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ABSTRACT

Animals are important reservoirs of zoonotic enteropathogens, and transmission to humans occurs more frequently in low- and middle-income countries (LMICs), where small-scale livestock production is common. In this study, we investigated the presence of zoonotic enteropathogens in stool samples from 64 asymptomatic children and 203 domestic animals of 62 households in a semirural community in Ecuador between June and August 2014. Multilocus sequence typing (MLST) was used to assess zoonotic transmission of *Campylobacter jejuni* and atypical enteropathogenic *Escherichia coli* (aEPEC), which were the most prevalent bacterial pathogens in children and domestic animals (30.7% and 10.5%, respectively). Four sequence types (STs) of *C. jejuni* and four STs of aEPEC were identical between children and domestic animals. The apparent sources of human infection were chickens, dogs, guinea pigs, and rabbits for *C. jejuni* and pigs, dogs, and chickens for aEPEC. Other pathogens detected in children and domestic animals were *Giardia lamblia* (13.1%), *Cryptosporidium parvum* (1.1%), and Shiga toxin-producing *E. coli* (STEC) (2.6%). *Salmonella enterica* was detected in 5 dogs and *Yersinia enterocolitica* was identified in 1 pig. Even though we identified 7 enteric pathogens in children, we encountered evidence of active transmission between domestic animals and humans only for *C. jejuni* and aEPEC. We also found evidence that *C. jejuni* strains from chickens were more likely to be transmitted to humans than those coming from other domestic animals. Our findings demonstrate the complex nature of enteropathogen transmission between domestic animals and humans and stress the need for further studies.

IMPORTANCE

We found evidence that *Campylobacter jejuni*, *Giardia*, and aEPEC organisms were the most common zoonotic enteropathogens in children and domestic animals in a region close to Quito, the capital of Ecuador. Genetic analysis of the isolates suggests transmission of some genotypes of *C. jejuni* and aEPEC from domestic animals to humans in this region. We also found that the genotypes associated with *C. jejuni* from chickens were present more often in children than were those from other domestic animals. The potential environmental factors associated with transmission of these pathogens to humans then are discussed.

Diarrheal diseases are a major cause of illness and death in low- and middle-income countries (LMICs), where there are over 1.5 billion diarrhea cases that occur annually among children less than 5 years old, resulting in nearly 700,000 deaths (1). Although the contribution of zoonotic pathogens to human diarrheal disease is significant (2), these pathogens are often overlooked, and their detection may be hindered by patterns of seasonality (3). Zoonotic enteropathogens comprise a large and diverse range of microorganisms that could be transmitted to humans by consumption of meat or dairy products, by direct contact with animals (or their feces) in the environment, or by consumption of food or water contaminated with animal feces (2, 4). In the United States, researchers estimated that 14% of enteric infections with 7 groups of zoonotic enteropathogens were attributable to direct contact with animals (5).

Most of the studies of zoonotic enteropathogens have taken place in high-income countries, and their results may not be applicable to LMICs with less developed sanitary infrastructure and different animal husbandry practices (4). LMICs report many zoonotic pathogens that are rare in industrialized nations and vice versa (4). For instance, *Campylobacter jejuni*, non-Typhi *Salmonella*, and enterohemorrhagic *Escherichia coli* (EHEC) are zoonotic pathogens associated with high morbidity in high-income countries (6, 7), but they are unusual causes of human disease in LMICs (8–10).

Although some zoonotic pathogens, such as *Campylobacter jejuni*, are recognized as the most frequent gastrointestinal bacterial pathogen in humans in industrialized countries (11, 12), the contribution of other zoonotic pathogens (such as enteropathogenic *E. coli* or many strains of Shiga-toxigenic *E. coli*) to human diarrhea is less understood (13–15).

Most zoonotic enteropathogens were thought to be generalists (able to infect a wide variety of animals, including humans); however, recent evidence suggests that some strains of *Cryptosporidium parvum*, *Campylobacter jejuni*, and *Giardia lamblia* are host adapted with low levels of transmission to humans (12, 16, 17). Furthermore, some domestic animals may harbor generalist strains while others carry more host-adapted strains (9).

TABLE 1 Frequency of zoonotic enteropathogens identified in both children and domestic animals

Source	No. of samples	No. (%) positive for:								
		C. jejuni	*C. coli*	aEPEC	*Campylobacter* spp.[a]	STEC	*Salmonella* spp.	*Yersinia*	*Giardia lamblia*	*Cryptosporidium parvum*
Children	64	7 (10.9)	3 (4.7)	11 (17.2)	1 (1.6)	1 (1.6)	0	0	22 (34.4)	2 (3.1)
Chickens	42	25 (59.5)	7 (16.7)	3 (7.1)	0	1 (2.4)	0	0	0	0
Guinea pigs	40	29 (72.5)	2 (5.0)	2 (5.0)	0	1 (2.5)	0	0	1 (2.5)	0
Dogs	40	10 (25.0)	1 (2.5)	4 (10.0)	0	0	5 (12.5)	0	5 (12.5)	0
Pigs	36	3 (8.3)	14 (38.9)	4 (11.1)	10 (27.8)	0	0	1 (2.8)	2 (5.6)	0
Rabbits	20	2 (10.0)	0	0	0	0	0	0	4 (20.0)	0
Cattle	7	1 (14.3)	1 (14.3)	2	0	4 (57.1)	0	0	0	0
Cats	6	2 (33.3)	1 (16.7)	0	0	0	0	0	0	0
Ducks	5	1 (20.0)	1 (20.0)	1	2 (40.0)	0	0	0	0	0
Quail	3	2 (66.7)	0	0	0	0	0	0	0	0
Sheep	2	0	1 (50.0)	1	0	0	0	0	1 (50.0)	1 (50.0)
Geese	1	0	0	0	0	0	0	0	0	0
Horses	1	0	0	0	0	0	0	0	0	0
Total	267	82 (30.7)	31 (11.6)	28	13 (4.9)	7 (2.6)	5 (1.9)	1 (0.4)	35 (13.1)	3 (1.1)

[a] *Campylobacter* non-*jejuni/coli* species included *C. hyointestinalis* (pigs and child), *C. lanienae* (pig), and *C. canadensis* (ducks).

The present study aimed to investigate the prevalence of 7 zoonotic enteropathogens (bacteria and protozoa) in children and domestic animals in a semirural community of Ecuador.

MATERIALS AND METHODS

Study location. The study was conducted in Otón de Vélez-Yaruquí, a low-income semirural community east of Quito, at an altitude of 2,527 m above sea level. The main economic activities are agriculture and animal husbandry, particularly intensive poultry production (four chicken industrial operations were present in the community). Of the sampled households, 68% had chickens, 64.5% had guinea pigs (raised for food), 64.5% had dogs, 58% had pigs, 32.3% had rabbits, and 11.3% had cattle, and cats, ducks, quails, sheep, geese, and horses were present in 10%, 8%, 5%, 3%, 1.6% and 1.6% of the households, respectively (see Fig. S1 in the supplemental material).

Ethical considerations. The study protocol was approved by the Institutional Animal Care and Use Committee at the George Washington University (IACUC number A296), as well as the Bioethics Committee at the Universidad San Francisco de Quito (2014-135M) and the George Washington University Committee on Human Research Institutional Review Board (IRB number 101355).

Sample collection. Sixty-five households were recruited randomly between June and August 2014, during the dry season. Fifty-nine of the households had domestic animals (1 to 8 animal species), 3 did not have animals, and 3 households did not provide samples. Eleven households reported having someone in the home who worked in the poultry industry. We collected 64 stool samples from asymptomatic children (47% female and 53% male; ages were 3 to 12 months old [n = 11], 1 to 3 years old [n = 31], 3 to 5 years old [n = 18], and 6 years old [n = 4]) (see Table S1 in the supplemental material) and 203 samples from 12 animal species (Table 1; also see Fig. S1). Animal fecal samples were obtained either directly from the rectus (dogs, cats, sheep, and quail) or from pooled fecal matter when animals were maintained in enclosures (pigs, chickens, and cows) or cages (guinea pigs, rabbits, and quails). The stool samples were placed in a cooler on ice for transportation to the laboratory. All bacterial culturing and sample preservation began less than 8 h after collection.

Identification of zoonotic enteropathogens. Fecal samples were analyzed for seven zoonotic enteropathogens: *Campylobacter* spp., atypical enteropathogenic *E. coli* (aEPEC), Shiga toxin-producing *E. coli* (STEC), *Salmonella* spp., *Yersinia* spp., *Cryptosporidium parvum*, and *Giardia lamblia*.

Pathotypes of *E. coli* were obtained by culturing samples on MacConkey lactose agar (Difco, Sparks, MD) (at 37°C for 18 h), and lactose-fermenting colonies were plated in Chromocult coliform agar (Merck KGaA, Darmstadt, Germany) to identify the ß-D-glucuronidase activity. Five lactose-positive isolates were pooled as a random sample, suspended in 300 µl of sterile distilled water, and boiled for 10 min to release the DNA. The resulting supernatant was used for PCR to identify *eae* (18), *bfpA* (19), *stx₁*, and *stx₂* as previously described (20). Isolates from positive pools for any loci were individually analyzed by PCR and cryopreserved in brain and hearth infusion medium (Difco, Sparks, MD) with 20% glycerol at −80°C for further analyses. aEPEC isolates were positive for *eae* (LEE gene) and negative for *bfpA* and *stx* genes (13); STEC isolates were positive for any of the *stx* genes by PCR but lacked the *eae* gene.

To isolate *Yersinia* spp., the samples were preenriched in 1× phosphate-buffered saline (PBS) for 21 days at 4°C and cultured in cefsulodin irgasan novobiocin agar (at 28°C for 24 and 48 h) (Oxoid Ltd., Basingstoke, Hampshire, England). Suspected colonies were confirmed with the following tests: oxidase test (Bactident oxidase; Merck), RapiD-20E (bioMérieux, Marcy l'Etoile, France) with identification percentages greater than 85%, lack of lactose fermentation on MacConkey lactose agar (Difco, Sparks, MD), and urease activity in Christenson urea agar (Difco, Sparks, MD).

To recover *Salmonella* spp., samples were preenriched in selenite broth (Merck KGaA, Darmstadt, Germany) (at 37°C for 18 h) and cultured in xylose-lysine-deoxycholate agar (Difco, Sparks, MD) (at 37°C for 18 h). Suspected colonies were subjected to RapiD-20E tests (bioMérieux, Marcy l'Etoile, France), with acceptable identification set at greater than 95%. The identification of serovars was performed by amplifying 10 pairs of primers by multiplex PCR in two separate reactions (assays STM and STY) as previously described (21). The STM amplification was performed in a 10-µl reaction mix with 1.4× PCR buffer, 2 mM MgCl₂, 0.2 mm deoxynucleoside triphosphates (dNTPs), 0.3 µM each primer (STM1, STM2, STM3, STM4, and STM5), 0.75 U GoTaq polymerase, and 1 µl of DNA (∼10 ng/µl). Furthermore, the STY amplification reaction was performed in a final volume of 10 µl with 1.6× reaction buffer; 2 mM MgCl₂; 0.2 mm dNTPs; 0.08 µM primers STY1, STY2, and STM6; 0.3 µM primer STY3; 0.1 µM primer STY4; 0.75 U GoTaq polymerase; and 1 µl of DNA. Both reactions used the same amplification program: initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min, and ending with a final extension at 72°C for 5 min. Electrophoresis conditions for displaying the results of STM and STY included a 2.5% agarose gel run for 2 h at 80 V.

To investigate thermophilic *Campylobacter* spp., samples were cul-

tured on *Campylobacter* agar with 5% lysed horse blood and modified Preston *Campylobacter* selective supplement (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated at 42°C during 48 h under microaerobic conditions using CampyGen CO_2 (Oxoid Ltd., Basingstoke, Hampshire, England). The colonies were Gram stained and tested for oxidase (Bactident oxidase; Merck). *Campylobacter jejuni/Campylobacter coli* were confirmed by PCR of hippuricase and aspartokinase genes according to the protocol developed by Persson and Olsen in 2005 (22). *Campylobacter* species not belonging to *C. jejuni/coli* were identified through 16S rRNA gene sequencing by Functional Biosciences (Madison, WI), and sequences were uploaded to GenBank.

Giardia lamblia and *Cryptosporidium parvum* were detected using an enzyme-linked immunosorbent assay (Ridascreen *Giardia* and Ridascreen *Cryptosporidium*; r-Biopharm, Darmstadt, Germany).

Transmission between humans and domestic animals. Transmission of bacterial pathogens (*C. jejuni* and aEPEC) between domestic animals and humans and between domestic animals was assessed by characterizing the clonal relationships of bacterial isolates using multilocus sequence typing (MLST). To detect clonally related bacteria, we screened *C. jejuni* isolates using nucleotide sequences of *pgm* genes and aEPEC isolates using nucleotide sequences of *fumC* genes; all isolates showing an identical DNA sequence were assumed to be clonally related and were subjected to a full MLST profiling to confirm this status.

MLST. DNA extraction of *C. jejuni* and aEPEC isolates was performed using DNAzol reagent (Invitrogen, Carlsbad, CA, USA) by following the manufacturer's protocol. We screened 72 *C. jejuni* isolates by amplifying and sequencing the *pgm* allele (23); 55 isolates with identical *pgm* DNA sequences were subjected to additional analysis of genes *glyA* and *tkt*, and only the isolates with identical DNA sequences for *pgm*, *glyA*, and *tkt* alleles (48 out of 72 isolates) were subjected to full MLST analysis (23). Similarly, aEPEC isolates with identical *fumC* DNA sequences (14 out of 28 isolates) were subjected to full MLST analysis (24). The PCR products were sequenced by Functional Biosciences (Madison, WI), and sequences were uploaded to the PubMLST website for *Campylobacter* spp. (http://pubmlst.org/campylobacter/) and *E. coli* (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/) to assign the allelic profiles.

Data analyses. Evolutionary relationships of sequence types were inferred using eBURST V3 (http://eburst.mlst.net/). To visualize the microevolutionary processes of the STs, minimum-spanning trees were constructed with Prim's algorithm in the BioNumerics software according to species source (version 7.5; Applied-Maths, Sint Martens-Latem, Belgium). Statistical analyses were performed using Microsoft Office Excel 2013. Geographic distribution maps were developed using BaseCamp software version 4.4.7. and GPSvisualizer (http://www.gpsvisualizer.com/).

Nucleotide sequence accession numbers. Sequences determined during the course of this work were deposited in GenBank under accession numbers KU362553 to KU362565.

RESULTS

Prevalence and geographic distribution of zoonotic enteropathogens. *Campylobacter jejuni* was the most prevalent pathogen in all samples (30.7%; $n = 82$), followed by *Giardia lamblia* (13.1%; $n = 35$), *C. coli* (11.6%; $n = 31$), aEPEC (10.5%; $n = 28$), *Campylobacter* non-*jejuni/coli* (5.2%; $n = 13$), STEC (2.6%; $n = 7$), *Salmonella* spp. (1.9%; $n = 5$), *Cryptosporidium parvum* (1.1%; $n = 3$), and *Yersinia enterocolitica* (0.4%; $n = 1$) (Table 1). The geographic distribution of isolates is shown in Fig. S2 in the supplemental material.

Campylobacter spp. were found in 17.2% of children (7 samples were *C. jejuni*, 3 were *C. coli*, and 1 was *C. hyointestinalis*), and 57.1% of samples ($n = 112$) were positive in domestic animals. High percentages of guinea pigs (77.5%) and chickens (76%) were positive for *Campylobacter* spp. (mostly *C. jejuni*) (chickens,

59.5%; guinea pigs, 72.5%). Also, 75% of pigs were positive for *Campylobacter* spp., including *C. coli* (38.9%) and *C. hyointestinalis* (27.8%). Dogs carried *Campylobacter* spp. (30%; $n = 12$), mainly *C. jejuni* (25%). In addition, *Campylobacter* also was present in rabbits, cows, cats, ducks, and quails but at a lower prevalence (Table 1). Other *Campylobacter* species identified were *C. canadensis* in ducks (2 out of 5 samples) and *C. lanienae* in a pig (1 out of 36 samples).

Atypical EPEC was present in a wide range of hosts, including children (17.2%; $n = 11$), dogs (10.0%; $n = 4$), pigs (11.1%; $n = 4$), chickens (7.1%; $n = 3$), guinea pigs (5.0%; $n = 2$), cattle (28.6%; $n = 2$), ducks (20.0%; $n = 1$), and sheep (50.0%; $n = 1$) (Table 1).

Seven STECs were isolated from a child ($n = 1$), cattle ($n = 4$), a guinea pig ($n = 1$), and a chicken ($n = 1$). *Giardia lamblia* was present mainly in children ($n = 22$) and was detected in 13 animal fecal samples from guinea pigs, dogs, pigs, rabbits, and a sheep (Table 1). *Salmonella* spp. were detected in 5 samples from dogs (*S. enterica* serovar Infantis), 2 children and 1 sheep were positive for *Cryptosporidium parvum*, and 1 pig was positive for *Yersinia enterocolitica* (Table 1).

The copresence of pathogens was found in 9 children, 7 pigs, 4 guinea pigs, 5 dogs, 2 cattle, 3 chickens, 1 sheep, 1 cat, and 1 duck (see Table S4 in the supplemental material). The most common one was *Campylobacter*-aEPEC, found in 11 (4.1%) samples, followed by *Campylobacter-Giardia* in 8 (3%) samples and aEPEC-*Giardia* in 7 (2.6%) samples.

Zoonotic enteropathogens were present in all age groups of children; however, most of the pathogens were detected in children aged 1 to 3 years old (56.4% of 47 positive samples; 27 of 31 children of this age group), followed by children aged 3 to 5 years old (31.9% of 47 positive samples; 15 of 18 children of this category) (see Table S1 in the supplemental material). The youngest children infected were one 3-month-old baby with aEPEC, a 3-month-old baby with *G. lamblia*, a 10-month-old infant with *C. coli*, a 13-month-old infant with coinfection of *C. parvum* and *C. jejuni*, a 4-year-old child with *C. hyointestinalis*, and a 5-year-old child with STEC (see Table S1 and Fig. S3). Interestingly, none of the subjects showed evidence of acute diarrhea.

***Campylobacter jejuni* MLST.** We identified 21 *C. jejuni* STs in the 48 analyzed isolates, of which 14 were novel: ST-7643, ST-7759, ST-7760, ST-7662, ST-7669, ST-7671, ST-7672, ST-7775, ST-7777, ST-7778, ST-7779, ST-7780, ST-7781, and ST-7789 (Table 2). Four of the STs were detected in both children and domestic animals: ST-137, ST-1233, ST-3515, and ST-7671 (Table 2 and Fig. 1). Ten STs belonged to 5 clonal complexes (CC). The most common was CC-353, comprised of 8 isolates (2 from children and 6 from domestic animals), followed by CC-607, comprised of 7 isolates from different domestic animal species but no humans, and CC-354, with 4 isolates from avian species (3 chickens and 1 quail). In three households we found animals that shared isolates with the same ST (1 guinea pig and 1 chicken, 1 rabbit and 1 chicken, and 1 dog and 1 quail). The rest of the STs and CCs were randomly distributed in the community (Table 2; also see Table S2 in the supplemental material). Further, guinea pigs were the major reservoir of *C. jejuni*; 21 isolates of this animal species were analyzed by MLST, and nine novel STs were identified solely in guinea pigs (25).

Atypical EPEC MLST. Fourteen aEPEC isolates belonged to 9 STs; 4 STs (ST-20, ST-137, ST-517, and ST-4550) were present in both children and domestic animals. Isolates from a sheep and a

TABLE 2 Number of isolates of *C. jejuni*, by sequence type, recovered from children 0 to 3 years of age and from each animal source

CC[a]	ST[b]	No. of isolates from:								
		Children	Chickens	Guinea pigs	Dogs	Pigs	Rabbits	Cattle	Cats	Quails
CC-607	607	0	2	0	2	0	0	0	0	0
	1212	0	1	0	0	1	0	1	0	0
CC-353	1233	1	2	1	0	0	0	0	0	0
	3515	1	1	0	0	0	0	0	0	0
	7643	0	0	0	1	0	0	0	0	1
CC-354	354	0	1	0	0	0	0	0	0	0
	7662	0	1	0	0	0	0	0	0	1
	7669	0	1	0	0	0	0	0	0	0
CC-464	464	0	1	0	0	0	1	0	0	0
CC-45	137	1	0	0	0	0	1	0	0	0
Unassigned CC	**7671**	1	0	0	1	0	0	0	0	0
	7672	0	0	0	1	0	0	0	1	0
	7759	0	0	6	0	0	0	0	0	0
	7760	0	0	1	0	0	0	0	0	0
	7775	0	0	4	0	0	0	0	0	0
	7777	0	0	1	0	0	0	0	0	0
	7778	0	0	1	0	0	0	0	0	0
	7779	0	0	2	0	0	0	0	0	0
	7780	0	0	2	0	0	0	0	0	0
	7781	0	0	3	0	0	0	0	0	0
	7789	0	0	1	0	0	0	0	0	0

[a] CC, clonal complex.
[b] The STs marked in boldface correspond to new STs.

duck belonged to ST-317 (Fig. 2). There were no predominant clonal complexes: 5 STs belonged to 5 different CCs, while 4 STs were not assigned to any CC. None of the STs shared by isolates from humans and domestic animals belonged to the same household; indeed, identical STs were found in households distantly located (Table 3; also see Table S3 in the supplemental material). ST-4550 was present in a child and a chicken living in close proximity and was genetically related to ST-29 identified in a child (identical at 6 of 7 loci).

DISCUSSION

Consistent with previous studies (26–29), our MLST analysis indicated that chickens were an important source of *C. jejuni* for both humans and domestic animals; this animal species commonly shared the same STs with other species (2 STs in 2 isolates from humans and 5 STs from 7 isolates of domestic animals) (Table 2 and Fig. 1). On the other hand, STs of *C. jejuni* from guinea pigs (the animal with the highest prevalence of *C. jejuni*) seemed to infect only this animal species (Table 2) (25). Previous MLST analyses have found that some *C. jejuni* strains may be more adapted to one animal species and less likely to infect humans or other hosts (25, 29, 30). The association of *C. jejuni*'s STs and CCs with specific animal species seemed to concur with findings previously reported (28, 30, 31).

Similarly, MLST analysis of aEPEC revealed that pigs, chickens, and dogs shared genotypes of aEPEC found in children; hence, these animals could be involved in the transmission of aEPEC to humans (Table 3 and Fig. 2). Additionally, several animal species, like guinea pigs, cattle, ducks, and sheep, carried aEPEC (Table 3) as reported in previous studies (32, 33). Most STs were not found

in animal species previously reported except for ST-4550 and ST-327, which were described previously in chickens and ruminants, respectively (http://mlst.warwick.ac.uk/mlst/).

Although our findings suggest zoonotic transmission of *C. jejuni* and aEPEC, they do not provide conclusive evidence for transmission from domestic animals to humans. This is especially critical for aEPEC, a pathogen of uncertain zoonotic potential, and it is possible that domestic animals became colonized by aEPEC from humans (14, 15, 34). It is important to note, however, that most households in this community have improved water and sanitation (i.e., piped drinking water and flush toilets inside the home), which prompt us to suggest that the main route of infection of these zoonotic pathogens for humans was contact with animals or a contaminated environment. In fact, we detected by PCR *C. jejuni* and *C. coli* in water from an irrigation channel in the community (data not shown).

The dissemination of zoonotic enteric pathogens could be influenced by the use of animal fecal matter to fertilize soils and the presence of four large poultry facilities (total capacity of ~200,000 chickens) within the community, which corresponds to the second largest conglomerate of poultry farms in Ecuador (http://www.conave.org/informacionlistall.php?pagina=2). Transmission could occur by workers in the poultry industry who subsequently expose their households to enteropathogens.

Additionally, *E. coli* (and probably pathogenic members of *Enterobacteriaceae*) grows massively in fresh fecal matter when exposed to oxygen in the environment (35, 36). A high prevalence of enteric pathogens in the environment may increase the possibility of crop contamination or the high presence of these pathogens in
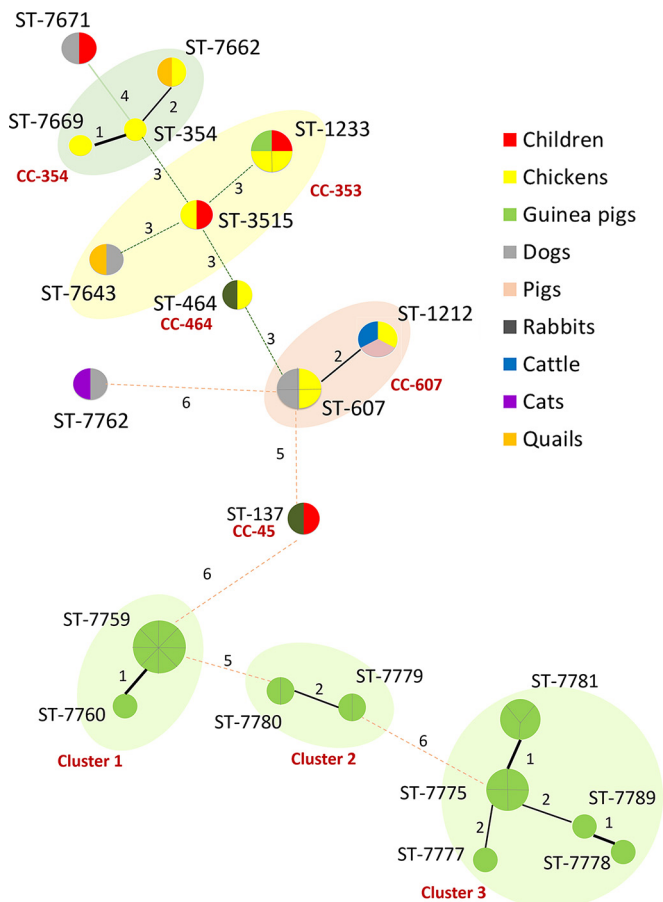
FIG 1 Minimum spanning tree analysis of 48 *C. jejuni* isolates based on MLST profile and according to source (the color of the circle indicates the species). Each circle represents the ST, and the size of the circle and circle divisions indicate the number of isolates within any given ST. Lines indicate strength length (distance) symbolized with numbers (number of nonshared alleles) and thickness. Clonal complexes and genetic clusters are labeled and represented by the surrounding shading.
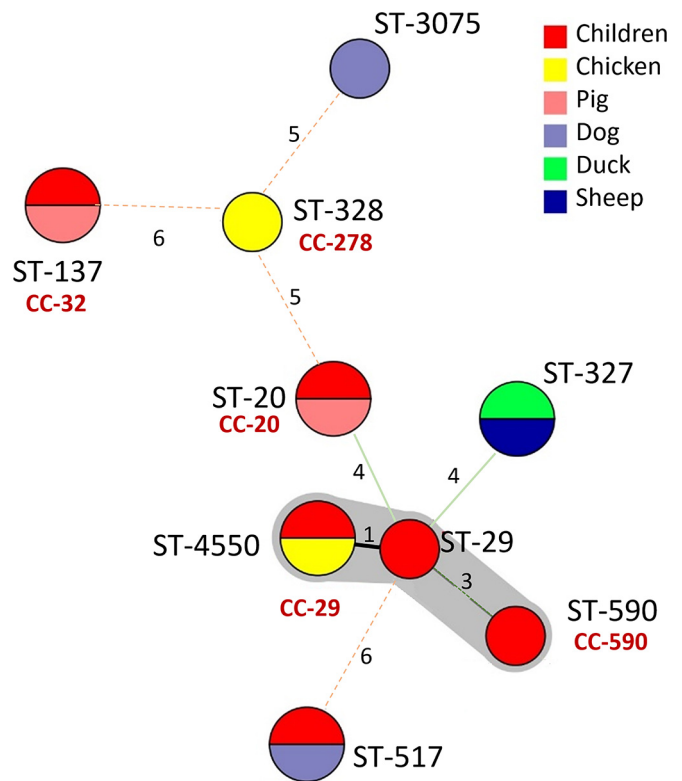

FIG 2 Minimum spanning tree analysis of 14 aEPEC isolates based on MLST profile and according to source (color of the circle indicates the species). Each circle represents the ST, and the size of the circle and circle divisions indicate the number of isolates within any given ST. Lines indicate strength length (distance) symbolized with numbers (number of nonshared alleles) and thickness. The genetic cluster is labeled and represented by the surrounding shading.

animal products. This area possibly represents a hot spot for zoonotic pathogens, especially for *Campylobacter* species, and their food products can represent a health risk to urban areas and may be associated with traveler's diarrhea when susceptible individuals visit this region, which is in close proximity to the Quito International airport.

Giardia lamblia was the most prevalent enteric pathogen among children (34.4%), and its level was higher than that in previous studies in Ecuador (ranging from 11 to 24%) (8, 37). Dogs, rabbits, pigs, guinea pigs, and sheep also carried *G. lamblia* in this location, which is an indication of transmission among animal species. However, we were not able to analyze genetic markers of these protozoa. Of the seven genotypes of *Giardia* (A to G), humans are susceptible to genotypes A and B, and its zoonotic transmission is mainly related to companion animals, such as dogs and cats, while livestock and contaminated water appear to be uncommon sources (16).

We also detected *Cryptosporidium parvum* in 2 samples from children (3.1%) and STEC in 1 sample from a child (1.6%). Both pathogens were detected in ruminants, and STEC was also detected in chickens and guinea pigs, which concurs with previous

studies (38). *Cryptosporidium* spp. are associated with morbidity and mortality in young children in developing countries (39) and may be an important cause of diarrhea in Ecuadorian rural villages (8). Although *Cryptosporidium* spp. are highly prevalent in livestock (40), several studies in developing countries suggest that zoonotic transmission of *Cryptosporidium* spp. is uncommon (17). Meanwhile, STEC is considered a food-borne disease with ruminants as the main reservoir (7); however, symptomatic disease in humans seems to be uncommon in Ecuador (8, 10).

TABLE 3 Number of isolates of aEPEC, by sequence type, recovered from children 0 to 5 years of age and from each animal source

CC	ST	No. of isolates from:					
		Children	Chickens	Dogs	Pigs	Ducks	Sheep
CC-20	20	1	0	0	1	0	0
CC-29	29	1	0	0	0	0	0
CC-32	137	1	0	0	1	0	0
CC-278	328	0	1	0	0	0	0
CC-590	590	1	0	0	0	0	0
Unassigned	327	0	0	0	0	1	1
	517	1	0	1	0	0	0
	3075	0	0	1	0	0	0
	4550	1	1	0	0	0	0

Despite the proximity of study households to poultry industrial operations, *Salmonella* was not detected in children, although it was isolated from five dogs (*S. enterica* serovar Infantis). *Yersinia enterocolitica* was present only in one pig fecal sample (pigs are known as the main reservoir for *Y. enterocolitica*) (7, 41).

The high prevalence of pathogens analyzed in this study of domestic animals (77% of the birds and 59% of the mammals) may contribute to environmental contamination and subsequent human infection. In fact, the distribution of certain species of animals in the community appeared to be related to the geographic presence of particular pathogens (see Fig. S1 and S2 in the supplemental material); for instance, the geographic location of guinea pigs and chickens corresponded to *C. jejuni*, pigs to *C. coli*, and ruminants to STEC (see Fig. S1 and S2).

Most children under 5 years of age (59.4%) carried intestinal pathogens but were asymptomatic (nondiarrheic stool), a phenomenon also observed with nonzoonotic human enteric pathogens in LMICs (42–46), and this may be due to herd immunity resulting from regular exposure to these pathogens (47); immune mothers may transfer immunoglobulins to their offspring through the placenta and breast milk. Another factor protecting people from symptomatic infection may be the microbiota composition (48). Despite the absence of diarrhea, asymptomatic infections, such as campylobacteriosis and cryptosporidiosis, may reduce growth in children (43, 44). The present work had some limitations; the first one was the small number of samples analyzed. The second limitation was the scheme used to assess clonality, as the initial selection of bacteria with one identical allele may have prevented us from detecting additional related clones (members of the same clonal complex). Finally, we were unable to carry out genotyping of *Giardia lamblia*, which was one of the most abundant enteric pathogens in the study.

The control of the dissemination of these pathogens calls for a comprehensive and multidisciplinary approach (49). It is necessary to have a complete analysis of the spatial, ecological, evolutionary, social, economic, and epidemiological aspects in order to reduce pathogen transmission. This report suggests that the area we investigated is heavily contaminated with zoonotic enteropathogens, which calls for additional research to detect pathogens in the environment (water, soil, and possibly crops).

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