Identifying Etiological Agents Causing Diarrhea in Low Income Ecuadorian Communities

Gabriela Vasco, Gabriel Trueba, Richard Atherton, Manuel Calvopiña, William Cevallos, Thamara Andrade, Martha Eguiguren, and Joseph N. S. Eisenberg*

Microbiology Institute, Universidad San Francisco de Quito, Quito, Ecuador; Centro de Biomedicina, Universidad Central del Ecuador, Quito, Ecuador; Department of Epidemiology, University of Michigan, Ann Arbor, Michigan; Centro de Salud de Guamaní, Ministerio de Salud Pública, Quito, Ecuador

Abstract. Continued success in decreasing diarrheal disease burden requires targeted interventions. To develop such interventions, it is crucial to understand which pathogens cause diarrhea. Using a case-control design we tested stool samples, collected in both rural and urban Ecuador, for 15 pathogenic microorganisms. Pathogens were present in 51% of case and 27% of control samples from the urban community, and 62% of case and 18% of control samples collected from the rural community. Rotavirus and Shigellae were associated with diarrhea in the urban community; co-infections were more pathogenic than single infection; *Campylobacter* and *Entamoeba histolytica* were found in large numbers in cases and controls; and non-typhi *Salmonella* and enteropathogenic *Escherichia coli* were not found in any samples. Consistent with the Global Enteric Multicenter Study, focused in south Asia and sub-Saharan Africa, we found that in Ecuador a small group of pathogens accounted for a significant amount of the diarrheal disease burden.

INTRODUCTION

Despite the decline in diarrhea-associated child mortality over the past four decades, diarrheal disease is still the second leading cause of death among children < 5 years of age in developing countries.^{1,2} To continue to reduce diarrhea-associated mortality, targeted interventions are needed. Informing the development of these interventions require an understanding of the social, environmental, and biological drivers of diarrheal disease. To this end, the Global Enteric Multicenter Study (GEMS) was conducted to ascertain the etiological agents causing the diarrhea in children < 5 years of age.³ The GEMS study sites were located in sub-Saharan Africa and south Asia, where diarrheal disease burden is highest in the world.^{1,2} The GEMS study, unfortunately, did not include study sites within Latin America, where improved water and sanitation facilities are lacking and diarrheal disease is a large concern. To complement the GEMS study, we conducted a case control study in a rural and urban community within Ecuador.

Clinical investigations of diarrheagenic microbial agents are time-consuming, expensive, difficult to achieve in the clinical setting, and the results seldom benefit the patient^{4,5}; nevertheless, understanding which agents cause disease can provide useful epidemiological information for designing intervention strategies.^{6,7} For example, *Vibrio* spp. infection has been associated with raw or undercooked shellfish⁶ and non-typhi *Salmonella* spp. is often linked to food-borne outbreaks.⁸ In addition to pathogen-specific transmission modes, diarrhea transmission also varies by social and environmental context. For example, it has been observed that the rate of salmonellosis decreases when the level of development increases in a country.⁹

Despite the importance of understanding the epidemiology of diarrheal disease, little is known about the etiological agents causing diarrhea in Latin American. For example, in Ecuador the reported incidence of diarrheal disease increased from 17/1,000 inhabitants in 1994 to 46/1,000 inhabitants in 2012,¹⁰ yet, there is limited researching describing the patho-

gen diversity of these diarrheal cases. Of the 12 studies published about diarrheic disease in Ecuador during the past 30 years (Table 1), seven studies presented prevalence data that contained scant etiologic information, whereas five studies analyzed data from case-control studies that highlighted the etiological relationship between disease and pathogens.^{23,24} Each study focused on at least one pathogen, with some studies identifying the viral causes of diarrhea (e.g., rotavirus and norovirus) and others describing bacterial and parasitic causes. Furthermore, each study was limited to either a rural or urban setting.

A few older studies provide relevant etiological data. In the early 1980s a prospective study in rural northeastern Brazil found that rotavirus, ETEC, STEC, Shigellae, and *Giardia lamblia* were important causes of the disease; rotavirus was more prevalent during dry seasons and ETEC during rainy seasons.²⁵ In the mid 1980s a case control study in urban Brazil revealed that EPEC, rotavirus, *Salmonella*, Shigellae, and ETEC and, co-infections with more than one pathogen were associated with disease.²⁶ More recently, a passive surveillance in Lima, Peru showed that the most common agents associated with diarrhea in < 1-year-old infants were EAEC, EPEC, *Campylobacter*, and rotavirus; EAEC, EPEC, and *Campylobacter* were frequently found in asymptomatic individuals.^{27,28}

Here, we present data from a comparative case-control study describing the etiology of diarrheal disease in an urban (low-income neighborhood in Quito) and a rural (low-income community in northwestern Ecuador) setting. Within each community, stool samples were collected from study participants and tested for 15 important viral, bacterial, and protozoan pathogens.

MATERIALS AND METHODS

Human subjects. We conducted a case-control study recruiting participants in two locations: Guamaní, a low-income urban neighborhood in the Ecuadorian capital city of Quito, and Borbón, a low-income rural community in northwestern Ecuador. Guamaní is located in the province of Pichincha at an altitude of 2,700 meters above sea level (m.a.s.l.). The population consists of 65,065 individuals; almost all (98%) households in this area have access to a drinking water source that is considered improved by the World Health Organization

*Address correspondence to Joseph N. S. Eisenberg, University of Michigan, 1415 Washington Heights, Ann Arbor, MI 48109. E-mail: jnse@umich.com

TABLE 1
Summary of published data on the presence of enteric pathogens in Ecuador*

	Year of publication	Number of tested samples	Age range	% positive cases	Measure of Association	Setting	Reference
<i>Campylobacter</i>	1987	100	0 to 24 months	23	—	Urban	11
<i>Campylobacter</i>	1994	177	Under 5 years	3.37	—	Urban	12
<i>Cryptosporidium</i>	1986	169	Under 5 years	11.24	—	Urban	13
Shigellae	1987	100	0 to 24 months	12	—	Urban	11
Shigellae	1994	177	Under 5 years	3.7	—	Urban	12
Shigellae	2007	915	All ages	0.9	0.6 (0.06, 2.7)†	Rural	14
Shigellae	2012	2936	All ages	—	1.6 (1.1–2.4)‡	Rural	15
Shigellae and EIEC	2012	3314	All ages	17.1	3.6 (2.4, 5.0)‡	Rural	16
<i>Entamoeba histolytica</i>	1987	100	0 to 24 months	1	—	Urban	11
Enteroinvasive <i>E. coli</i>	2007	915	All ages	8.9	3.1 (1.6, 6.0)†	Rural	14
Enteropathogenic <i>E. coli</i>	2007	915	All ages	1.7	1.9 (0.4, 8.2)†	Rural	14
Enterotoxigenic <i>E. coli</i>	2007	915	All ages	7.6	6.9 (2.8, 18.6)	Rural	14
<i>Giardia lamblia</i>	1987	100	0 to 24 months	5	—	Urban	11
<i>Giardia lamblia</i>	1994	177	Under 5 years	63	—	Urban	12
<i>Giardia lamblia</i>	2012	244	Under 5 years	18.3	—	Urban	17
<i>Giardia lamblia</i>	2012	3314	All ages	31.5	2.6 (2.1, 3.2)‡	Rural	16
<i>Giardia lamblia</i>	2012	2936	All ages	—	1.5 (1.0, 2.2)‡	Rural	15
Norovirus	2012	244	Under 5 years	29.5	—	Urban	17
Rotavirus	1981	702	Under 3 years	21.1	—	Urban	18
Rotavirus	1986	1722	Under 3 years	21.8	—	Urban	19
Rotavirus	1987	100	0 to 24 months	21	—	Urban	11
Rotavirus	1994	177	Under 5 years	7.5	—	Urban	12
Rotavirus	2007	1656	All ages	23.35	9.2 (6.1, 13.9)†	Rural	20
Rotavirus	2008	728	Under 5 years	37	—	Urban	21
Rotavirus	2009	3300	All ages	22.3	10.6 (7.9, 14.3)†	Rural	22
Rotavirus	2012	3314	All ages	22.2	10.7 (7.9, 15.1)‡	Rural	16
Rotavirus	2012	2936	All ages	—	1.7 (1.1, 2.5)‡	Rural	15
<i>Salmonella</i> spp.	1987	100	0 to 24 months	3	—	Urban	11

*Studies that reported % positive cases used a cross-sectional design, and studies that reported an odds ratio or risk ratio used a case-control design.

†Odds ratio (OR) (95% confidence interval [CI]), OR compared the odds of having diarrhea given infection with the odds of not having diarrhea given infection.

‡Crude risk ratio (95% CI).

(WHO) criteria. Borbón is a small town in Esmeraldas province of northwestern Ecuador, at an approximate altitude of 15 m.a.s.l. Borbón has a population of 7,696 people. In contrast to Guamaní, < 3 in 5 households in Borbón (58%) have access to an improved drinking water source.²⁹

Guamaní study. A case was defined as anyone who came to the local health center suffering from diarrhea (three loose stools in 24 hours) and had not taken antibiotic or similar medication in the 2 weeks before enrollment. Controls were defined as those who came to the health center for another reason, and did not have diarrheal symptoms during the past 2 weeks and had not taken antibiotics in the 2 weeks before enrollment. Each enrolled case was matched by age (within 1 month for those < 1 year of age, within 6 months for those between 1 and 10 years of age, and within 1 year for those > 10 years of age) and gender to an appropriate control. The recruitment took place between March and May 2012 during the rainy season (median rainfall is 145.8, 372, and 55 mm during March, April, and May, respectively; mean temperature is 13.9, 13.9, and 15.5°C during March, April, and May, respectively).^{30–32}

Borbón study. Using a cohort of 400 households that were being followed in a multiyear observational study, each household was visited daily for a period of 2 weeks. During each visit, a household respondent was asked if any household member had diarrhea (three loose stools in 24 hours). If more than one household member were case candidates, only one randomly selected case was included in the study. For every case identified three randomly selected controls (individuals without diarrheal symptoms during the past week) were selected among the 400 households in the cohort. Stool samples were collected between July and August 2012, which

corresponds to the dry season (median rainfall is 18.2 and 25.6 mm during July and August, respectively, mean temperature is 25.7 and 25.6°C during July and August, respectively).^{33,34} All the participants declared that they did not take antibiotics in the past week. Before enrollment in the Guamaní and Borbón studies, all individuals signed a consent document outlining the study procedures, which were approved by the Ethics Committees of University Michigan and Universidad San Francisco de Quito, with local and national government approval by the Ecuadorian Public Health Ministry.

Laboratory procedures. All participants provided stool samples to the study team within 1 hour of the bowel movement; samples were placed in a cooler if the transport to the laboratory took over an hour. All bacteria culturing and sample preservation began < 8 hours after collection.

Identification of bacteria. To identify diarrheagenic enterobacteria, stool samples were cultured in four agar media: MacConkey Lactose agar (Difco, Sparks, MD); MacConkey Sorbitol agar (Difco); *Salmonella-Shigella* agar (Difco); and Xylose Lysine Deoxycholate agar (Difco). To investigate *Campylobacter* sp., samples were cultured in *Campylobacter* Oxoid agar (Oxoid Ltd., Basingstoke, Hampshire, England) using CampyGen CO₂ (Oxoid Ltd.). *Campylobacter jejuni*-coli suspicious colonies were Gram-stained, tested for oxidase, and confirmed by polymerase chain reaction (PCR) (hippuricase and aspartokinase) genes.^{35,36} To classify *Vibrio* spp., samples were cultured in TCBS agar (Difco) sucrose positive and sucrose negative colonies were subjected to API-20E (bio Merieux, Marcy l'Etoile, France) tests. Lactose fermenting and β-D-glucuronidase positive colonies (using Chromocult agar from Difco) were subjected to PCR for the presence of

TABLE 2
Number of infections by case/control status and odds ratios for diarrhea relative to case status in Guamaní and Borbón, Ecuador*

	GUAMANÍ (100 cases and 100 controls)				BORBÓN (39 cases and 112 controls)			
	Total (%)	Cases (%)	Controls (%)	Pair-matched odds ratio (95% CI)	Total (%)	Cases (%)	Controls (%)	Mantel-Haenszel odds ratio (95% CI)
Zero Pathogens	122 (61)	49 (49)	73 (73)	0.74 (0.5–1.04)	107 (71)	15 (38)	92 (82)	0.22 (0.8–0.61)
Pathogens (including co-infections)	78 (39)	51 (51)	27 (27)	4.4 (1.9–12)	44 (29)	24 (62)	20 (18)	7.36 (3.2–16)
1 pathogen	61 (30.5)	35 (35)	26 (26)	3.14 (1.3–8.7)	32 (21)	15 (38)	17 (15)	3.5 (1.5–8)
Co-infection	17 (8.5)	16 (16)	1 (1)	19 (1.1–326)	12 (8)	9 (23)	3 (2.6)	11 (2.7–43)
2 pathogens	9 (4.5)	8 (8)	1 (1)	13 (0.7–230)	9 (6)	7 (18)	2 (1.8)	16 (3.2–86)
3 pathogens	8 (4)	8 (8)	0 (0)	7 (0.3–135)	3 (2)	2 (5)	1 (0.9)	6 (0.5–68)

* Estimates in bold are statistically significant ($P < 0.05$).

the *ipaH*, *estA*, *eltA*, *bfpA*, *stx-1*, *stx-2* genes to detect *E. coli* pathotypes enteroinvasive (EIEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), and shiga-toxigenic (STEC), respectively.^{37–39} The β -D-glucuronidase negative *E. coli* were also tested for the presence of *stx-1* and *stx-2* genes. Lactose negative and xylose negative colonies were tested using API-20E tests to determine whether colonies were Shigellae and *Salmonella* spp. Suspect Shigellae were confirmed by the presence of the *ipaH* gene.

Identification of viruses. Immunochromatographic tests were used for detection of norovirus (Rida Quick Norovirus, r-Biopharm, Darmstadt, Germany) and rotavirus (RidaQuick Rotavirus, r-Biopharm).

Identification of parasites. Enzyme-linked immunosorbent assay was used to detect *Giardia lamblia* (Ridascreen *Giardia*, r-Biopharm) and *Cryptosporidium parvum* (Ridascreen *Cryptosporidium*, r-Biopharm). Identification of amoeba: PCR from stool samples was performed to detect *Entamoeba histolytica*.⁴⁰ We also used microscopy to detect protozoa (including Kinyoun's acid-fast stain).

Statistical analyses. Statistical analyses were performed using Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA) and Center for Disease Control and Prevention (CDC) Epi Info 7.1.0.6. We calculated the odds ratio (OR) to compare pathogens present in case and control samples. A matched pair OR and the exact confidence intervals (CIs) were calculated for the Guamaní analyses. For the Borbón analyses, a weighted stratified Mantel Haenszel OR was calculated by using three age groups: 0 to 4.9 years, 5 to 13 years, and > 13 years of age.

RESULTS

Participants. In Guamaní, the sample size was 200 (100 cases and 100 controls). The median age was 14 years for both the cases and controls (SD = 15.6 and 16.2 for the cases and controls, respectively). Children < 5 years of age represented 49 cases and 49 controls and males represented 54 cases and 54 controls. In Borbón, the sample size was 151 (39 cases and 112 controls). The median age was 13 (SD = 19) and, 24 (SD = 19) years among cases and controls respectively. Males represented 23 cases and 57 controls.

Pathogens. Pathogens were isolated from case samples more often in Borbón (62%) than in Guamaní (51%). Moreover, among case samples, co-infections were more prevalent in Borbón (23%) compared with Guamaní (16%) (Table 2). The odds of pathogen-associated diarrhea, including both single infections and co-infections, were also higher in the rural than in the urban settings (Borbón OR 7.36, 95% CI

3.2–16 and Guamaní OR 4.4, 95% CI 1.9–12). The presence of each specific microorganism, as an agent of a single infection and a co-infection, isolated from both urban and rural locations is shown in Table 3. The pathogens associated with diarrhea were Shigellae and rotavirus in Guamaní, and rotavirus and *Giardia lamblia* in Borbón. *Salmonella* spp. and EPEC were not found in any stool sample collected from either location, whereas *Cryptosporidium* was found in four case samples in Borbón and two case samples in Guamaní, and was not found in any control samples. All *Cryptosporidium* positive cases were found in children < 5 years of age (4 of 21 in Borbón and 2 of 49 in Guamaní). Higher rates of *Campylobacter* and *Entamoeba histolytica* were found in samples from the urban site in both cases and controls, in comparison to the rural site.

Co-infections. Co-infections, defined as the presence of two or more pathogens in one stool sample, were found in both the urban and rural communities. The presence of more than one pathogen increased the odds of having diarrhea in Borbón for any infection OR 7.36, 95% CI 3.2–16, an infection with only one pathogen OR 3.5, 95% CI 1.5–8, and an infection with 2 pathogens OR 16, 95% CI 3.2–86 (Table 2). We found that rotavirus had higher odds for causing diarrhea in both the urban sites (OR 12, CI 95% 1.23–358 and OR 14.8, CI 95% 1.7–132, respectively) if the sample was co-infected. This was also true for *Giardia lamblia* samples from the rural site (OR 8, CI 95% 2–32). Within Borbón co-infections of rotavirus/*G. lamblia* were associated with diarrhea (OR 24, CI 95% 1.9–302). Table 4 presents a complete list of co-infections identified in the stool samples from the studied populations.

DISCUSSION

A vast number of pathogens can cause diarrheal disease; however, most studies suggest that only a few pathogens account for a majority of the disease burden.^{3,6,41} By in large, these same etiological agents have been consistently associated with disease over time, although there are a few important emerging pathogens such as norovirus and *Cryptosporidium parvum*. Our study in rural and urban Ecuador complements the information provided by GEMS carried out in sub-Saharan Africa and Asia.

Consistent with GEMS, rotavirus was associated with diarrheal disease in both our urban and rural low-income community, whereas Shigellae was only important in our urban setting. *Giardia* was found in a large number of samples in both our urban and rural settings and in cases and controls. The higher prevalence of *Giardia* in Borbón is likely a result of the study design differences between the

TABLE 3

Pathogen distribution and odds ratio for diarrhea reported as a crude estimate and stratified by single infection and co-infection (two or more infections) in Guamaní and in Borbón*

	Total	Single infections		Co-infections		All Infections	Single infections	Co-infections
		Case	Controls	Case	Controls	Odds ratio (exact 95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
GUAMANÍ								
						Matched OR		
Rotavirus	10	0	0	10	0	21 (1.23–358.4)	1 (0.01–50)	12 (1.23–358)
Norovirus	5	2	1	2	0	4 (0.4–197)	2 (0.1–117)	5 (0.24–104)
Shigellae	11	3	0	8	0	23 (1.35–390)	7 (0.36–135)	17 (0.98–294)
<i>Campylobacter jejuni</i>	11	7	4	0	0	2 (0.5–8)	2 (0.5–8)	1 (0.02–50)
<i>Campylobacter coli</i>	5	3	2	0	0	1.5 (0.25–9)	7 (0.36–136)	1 (0.02–50)
EIEC	8	3	2	3	0	5 (0.55–236)	2 (0.18–22)	7 (0.36–136)
ETEC (<i>eltA</i>)	3	0	1	1	1	0.5 (0.008–9.6)	0.33 (0.0002–15)	1 (0.1–78)
ETEC (<i>estA</i>)	3	2	0	1	0	7 (0.36–135)	5 (0.24–104)	3 (0.1–74)
STEC (<i>stx-1</i>)	3	1	1	1	0	3 (0.12–73)	1 (0.02–50)	1 (0.02–50)
<i>Vibrio parahaemolyticus</i>	1	1	0	0	0	3 (0.12–73)	3 (0.12–74)	1 (0.02–50)
<i>Giardia lamblia</i>	15	2	7	6	0	1.25 (0.27–6.3)	0.16 (0.004–1.37)	13 (0.73–231)
<i>Cryptosporidium parvum</i>	2	2	0	0	0	8 (0.24–104)	5 (0.24–104)	1 (0.02–50)
<i>Entamoeba histolytica</i>	26	9	8	8	1	2.1 (0.82–6.2)	1.1 (0.36–3.7)	15 (0.85–262)
BORBÓN†								
						Mantel-Haenszel OR		
Rotavirus	9	2	1	5	1	12 (2.4–60)	6 (0.52–68)	14.8 (1.7–132)
Norovirus	1	0	1	0	0	NA	NA	NA
Shigellae	2	0	0	1	1	2.92 (0.18–48)	NA	2.92 (0.18–48)
<i>Campylobacter jejuni</i>	1	0	1	0	0	NA	NA	NA
<i>Campylobacter coli</i>	1	0	0	1	0	NA	NA	NA
EIEC	6	1	2	1	2	1.46 (0.25–8.3)	NA	1.44 (0.13–16)
ETEC (<i>eltA</i>)	2	2	0	0	0	NA	NA	NA
ETEC (<i>estA</i>)	3	1	1	1	0	6 (0.53–68)	2.9 (0.18–48)	NA
<i>Giardia lamblia</i>	25	6	9	7	3	4.16 (1.7–10.2)	2 (0.7–6.3)	8 (2–32)
<i>Cryptosporidium parvum</i>	4	2	0	2	0	NA	NA	NA
<i>Entamoeba histolytica</i>	5	1	2	2	0	4.6 (0.74–28)	1.4 (0.12–16)	NA

* Estimates in bold are statistically significant ($P < 0.05$).

† Two samples from Borbón were not tested for *Entamoeba histolytica*.

NA = not enough data for analyses.

TABLE 4

Co-infections found in Guamaní and in Borbón*

	Total (%)	Number cases	Number controls	OR (95% CI)
Guamaní				
Shigellae/Rotavirus/EIEC	2	2	0	5 (0.24–104)
Shigellae/Rotavirus/ <i>Entamoeba histolytica</i>	1	1	0	3 (0.12–74)
Shigellae/EIEC	1	1	0	3 (0.12–74)
Shigellae/ <i>Entamoeba histolytica</i>	1	1	0	3 (0.12–74)
ETEC <i>elt</i> / <i>Entamoeba histolytica</i>	1	0	1	0.3 (0.01–8)
ETEC <i>est</i> / <i>Entamoeba histolytica</i>	1	1	0	3 (0.12–74)
<i>Giardia lamblia</i> /Shigellae/Rotavirus	3	3	0	7 (0.36–136)
<i>Giardia lamblia</i> /ETEC <i>elt</i> / <i>Entamoeba histolytica</i>	1	1	0	3 (0.12–74)
<i>Giardia lamblia</i> /Rotavirus	2	2	0	5 (0.24–104)
Norovirus/ <i>Entamoeba histolytica</i>	1	1	0	3 (0.12–74)
Norovirus/STEC/ <i>Entamoeba histolytica</i>	1	1	0	3 (0.12–74)
Rotavirus/ <i>Entamoeba histolytica</i>	2	2	0	5 (0.24–104)
Total	17	16	1	19 (1.1–326)
Aggregated† Shigellae/Rotavirus	6	6	0	13 (0.73–231)
Aggregated <i>Giardia lamblia</i> /Rotavirus	5	5	0	11 (0.6–199)
Borbón				
Shigellae/EIEC	1	1	0	NA
<i>Giardia lamblia</i> / <i>Campylobacter coli</i>	1	1	0	NA
<i>Giardia lamblia</i> / <i>Cryptosporidium</i>	1	1	0	NA
<i>Giardia lamblia</i> /Shigellae/EIEC	1	0	1	NA
<i>Giardia lamblia</i> /EIEC	1	0	1	NA
<i>Giardia lamblia</i> /ETEC <i>est</i>	1	1	0	NA
<i>Giardia lamblia</i> /Rotavirus	3	2	1	6 (0.5–68)
<i>Giardia lamblia</i> /Rotavirus/ <i>Entamoeba histolytica</i>	2	2	0	NA
Rotavirus/ <i>Cryptosporidium</i>	1	1	0	NA
Total	12	9	3	17 (3.2–88)
Aggregated <i>Giardia lamblia</i> /Rotavirus	5	4	1	24.13 (1.9–302)
Aggregated Shigellae/EIEC	2	1	1	5.12 (0.33–79)

* Estimates in bold are statistically significant ($P < 0.05$).

† Includes all co-infections that contain both pathogens.

OR = odds ratio; CI = confidence interval; NA = insufficient data for analysis.

sites. The Guamaní samples came from individuals attending a health center; whereas samples from Borbón came from an active surveillance within the community. Moreover, a single infection with *Giardia* was not associated with diarrhea in either setting, which is consistent with findings from a recently published systematic review.⁴² Unlike single infections, a co-infection with *Giardia* and rotavirus was associated with diarrhea in our rural setting. This is in agreement with two prior studies, one performed in our study region¹⁶ and the other in Israel.⁴³ *Giardia* infections also occur in a high proportion of individuals with subclinical or mild symptoms.

Campylobacter coli and *C. jejuni* were isolated in higher numbers in the urban community in both cases and controls, and therefore were not associated with diarrhea. Neither ETEC nor Shigellae and EPEC were significantly associated with diarrhea in our rural setting. Data collected from the past 10 years in the same rural site showed consistent association of these three pathogens with diarrhea.¹⁴ Environmental factors, such as climate^{14,44} or reduced virulence of pathovars circulating at the time of the study, could also provide an explanation for the data's presentation. The greater role of Shigellae in Guamaní may be caused by the high population density in the urban setting.

Interestingly, we did not find any non-typhi *Salmonella*. Although this is consistent with the GEMS study, our low sample size does not preclude the fact that non-typhi *Salmonella* occurs at lower levels. In fact, similar studies in Brazil found non-typhi *Salmonella* spp. in diarrheagenic stool samples.^{45–48} These discrepancies may result from differences in animal husbandry of livestock, or from other environmental characteristics that may affect transmission of this intestinal pathogen.

Although we only isolated *C. parvum* in two samples in Guamaní and four samples in Borbón, it is of interest that all six samples were from cases of children < 5 years of age. Therefore, in Borbón, 4 of the 21 cases were positive for *C. parvum*. In comparison, 3 of the 21 cases were positive for rotavirus, suggesting that *C. parvum* is an important pathogen in this rural area of Ecuador. This is in general consistent with the GEMS study, which focused on children < 5 years of age. The importance of *C. parvum* as a cause of diarrhea dropped off significantly after the age of 2 in the GEMS data, showing that this pathogen is less likely to cause disease in older children. An important distinction between the GEMS study and ours is that we did not use a severity scale in isolating cases; however, we assume that the Guamaní (urban) clinical cases were generally more severe than the Borbón (rural) community cases.

High rates of asymptomatic carriage of intestinal pathogens were observed in the two locations (27% in Guamaní and 18% in Borbón). Asymptomatic carriage may be caused by several factors, including strain pathogenicity, host immunity against pathogenic factors, intestinal microbiota, and herd immunity.^{5,49,50} Asymptomatic *Campylobacter* spp. carriage among people living in Latin America countries has been documented since the late 1980s⁵¹; specifically, *C. coli* carriage has been documented among people living or handling with pigs, sheep, or chickens.^{52,53} These findings support the hypothesis that *Campylobacter* spp. is present in environments where people live in close proximity with animals and under poor sanitation conditions.⁵¹

Some of the discrepancies between rural and urban settings could be explained by ecologic differences. Guamaní is located in the Andes at an elevation of 2,700 m, has low humidity, and samples were collected during the rainy season. Borbón is located near the coast, (low elevation and high humidity) and samples were obtained during the dry season.

Our pathogen detection rate among cases in this study, 51% in the urban and 62% in the rural settings, is consistent with similar studies in South America (13.1% to 62.2%)^{46,54} and Ecuador (27% to 65%, Table 1). There are a variety of reasons for not being able to explain 100% of the diarrhea cases. First, not all cases were infectious in origin. Second, we used diagnostic procedures that had sensitivity limitations. Our results differed from GEMS, possibly because our procedures and reagents (such as EIA kits) were different and our sample sizes were significantly smaller. Additionally, we did not investigate all potential pathogens included in the GEMS study such as adenovirus, *Yersinia enterocolitica*, *Clostridium perfringens* infections, or enterotoxins (*Staphylococcus aureus* or *Bacillus cereus*).

This study confirms that in Ecuador, a small and consistent subset of pathogens, including rotavirus and pathogenic *E. coli*, are the major causes of diarrhea. The specific set of important etiologic agents, however, varies depending on the urban/rural setting, socioeconomic level, and other important environmental drivers. Thus, site-specific etiological studies can help inform targeted interventions and control strategies.

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Authors' addresses: Gabriela Vasco, Gabriel Trueba, and Tamara Andrade, Microbiology Institute, Universidad San Francisco de Quito, Quito, Ecuador, E-mails: piavas_rc@hotmail.com, gtrueba@usfq.edu.ec, and tamaraandrade@yahoo.com. Richard Atherton, Manuel Calvopiña, and William Cevallos, Centro de Biomedicina, Universidad Central del Ecuador, Quito, Ecuador, E-mails: richard.atherton@hotmail.co.uk, mcalvopina@gmail.com, and wcalvopina@gmail.com. Martha Eguiguren, Centro de Salud Guamaní, Ministerio de Salud Pública, Quito, Ecuador, E-mail: m_eguiguren@hotmail.com. Joseph N. S. Eisenberg, School of Public Health, University of Michigan, CA, E-mail: jnse@umich.edu.

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