Fecal Leukocytes in Children Infected with Diarrheagenic *Escherichia coli*[▼]

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The purpose of this study was to determine the presence and quantity of fecal leukocytes in children infected with diarrheagenic *Escherichia coli* and to compare these levels between diarrhea and control cases. We analyzed 1,474 stool samples from 935 diarrhea episodes and 539 from healthy controls of a cohort study of children younger than 2 years of age in Lima, Peru. Stools were analyzed for common enteric pathogens, and diarrheagenic *E. coli* isolates were studied by a multiplex real-time PCR. Stool smears were stained with methylene blue and read by a blinded observer to determine the number of polymorphonuclear leukocytes per high-power field (L/hpf). Fecal leukocytes at >10 L/hpf were present in 11.8% (110/935) of all diarrheal episodes versus 1.1% (6/539) in controls ($P < 0.001$). Among stool samples with diarrheagenic *E. coli* as the only pathogen isolated (excluding coinfection), fecal leukocytes at >10 L/hpf were present in 8.5% (18/212) of diarrhea versus 1.3% (2/157) of control samples ($P < 0.01$). Ninety-five percent of 99 diarrheagenic *E. coli* diarrhea samples were positive for fecal lactoferrin. Adjusting for the presence of blood in stools, age, sex, undernutrition, and breastfeeding, enterotoxigenic *E. coli* (ETEC) isolation as a single pathogen, excluding coinfections, was highly associated with the presence of fecal leukocytes (>10 L/hpf) with an odds ratio (OR) of 4.1 (95% confidence interval [CI], 1.08 to 15.51; $P < 0.05$). Although diarrheagenic *E. coli* was isolated with similar frequencies in diarrhea and control samples, clearly it was associated with a more inflammatory response during symptomatic infection; however, in general, these pathogens elicited a mild inflammatory response.

Diarrheagenic *Escherichia coli* strains as a group are the most common enteric pathogens in children in developing countries, responsible for 30% to 40% of all diarrhea episodes (20). However, some of these pathogens can be found with similar frequency in asymptomatic controls, depending on several factors, such as the age of the patient and host susceptibility. The presence of fecal leukocytes in stool samples is used as an indicator of inflammatory diarrhea. A brisk inflammatory response is associated with invasive pathogens, such as *Shigella*, *Salmonella*, or *Campylobacter* (4). However, other, noninvasive pathogens can elicit a mild inflammatory response as a result of the interaction of the pathogen with the host's enteric cells. An additional method to determine an inflammatory response in the gut is the measurement of fecal lactoferrin, an antimicrobial protein present in several human secretions (milk, saliva, tears, etc.) and in the granules of neutrophils (6).

Remarkable progress has been made in identifying virulence determinants required to mediate the pathogenesis of the different diarrheagenic *E. coli* pathotypes. However, there are few data on the level of fecal leukocytes and fecal lactoferrin as markers of inflammatory response in children infected with these pathogens. Although the mechanisms of action and the

pathogenesis of these bacteria are diverse, our hypothesis was that children infected with diarrheagenic *E. coli* who develop diarrhea elicit an inflammatory response greater than that of children with asymptomatic colonization with these pathogens. Therefore, we conducted this study to determine the presence and quantity of fecal leukocytes and lactoferrin in children infected with diarrheagenic *E. coli* and to compare these levels between diarrhea and control cases.

MATERIALS AND METHODS

Patients. This study was part of a prospective passive-surveillance cohort diarrhea study in children followed from 2 to 24 months of age. The study was conducted in periurban communities of Lima, Peru, between September 2006 and December 2007 (first cohort; 1,034 children) (19) and from January to July 2008 (second cohort; 529 children). Diarrhea was defined as the presence of ≥ 3 liquid or semiliquid stools in 24 h or ≥ 1 bloody stool. Control stool samples were collected from randomly selected healthy children without diarrhea 7 days before and after the stool sample collection. Clinical information on the diarrheal episodes was obtained from the medical records filled out by study doctors. We used a modified Vesikari score to determine the severity of the diarrhea episodes (19, 23). Weight and height measurements were obtained at the study clinic for all children at 12 months of age.

Pathogen determination. Stool samples were analyzed for common enteric pathogens; enzyme-linked immunosorbent assay (ELISA) was used for rotavirus; routine stool cultures were used to detect *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio* spp.; and the DNAs from five lactose-positive *E. coli* colonies were studied by a multiplex real-time PCR to identify enterotoxigenic (ETEC), enteropathogenic (EPEC), Shiga toxin-producing (STEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent (DAEC) *E. coli* by searching virulence genes with primers described previously (Table 1) and following reported methods (1, 7). In this study, we have included data on *Campylobacter* and rotavirus, the two most commonly isolated pathogens after diarrheagenic *E. coli*,

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TABLE 1. Virulence genes and primers for multiplex real-time PCR for diarrheagenic *E. coli*^a

Diarrheagenic <i>E. coli</i>	Gene	Orientation ^b	Primer sequence (5'→3')	Amplicon size (bp)	Amplicon T_m ^c (°C) (mean ± SD)
EAEC	<i>aggR</i>	F R	CGAAAAAGAGATTATAAAAATTAAC GCTTCCTTCTTTTGTGTAT	100	77.07 ± 0.68
ETEC	<i>st</i> (<i>stIa</i>)	F	TTTCCCCTCTTTTAGTCAGTCAA	159	81.45 ± 0.27
		F	TGCTAAACCAGTAGAGTCTTCAAAA	138	81.45 ± 0.27
	<i>st</i> (<i>stIb</i>)	R	GCAGGATTACAACACAATTACAGCAG		
		R	TCTCTATGTGCATACGGAGC CCATACTGATTGCCGCAAT	322	85.88 ± 0.34
EPEC	<i>eaeA</i>	F	ATGCTTAGTGCTGGTTTAGG	248	83.93 ± 0.31
		R	GCCTTCATCATTTTCGCTTTC		
STEC/EHEC ^d	<i>stx</i> ₁	F	CTGGATTAAATGTCGCATAGTG	150	87.37 ± 0.32
		R	AGAACGCCCCACTGAGATCATC		
	<i>stx</i> ₂	F	GGCACTGTCTGAAACTGCTCC	255	89.65 ± 0.33
		R	TCGCCAGTTATCTGACATTCTG		
EIEC	<i>ipaH</i>	F	GTTCCCTTGACCGCCTTTCCGATACCGTC	619	91.54 ± 0.26
		R	GCCGGTCAGCCACCCCTCTGAGAGTAC		
DAEC	<i>daaD</i>	F	TGAACGGGAGTATAAGGAAGATG	444	93.81 ± 0.4
		R	GTCCGCCATCACATCAAAA		

^a Modified from reference 7.^b F, forward; R, reverse.^c T_m , melting temperature.^d EHEC, enterohemorrhagic *E. coli*.

for comparison. We did not include samples positive for *Shigella* or *Salmonella*, since these pathogens were found at low frequency (19).

Fecal leukocytes. Fresh stool samples were examined for the presence of fecal leukocytes on smears made in the field 2 to 4 h after the stools were collected (13). The stools for microscopic examination were chosen from an area with blood or mucus, if present. Each sample was stained with methylene blue (Hi-media, Mumbai, India) and read by an experienced technician who was blinded to the source of the sample (diarrhea or control) or the isolated pathogen. The reading was done for 10 min using an optical light microscope. The numbers of leukocytes per high-power field (L/hpf) (magnification, ×1,000) were determined in at least 50 fields. The results were categorized as follows: 1 to 10 L/hpf, 11 to 20 L/hpf, 21 to 49 L/hpf, or >50 L/hpf. Based on previous studies, we chose a cutoff point of >10 L/hpf to determine the presence of an inflammatory process associated with an infectious agent (3, 15, 17, 21, 27).

Fecal lactoferrin. We randomly selected 99 stool samples from diarrheal cases in children not breastfeeding at the time of the diarrhea episode, including 43 EAEC, 29 EPEC, 9 ETEC, 9 DAEC, 1 STEC, and 8 coinfections, to determine the presence of fecal lactoferrin as measured by an immunochromatographic qualitative test performed according to the manufacturer's instructions (Leuko ez Value; Techlab, VA).

Ethical aspects. The study was approved by the Institutional Review Boards of the Universidad Peruana Cayetano Heredia, Instituto de Investigación Nutricional, and Instituto Nacional de Salud del Niño, all in Lima, Peru.

Statistical analysis. Differences between isolation rates, clinical characteristics, and fecal leukocytes among diarrhea and control samples were evaluated by chi-square or Fisher exact test. Anthropometric data (height-for-age and weight-for-height z scores) were calculated according to the World Health Organization Child Growth Standards for 2006. Partial correlations between fecal leukocytes and z scores for height for age, weight for height, and weight for age were performed, controlling for full breastfeeding duration, number of diarrhea episodes, and presence or absence of coinfections. To take into account within-individual correlation of stool samples and diarrheal episodes, we used random-effects models. To test the odds of positive leukocyte counts (>10 L/hpf) given the isolation of each pathogen and adjusting for possible modifiers (blood in stools, age, sex, undernutrition, and breastfeeding), we used random-effects logistic regressions. All statistical analyses were performed using STATA version 10.1 (Stata Corp). The significance level was set at a *P* value of <0.05.

RESULTS

Samples and pathogens. We analyzed 1,474 samples from 935 diarrhea episodes and 539 healthy controls. We found that diarrheagenic *E. coli* was isolated from diarrhea samples (30.9%) as often as from control samples (33.8%). The most common diarrheagenic *E. coli* strains were EAEC (14.1% and 15.4%) and EPEC (9.8% and 13.0%) in diarrhea and control samples, respectively, including coinfections (Table 2). The prevalence of *Campylobacter*, including coinfections, was 17.5% in diarrhea samples and 14.5% in controls. The prevalence of rotavirus, including coinfections, was 13.1% (61/465) in diarrhea samples. Control samples were not tested for rotavirus.

Fecal leukocytes and pathogens. There were no fecal leukocytes (0 L/hpf) in 73.7% of diarrhea samples and 89.8% of healthy control samples. Fecal leukocytes (>10 L/hpf) were present in 11.8% (110/935) of all diarrheal episodes versus 1.1% (6/539) of healthy controls (*P* < 0.001) (Table 3). Among stool samples with diarrheagenic *E. coli* as the only pathogen isolated (excluding coinfection with other bacteria or viruses), fecal leukocytes at >10 L/hpf were present in 8.5% (18/212) of diarrhea samples versus 1.3% (2/157) of controls (*P* < 0.01). The highest inflammatory response (>50 L/hpf) was present in only 1% of all diarrheagenic *E. coli* and 4% of *Campylobacter* isolates among diarrheal samples. EPEC as the sole pathogen isolated was associated with the presence of fecal leukocytes (>10 L/hpf) in diarrhea samples but not asymptomatic controls (8.3% versus 0%, respectively; *P* < 0.05) (Table 4). The presence of fecal leukocytes (>10 L/hpf) was significantly more common among diarrhea cases than in healthy controls in all three age groups (Table 4). For comparison, in a different

TABLE 2. Frequencies of pathogen isolation

Pathogen	No. of samples in indicated category/ total no. of samples (%)	
	Diarrhea	Control
Diarrheagenic <i>E. coli</i> (DEC)	212/935 (22.5)	157/539 (29.5)
EAEC ^a	89/935 (9.5)	68/539 (12.6)
EPEC ^a	60/935 (6.4)	61/539 (11.3)
ETEC ^a	29/935 (3.1)	14/539 (2.6)
DAEC ^a	18/935 (1.9)	8/539 (1.5)
STEC ^a	4/935 (0.4)	4/539 (0.7)
Multiple DEC ^b	12/935 (1.3)	2/539 (0.4)
<i>Campylobacter</i> sp. ^a	99/935 (10.6)	53/539 (9.8)
<i>Campylobacter</i> sp. with DEC	55/935 (5.9)	25/539 (4.6)
Rotavirus ^a	31/465 (6.7)	NA ^c
Rotavirus with <i>Campylobacter</i>	8/465 (1.7)	NA
Rotavirus with DEC	20/465 (4.3)	NA
Rotavirus with <i>Campylobacter</i> and DEC	2/465 (0.4)	NA
No pathogen identified	508/935 (54.3)	304/539 (56.4)

^a As the only pathogen isolated.^b Coinfection with diarrheagenic *E. coli*. Diarrhea samples (12 cases): DAEC/EAEC, 1; EAEC/STEC, 1; EAEC/EPEC, 6; EAEC/ETEC, 1; EPEC/EIEC, 1; and EPEC/ETEC, 2. Controls (2 cases): EAEC/EPEC, 1; EAEC/ETEC/DAEC, 1.^c NA, not applicable. Control samples were not tested for rotavirus.

TABLE 4. Comparison of fecal leukocytes among diarrhea and control samples by pathogen and age

Sample characteristic	Fecal leukocytes (>10 L/hpf) [no. of samples in indicated category/total no. of samples (%)]	
	Diarrhea	Control
Pathogen		
Diarrheagenic <i>E. coli</i> (DEC)		
EAEC ^a	5/89 (5.6)	1/68 (1.5)
EPEC ^a	5/60 (8.3)	0/61 (0.0) ^b
ETEC ^a	4/29 (13.8)	1/14 (7.1)
DAEC ^a	3/18 (16.7)	0/8 (0.0)
STEC ^a	1/4 (25.0)	0/4 (0.0)
<i>Campylobacter</i> sp. ^a	25/99 (25.3)	0/53 (0.0) ^c
<i>Campylobacter</i> sp. with DEC	8/55 (14.5)	0/25 (0.0) ^b
No pathogen identified	51/508 (10.0)	4/304 (1.3)
Age group		
2–6 mo	41/296 (13.9)	1/102 (1.0) ^c
7–12 mo	59/450 (13.1)	3/224 (1.3) ^c
13–24 mo	10/189 (5.3)	2/213 (0.9) ^b
All ages	110/935 (11.8)	6/539 (1.1) ^c

^a As a single pathogen.^b $P < 0.05$.^c $P < 0.001$.

ongoing cohort study in children, among *Shigella* samples, fecal leukocytes at >10 L/hpf were present in 35% (18/52) of diarrhea samples versus 0% (0/21) of controls; the highest inflammatory response (>50 L/hpf) was present in only 15% (8/52) of

diarrhea samples associated with *Shigella* infections. Similarly, among 14 *Salmonella* samples (6 from diarrhea cases and 8 from controls), none had fecal leukocytes at >10 L/hpf.

TABLE 3. Distribution of fecal leukocytes in diarrheagenic *E. coli* infections among diarrhea and control samples

Pathogen	Fecal leukocytes [no. of samples in indicated category/total no. of samples (%)]				
	0 L/hpf	1–10 L/hpf	11–20 L/hpf	21–50 L/hpf	>50 L/hpf
Diarrhea					
All diarrhea samples	689/935 (73.7)	136/935 (14.5)	69/935 (7.4)	31/935 (3.3)	10/935 (1.1)
Diarrheagenic <i>E. coli</i>					
EAEC ^a	69/89 (77.5)	15/89 (16.9)	2/89 (2.2)	2/89 (2.2)	1/89 (1.1)
EPEC ^a	49/60 (81.7)	6/60 (10.0)	4/60 (6.7)	1/60 (1.7)	
ETEC ^a	22/29 (75.9)	3/29 (10.3)	3/29 (10.3)		1/29 (3.4)
DAEC ^a	13/18 (72.2)	2/18 (11.1)	3/18 (16.6)		
STEC ^a	1/4 (25.0)	2/4 (50.0)	1/4 (25.0)		
Multiple DEC ^b	6/12 (50.0)	6/12 (50.0)			
<i>Campylobacter</i> sp. ^a	61/99 (61.6)	13/99 (13.1)	13/99 (13.1)	8/99 (8.1)	4/99 (4.0)
<i>Campylobacter</i> sp. with DEC	40/55 (72.7)	7/55 (12.7)	7/55 (12.7)	1/55 (1.8)	
No pathogen identified	376/508 (74.0)	81/508 (15.9)	29/508 (5.7)	19/508 (3.7)	3/508 (0.6)
Control					
All control samples	484/539 (89.8)	49/539 (9.1)	5/539 (0.9)	1/539 (0.2)	
Diarrheagenic <i>E. coli</i>					
EAEC ^a	62/68 (91.2)	5/68 (7.4)	1/68 (1.5)		
EPEC ^a	54/61 (88.5)	7/61 (11.5)			
ETEC ^a	11/14 (78.6)	2/14 (14.3)		1/14 (7.1)	
DAEC ^a	8/8 (100.0)				
STEC ^a	4/4 (100)				
Multiple DEC ^b	2/2 (100.0)				
<i>Campylobacter</i> sp. ^a	49/53 (92.5)	4/53 (7.5)			
<i>Campylobacter</i> sp. with DEC	24/25 (96.0)	1/25 (4.0)			
No pathogen identified	270/304 (88.8)	30/304 (9.9)	4/304 (1.3)		

^a As the only pathogen isolated.^b Only coinfection among DEC. Diarrhea samples (12 cases): DAEC plus EAEC, 1; EAEC plus STEC, 1; EAEC plus EPEC, 6; EAEC plus ETEC, 1; EPEC plus EIEC, 1; and EPEC plus ETEC, 2. Control (2 cases): EAEC plus EPEC, 1; EAEC plus ETEC plus DAEC, 1.

TABLE 5. Bivariate analysis of the odds of a positive fecal leukocyte count (>10 L/hpf) by factor and pathogen

Characteristic	OR (95% CI)	P
Factor		
Age (<12 mo)	3.63 (1.95–6.76)	0.000
Sex (male)	0.86 (0.57–1.30)	0.477
Undernutrition	0.88 (0.55–1.40)	0.594
Breastfeeding	2.56 (1.69–3.88)	0.000
Diarrhea severity (Vesikari)	0.80 (0.45–1.42)	0.450
Blood in stools	6.50 (3.39–12.43)	0.000
Pathogen		
EAEC	0.51 (0.26–1.02)	0.056
EPEC	0.52 (0.20–1.33)	0.173
ETEC	2.00 (0.87–4.61)	0.102
DAEC	1.68 (0.61–4.61)	0.317
<i>Campylobacter</i> sp.	2.20 (1.39–3.51)	0.001
Rotavirus	0.79 (0.74–0.84)	0.000

Fecal leukocytes and clinical data. Clinical information was available on 626 diarrhea episodes; 72.7% (455 episodes) were mild, 25.6% (160 episodes) were moderate, and 1.8% (11 episodes) were severe, according to the modified Vesikari score. The presence of fecal leukocytes (>10 L/hpf) was not associated with severity (14.5% in mild cases, 13.1% in moderate cases, and 0% in severe cases). Information on the presence of fecal blood was available for 750 diarrhea cases; 12.9% (97 samples) had visible blood, including 7 DAEC, 3 EPEC, 2 EAEC, 29 *Campylobacter*, and 14 coinfections with *Campylobacter* and a diarrheagenic *E. coli* strain (with 6 EAEC, 2 EPEC, 2 ETEC, 3 DAEC, and 1 EPEC plus ETEC). As expected, the presence of fecal leukocytes (>10 L/hpf) was significantly more common among stool samples with visible blood (35.1%; 34/97) than among samples without visible blood (9.2%; 60/653) ($P < 0.001$). We did not test for occult blood. Partial correlations between fecal leukocytes and anthropometric z scores did not reveal any relevant association.

Fecal lactoferrin. Among the 99 randomly selected diarrhea samples analyzed for the presence of fecal lactoferrin, 11 samples had fecal leukocytes at >10 L/hpf, all of which were lactoferrin positive (100%), and 88 samples had ≤ 10 L/hpf, 83 of which were lactoferrin positive (94%). Overall, 95% of all diarrheagenic *E. coli* diarrhea samples analyzed were positive for fecal lactoferrin in the stools.

Multivariate analysis. In the bivariate analysis, blood in stool samples was highly associated with the presence of fecal leukocytes (>10 L/hpf), with an odds ratio (OR) of 6.5 (95% confidence interval [CI], 3.39 to 12.43; $P < 0.001$) (Table 5). This association persisted in the multivariate analysis by pathogen. Children less than 12 months of age had a higher risk of having fecal leukocytes, when the presence of blood in stools and breastfeeding were adjusted for, with an OR of 5.2 (95% CI, 1.95 to 13.79; $P = 0.001$). This association persisted in the multivariate analysis by pathogen. No association was found between the clinical severity score, breastfeeding, or undernutrition and the presence of fecal leukocytes. Adjusting for the presence of blood in stools, age, sex, undernutrition, and breastfeeding, ETEC isolation in stool samples was highly associated with the presence of fecal leukocytes, with an OR of 3.1 (95% CI, 1.01 to 9.72; $P < 0.05$). This association increased

TABLE 6. Multivariate analysis^a of the odds of a positive fecal leukocyte count (>10 L/hpf) by pathogen

Pathogen	Fecal leukocytes by pathogen		Fecal leukocytes by single-pathogen infection ^b	
	OR (95% CI)	P	OR (95% CI)	P
EAEC	0.50 (0.21–1.16)	0.105	0.56 (0.19–1.63)	0.289
EPEC	0.54 (0.17–1.69)	0.288	0.64 (0.13–3.18)	0.585
ETEC	3.13 (1.01–9.72)	0.048	4.09 (1.08–15.51)	0.039
DAEC	1.35 (0.37–4.95)	0.649	3.13 (0.50–19.60)	0.224
<i>Campylobacter</i> sp.	1.55 (0.85–2.85)	0.154	1.63 (0.80–3.34)	0.178
Rotavirus	0.94 (0.83–1.07)	0.352	0.54 (0.18–1.67)	0.285

^a Using random-effects logistic regressions and adjusting for possible modifiers (blood in stools, age, sex, undernutrition, and breastfeeding).

^b Excluding coinfections.

when we made an analysis of single pathogens, excluding coinfections with other pathogens, to an OR of 4.1 (95% CI, 1.08 to 15.51; $P < 0.05$). No other pathogens were associated with the presence of fecal leukocytes (Table 6).

DISCUSSION

Although diarrheagenic *E. coli* was isolated with similar frequencies in diarrhea and control samples, illnesses were associated with a more inflammatory response. However, these pathogens elicited a mild inflammatory response. Fecal leukocytes as a marker of inflammatory response have different sensitivities and specificities in outpatients and hospitalized children (25), and also in developed or resource-poor countries (4). In general, fecal leukocytes have limited value in discriminating between pathogens causing watery diarrhea when the inflammatory response is mild. Patients with presumably non-inflammatory diarrheal pathogens, such as rotavirus, ETEC, and cholera, may have a mild inflammatory response with fecal leukocytes (11 to 20 L/hpf), suggesting that the threshold for separating patients with primary inflammatory diarrhea from those with noninflammatory diarrhea may be higher in areas where multiple bacterial and parasitic infections are common (6, 11, 12). On the other hand, typical invasive pathogens, such as *Shigella* and EIEC, have been associated with a higher inflammatory response (>50 L/hpf) (8, 27), suggesting that these levels may be useful for discriminating invasive bacteria at the emergency room or outpatient consultation.

In this study, EAEC, EPEC, and ETEC were the most prevalent *E. coli* pathogens. Fecal leukocytes (>10 L/hpf) were found in 5.6% of EAEC diarrhea cases; this is lower than previously reported (~28 to 40%) in EAEC traveler's diarrhea (2, 3, 10). In a study in Brazil, children with malnutrition and persistent diarrhea due to EAEC had elevated levels of fecal lactoferrin and proinflammatory cytokines (interleukin-8 [IL-8] and IL-1 β) in their stool samples (26). Interestingly, patients infected with EAEC and carrying a group of virulence genes (*aggR*, *aap*, *aatA*, *astA*, or *set1A*) were associated with the presence of fecal leukocytes and increased production of fecal cytokines (IL-8, gamma interferon [IFN- γ], IL-1 β , and IL-1 receptor antagonist [IL-1ra]) (3, 9, 14).

The second most commonly isolated pathogen was EPEC. Fecal leukocytes were found in 8.3% of EPEC infections and were significantly associated with diarrhea cases. Previous

studies in children have shown higher frequencies of fecal leukocytes in stool samples (19%) (15). Although EPEC strains are not invasive pathogens, they induce an inflammatory response in the gut epithelium *in vivo* by triggering production of cytokines and chemokines, including IL-8, which recruits polymorphonuclear leukocytes to the infection site (24). *In vitro* studies have shown that intestinal epithelial cells infected with EPEC trigger IL-8 release through Toll-like receptor 5 (TLR-5) and activation of NF- κ B, mediated by flagellin, the secreted protein of the EPEC *fliC* gene (16). In addition, NleE, a type 3 secretory system (T3SS) effector, is required for EPEC-induced polymorphonuclear leukocyte migration (28).

Fecal leukocytes were found in 13.8% of ETEC diarrhea cases; this was similar to other studies in children (10 to 34%) (15, 18, 27). Interestingly, ETEC isolation in stool samples was highly associated with the presence of fecal leukocytes, and this association increased when we analyzed ETEC isolation as a single pathogen, adjusting for the presence of blood in stools, age, sex, undernutrition, and breastfeeding. Infection with ETEC has traditionally been considered a secretory diarrhea with little or no inflammatory response. However, several studies have shown that tissue culture cells infected with ETEC cause disruption of the membrane barrier plus increase of IL-8 expression, especially with heat-stable enterotoxin strains (ETEC-ST) (9, 22). Similarly, increased levels of IL-8, IL-1 β , and IL-1 α were found in fecal samples from travelers with ETEC infection (5), although these levels were lower than those in *Shigella* infection. Travelers with ETEC diarrhea were found to have markers of enteric inflammation, such as the presence of occult blood in 30%, fecal leukocytes in 27%, and fecal lactoferrin in 27% (2). However, there are few data on the inflammatory response to ETEC infection in children.

The relatively high presence of fecal leukocytes in this study compared to others is almost certainly due to careful and rapid screening, as opposed to “real-world” situations, where samples sit for too long before being analyzed. However, these pathogens in general elicited a mild inflammatory response, measured by the number of fecal leukocytes per high-power field (most samples had between 11 and 20 L/hpf); the vast majority (95%) of diarrheagenic *E. coli* diarrhea samples were positive for fecal lactoferrin. Screening for fecal lactoferrin is a highly sensitive method to detect an inflammatory process. At the screening dilution, the assay detects as little as 15 ng of lactoferrin per μ l, or about 3,000 leukocytes/ μ l, which is equivalent to >1 L/hpf (6). Further studies are needed to confirm our findings and to compare the prevalences of fecal lactoferrin in control samples (stool samples from children without diarrhea and with and without infection by diarrheagenic *E. coli* or other enteric pathogens). It is possible that Peruvian children, like children from developing countries in general, have a chronic mild inflammation in the gut (high rates of fecal lactoferrin), due to frequent and recurrent exposure to enteric pathogens. It is important to clarify this in order to determine the screening value of this test in developing countries.

As far as our partial correlation analyses revealed, it seems that fecal leukocytes are not associated with significant variations in anthropometric indicators. However, particularly in respect to association with the height-for-age metric, further longitudinal studies seem to be warranted before reaching a

definitive conclusion as to the effects of inflammatory diarrhea, particularly in the context of multiple inflammatory diarrhea episodes during the period of follow-up.

The presence of blood in stool samples was highly associated with the presence of fecal leukocytes (>10 L/hpf) ($P < 0.001$), and this association persisted in the multivariate analysis. In addition, children less than 12 months of age had a higher risk of having fecal leukocytes, after adjustment for the presence of blood and breastfeeding ($P = 0.001$).

There were several limitations in our study. First, we did not search for viral pathogens other than rotavirus (calicivirus, enteric adenovirus, or astrovirus), and therefore, the samples considered “single-pathogen infections” may have included some cases of coinfections with other viral pathogens. Second, we did not evaluate for fecal lactoferrin in all samples. Third, we used a qualitative method to determine the presence of fecal lactoferrin. A quantitative method to correlate the level of lactoferrin with the amount of fecal leukocytes and the clinical information might be more informative. Nevertheless, this study provides important information on fecal leukocytes and lactoferrin in all currently recognized groups of diarrheagenic *E. coli* pathogens diagnosed by molecular methods. Further studies are needed to confirm the association of ETEC with the presence of fecal leukocytes and to determine the levels of fecal lactoferrin and other inflammatory markers in stool samples of children infected with the pathogen.

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We have no conflict of interest.

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