
Kenya	7 (4–11)	5 (4–11)	.28
Mali	6 (5–8)	4 (3–7)	.22
The Gambia	6 (4–9)	5 (4–8)	.19
Shigella detected			
No	6 (5–11)	6 (4–10)	.09
Yes	6 (4–9)	5 (3–8)	**.03**[a]
Stunting (HAZ >−2)			
Yes	7 (5–11)	5 (3–10)	**.02**[a]
No	6 (4–9)	5 (4–8)	**.02**[a]
Watery diarrhea	5 (4–8)	5 (3–7)	.06
Age group			
0–11 m	6 (4–8)	6 (4–8)	.89
12–23 m	5 (4–8)	5 (3–8)	**.05**[a]
24–59 m	5 (3–7)	4 (3–6)	.10
Site			
Kenya	6 (4–10)	7.5 (4–9)	.86
Mali	4 (3–6)	5 (3–7)	.29
The Gambia	5 (4–9)	5 (3–8)	**.03**[a]
Shigella detected			
No	5 (4–8)	5 (4–7)	.57
Yes	5 (4–8)	5 (3–7)	**.02**[a]
Stunting (HAZ >−2)			
Yes	5 (4–9)	5 (4–8)	.30
No	5 (4–8)	5 (3–7)	.12

Abbreviations: HAZ, height-for-age *z* score; IQR, interquartile range.

[a]Significant at $P < .05$ (P values based on Wilcoxon rank sum test).

The AF of *Cryptosporidium* was comparable to rotavirus among infants with AWD and was a leading cause of persistent WD at all ages, as reported elsewhere [31, 44, 45]. Although not significant in multivariable analysis, we and others found additional pathogens that were associated with PD in bivariate analysis, including ST-ETEC [31, 39], *Shigella* [39, 46], norovirus, and *Campylobacter* spp. [46]. Norovirus was associated with persistent WD in VIDA but not in GEMS [11].

Antibiotics appeared to confer a significant reduction in the duration of both bloody diarrhea and WD that seemed to be driven by the impact on shigellosis. This presents a dilemma, since the risk of PD among children with WD (9.7%), for which no recommendation for the use of antibiotics exists, is comparable to that among children with bloody diarrhea (9.4%), which has a treatment indication. Although our findings provide an argument for expanded treatment of diarrheal diseases in sub-Saharan Africa, the risk of emerging antibiotic resistance remains problematic. Regardless, innovative nutritional rehabilitation [47], accelerated development of *Shigella* vaccines [48–50], and enhanced messaging to encourage the use of zinc for diarrheal diseases is warranted.

In this population, children with persistent bloody diarrhea were no more likely to have growth faltering than controls. Those with bloody diarrhea had a high rate (62.4%) of treatment with antibiotics, and our group has previously demonstrated that that children with *Shigella* who receive antibiotics grow better [51]. Thus, the lack of association between bloody diarrhea and growth faltering may well be the result of antibiotic use.

The current study had several strengths. While community-based studies are ideal for studying risk factors of PD, there have been few since the 1990s [35], or in Africa [34, 38]. VIDA's design optimized our ability to assess risk factors and etiology by collecting data within 7 days of episode onset and analyzing linked specimens using highly sensitive qPCR tests.

Table 4. Odds of Persistent Diarrhea and Adjusted Odds Ratio for Persistent Diarrhea Comparing the Vaccine Impact on Diarrhea in Africa Study With the Global Enteric Multicenter Study

Diarrhea Type by Location and Study	Odds of PD	aOR (95% CI)[a]
Watery diarrhea		
The Gambia		
GEMS	0.06	Reference
VIDA	0.11	2.2 (1.6–3.2)
Mali		
GEMS	0.11	Reference
VIDA	0.04	0.4 (.3–.5)
Kenya		
GEMS	0.25	Reference
VIDA	0.19	0.8 (.6–.9)
Bloody diarrhea		
The Gambia		
GEMS	0.06	Reference
VIDA	0.09	1.5 (.9–2.3)
Mali		
GEMS	0.15	Reference
VIDA	0.09	0.3 (.2–.4)
Kenya		
GEMS	0.39	Reference
VIDA	0.15	0.5 (.3–.7)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; GEMS, Global Enteric Multicenter Study; PD, persistent diarrhea; VIDA, Vaccine Impact on Diarrhea in Africa.

[a]Adjusted using logistic regression for odds of PD in VIDA versus GEMS, including interaction terms for bloody versus watery diarrhea and site and adjustment for stunting, antibiotic prescription, fever, vomiting, stool frequency, lethargy, dehydration, caregiver educational level, and household electricity.

Table 5. Association Between Change in Height-for-Age *z* Score and Diarrheal Syndrome From Adjusted Linear Regression Model

Characteristic	Change in HAZ Compared With Reference Group	Standard Error	*P* Value
Diarrheal syndrome			
Controls	Reference	…	…
Case patients			
Acute bloody diarrhea	−0.036	0.015	.02
Acute watery diarrhea	−0.076	0.008	<.001
Persistent bloody diarrhea	0.060	0.043	.16
Persistent watery diarrhea	−0.076	0.019	<.001
Age group			
0–11 m	Reference	…	…
12–23 m	0.136	0.009	<.001
24–59 m	0.231	0.009	<.001
Study site			
The Gambia	Reference	…	…
Kenya	0.054	0.010	<.001
Mali	0.084	0.009	<.001
Enrollment HAZ[a]	−0.067	0.003	<.001
Caregiver educational level			
Less than primary school	Reference	…	…
At least primary school	0.030	0.009	<.001

Abbreviation: HAZ, height-for-age *z* score.

Adjusted for age, site, enrollment HAZ, caregiver education level, and follow-up time.

[a]Values for enrollment HAZ indicate the effect of enrollment HAZ on change in HAZ.

Longitudinal follow-up of case patients and controls using a memory aid allowed prospective detection of episodes that became PD. We were able to compare our findings from VIDA and GEMS, a study conducted 10 years earlier using the same study sites and nearly identical methods to assess temporal changes.

Nonetheless, this study has several limitations. The definition of PD relied on the accurate completion of our memory aid by caretakers with high illiteracy levels. Fortunately, compliance was high, and data integrity was optimized using training and the involvement of literate family members. Our study was not designed to assess compliance with antibiotic or zinc treatment, so we performed an "intent-to-treat" analysis. It is possible that the PD cases included may not be generalizable to PD episodes that do not result in medical care. Attribution of a diarrheal episode to an individual pathogen is difficult, as many cases had multiple pathogens detected, and qPCR cannot ensure the presence of a clinically significant infection. However, our ability to compare case patients with age- and site-matched controls improves the accuracy of identification of likely etiologic agents. Finally, owing to the small number of deaths in the study, we were not able to adjust for likely confounders, and our power to detect an association was limited.

In conclusion, our findings demonstrate that the burden of diarrheal disease continues in sub-Saharan Africa, with nearly 10% of episodes of WD and bloody diarrhea becoming persistent. After several decades in which research on diarrheal syndromes have paused, we have characterized clinical presentations, sociodemographic risk factors, and etiologic agents that can help build a contemporary knowledge base to inform interventions and case management strategies.

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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