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Zoonotic transmission of *Campylobacter jejuni* and Atypical Enteropathogenic *Escherichia coli* (aEPEC) in peri-urban Quito, Ecuador

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DEDICATORIA

A los niños ecuatorianos, por su presente y futuro.

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RESUMEN

Los patógenos zoonóticos son comunes en países de medianos y bajos recursos como el Ecuador. En el presente estudio se investigó la presencia de varios enteropatógenos zoonóticos en 267 muestras de heces de niños y animales domésticos de 62 hogares en una comunidad semirural del Ecuador, entre junio y agosto de 2014. Mediante Multilocus Sequence Typing (MLST) se determinó la transmisión zoonótica de *C. jejuni* y aEPEC, que fueron los patógenos bacterianos más prevalentes en niños y animales domésticos (30.7% y 10.5% respectivamente). Cuatro secuencia-tipos (STs) de *C. jejuni* y cuatro STs de aEPEC fueron idénticos en niños y animales domésticos, y los pollos, perros, cuyes y conejos podrían haber sido fuentes de *C. jejuni*, mientras que cerdos, perros y pollos parecieron ser fuentes de aEPEC de humanos. Otros enteropatógenos detectados en niños y animales domésticos fueron *Giardia lamblia* (13.1%), *Cryptosporidium parvum* (1.1%) y *E. coli* productora de Shiga-toxinas (STEC) (2.6%). *Salmonella* no-Typhi y *Yersinia enterocolitica* se detectaron en 5 perros y 1 cerdo, respectivamente.

Palabras clave: Zoonosis, niños, diarrea, animales, MLST, *Campylobacter*, *Escherichia coli, Giardia, Salmonella, Cryptosporidium, Yersinia.*

ABSTRACT

Zoonotic pathogens are common in low- and middle- income countries (LMICs) such as Ecuador. In the present study, we investigated the presence of zoonotic enteropathogens in stool samples from 267 children and domestic animals of 62 households in a semi-rural community in Ecuador between June and August 2014. Multilocus Sequence Typing (MLST) was used to assess *C. jejuni* and aEPEC zoonotic transmission, 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 sources of *C. jejuni* seemed to be chickens, dogs, guinea pigs and rabbits, while the sources of aEPEC seemed to be pigs, dogs and chickens. 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%). Non-Typhi *Salmonella* and *Yersinia enterocolitica* were detected in 5 dogs and 1 pig respectively.

Key words: Zoonoses, children, diarrhea, animals, MLST, *Campylobacter, Escherichia coli, Giardia, Salmonella, Cryptosporidium, Yersinia.*

TABLA DE CONTENIDO

ÍNDICE DE TABLAS

ÍNDICE DE FIGURAS

PART I

INTRODUCTION

Zoonotic diseases overview

Zoonoses are a major hazard to public health and result in hundreds of billions of US dollars spent globally each year (1). Zoonotic diseases are estimated to comprise above 60.3% of novel emerging infectious illnesses globally (2), while endemic and enzootic zoonoses are responsible for over a billion infections in humans and millions of deaths annually (2). In addition, misdiagnosed infections, mainly diarrheal and respiratory, are killing thousands of children and adults in low- and middle- income countries (LMICs) (3, 4).

The transmission of pathogens between species can be augmented when human and animal populations increase, environments change and individuals travel (5). Anthropogenic practices may also influence zoonotic disease transmission, including modifications in land use, new animal production systems, extractive industry and extensive antimicrobial use (1). Commercial and backyard livestock production has intensified, in size and density, and increasingly people are living in close contact with animals, disturbing the ecological balance between pathogens and hosts (6, 7).

The prevention and control of zoonoses requires comprehensive analysis of the spatial, ecological, evolutionary, social, economic, and epidemiological aspects. Multidisciplinary collaboration is crucial, including veterinarians, physicians, clinicians, public health scientists, ecologists, economists, and others (8). This coordinated,

collaborative and cross-sectoral approach has resulted in the 'One Health' initiative, which aims to link human, animal and environmental health (8).

Zoonotic enteric pathogens

Diarrheal disease is the second leading cause of death in children under five years old and it is both preventable and treatable (9). Most of the burden falls on children, who average 3.2 episodes of diarrhea per child per year (10). Zoonotic pathogens causing diarrhea comprise protozoa, bacteria, and viruses. The most relevant are: *Campylobacter jejuni/coli*, non-Thyphi *Salmonella*, Shiga Toxin- producing *E. coli* (STEC/VTEC), Enterohemorrhagic *E. coli* (EHEC), Atypical Enteropathogenic *Escherichia coli* (aEPEC), *Yersinia enterocolitica, Yersinia pseudotuberculosis*, *Giardia lamblia* genotypes A and B, and *Cryptosporidium* spp. (11). Viruses like Norovirus and Rotavirus have been isolated from other animals, however, the risk of cross-species infections have not yet been determined (12-14).

The transmission of zoonotic enteropathogens includes ingestion of contaminated animal products, contaminated water, close proximity to infected animals and in some cases transmission from person-to-person (15). The severity of infection depends on the pathogenicity of the microorganisms, and probably the number of infecting organisms (inoculum). Infectious doses also vary among infectious agents: *Giardia* spp. and *Cryptosporidium parvum* 10-100, Shiga toxin *E. coli* O157:H7 10-100, *Salmonella* 10^3-10^5, *Campylobacter* 10^3-10^6 and *Yersinia enterocolitica* 10^8-10^9 (11).

Pathogenic bacteria, including pathotypes of *Escherichia coli* and *Salmonella* spp. for example, attach to mucosal epithelial cells and colonize the mucosa through diverse mechanisms for adherence (e.g. adhesins) that are commonly encoded by mobile genetic

elements (MGEs) (11, 16). Enterotoxins, such as heat-labile toxins (LTs) produced by *Salmonella* spp. and *Campylobacter jejuni*, or heat-stable toxin (ST) produced by *Yersinia* spp. causes secretory or watery diarrhea. Enteric cytotoxins, such as Shiga-like toxin produced by STEC, EHEC and *Salmonella* strains, stimulate secretions through mucosal inflammation, often disrupting the protein synthesis in the ribosome (17). The invasiveness relates to adhesion and invasion proteins often carried on MGEs. Non-Typhi *Salmonella*, *C. jejuni* and *Yersinia* spp. usually produce bacteremia after the initial invasion (11).

Protozoa, such as *Giardia* spp. and *Cryptosporidum* spp. may cause diarrhea by similar mechanisms. *Giardia lamblia* can generate acute episodes of watery diarrhea, epigastric pain, nausea and vomiting. *Giardia* species attaches to enterocytes by a ventral disk, inducing apoptosis of enteric cells, mucosal inflammation, inhibition of trypsin and brushborder enzymes, bile salt deconjugation and uptake, and increased gastrointestinal transit by smooth-muscle contractility (18). Chronic giardiasis leads to villus blunting causing malabsorption, weight loss and gastrointestinal disorders (19, 20).

Zoonotic cryptosporidiosis in humans is commonly caused by *Cryptosporidium parvum,* which comprises the ~47% of the cases of cryptosporidiosis in developed countries and the ~18% in LIMCs (21-23). The clinical manifestations depend on the host response. In immunocompetent hosts, acute watery diarrhea is common, and typically resolves in 10–14 days (24). While in immunocompromised hosts, it is usually debilitating and watery diarrhea can occur over weeks to months (24). The molecular pathogenic process is not well understood, but putative virulence factors, such as genes for motility, attachment, invasion, parasitophorous vacuole formation, intracellular maintenance and host cell damage has been identified (21).

Epidemiology of enteric zoonoses.

The impact of each zoonotic pathogen is partially dependent upon the host species that it can colonize (25). Enteropathogens are usually generalists but some animal species are attributed as the main source for human disease; for example, chickens and cattle are the main sources for *Campylobacter* and *Salmonella* species, companion animals such as dogs and cats for *Giardia* sp., ruminants for STEC and *Cryptosporidium parvum,* and pigs for *Yersinia enterocolitica* (26).

The seasonality of enteric pathogens and subsequently, enteric infections, varies significantly. A systematic review, with data from countries with four distinct seasons (northern and southern hemisphere), shows peaks of certain enteropathogens. During the summer there are peaks for: Campylobacteriosis, salmonellosis and STEC (27). The majority of *Yersinia* infections occur in the winter (28, 29). While, *Cryptosporidium* infection present a bi-modal peak, in spring and late summer-early autumn and *Giardia* showed a small summer peak (27).

The epidemiology of some zoonotic enteric pathogens is reviewed here.

Campylobacter. Worldwide *Campylobacter* is the most frequently reported gastrointestinal bacterial pathogen in humans (30). The Center for Disease Control and Prevention (CDC) of United States of America (USA) reported 13.82 cases per 100,000 U.S. population in 2013 (31), and estimated that for every *Campylobacter* case reported there were 30 cases that went undiagnosed. Whereas, in 2013 the European Union (EU) notification rate was 64.8 per 100,000 population (26). The principal sources identified in outbreaks were contaminated poultry, milk and mixed food (26). Reports or studies of *Campylobacter* infections are scarce and fragmented in developing countries, but these

bacteria are considered endemic, with young children most at risk for symptomatic infections (30). Nevertheless, the Global Enteric Multicenter Study (GEMS) determined that *C. jejuni* was significantly associated with moderate-to-severe diarrhea in at least one age stratum of three Asian study sites (32).

Enteropathogenic *E. coli.* Typical EPEC (tEPEC) is not considered a zoonosis but is associated with 30–40% of infant diarrhea according to studies taking place in America and Africa (33-35). Atypical EPEC (aEPEC) are prevalent in both developed and developing countries; nevertheless, its pathogenicity is still controversial. In 2006, Nguyen and colleagues, associated atypical EPEC with prolonged diarrhea (35), as well as in Mexico, aEPEC was the only pathotype of *E. coli* associated with acute diarrhea episodes lasting 7 to 12 days (34). The potential zoonotic transmission (from domestic and wild animals) has been suggested (36), and human cases have been linked to aEPEC strains from cattle and dogs (37, 38).

Shiga Toxin- producing *E. coli* (STEC/VTEC and EHEC). STEC are the most frequent *E. coli* organisms associated with acute diarrhea and increased risk of death in infants aged 0–11 months in developed countries (32). In 2013, the EU notification rate was 1.59 cases per 100,000 population and 13 deaths due to VTEC infection were reported (26). VTEC serogroup O157:H7 was primarily detected in ruminants (cattle, sheep and goats) and meat, followed by vegetables, juices and cheese (26). In 2013, the USA notification rate of *E. coli* O157:H7 was 1.15 per 100,000 population. In addition, CDC estimates that for every *E. coli* O157 case reported, there are 26 cases that go undiagnosed (31).

Non-Typhi *Salmonella*. There is an estimated of 1.3 billion cases of non-typhoid salmonellosis worldwide each year (4). In 2013, the EU notification rate was 20.4 cases per

100,000 population and 59 fatal cases were reported (26). *Salmonella* was frequently detected in poultry meat, and less often in pork or beef. However, the most important source of *Salmonella* spp. outbreaks was eggs and egg products (26). In 2013, the USA rate was 15.19 per 100,000 and the CDC estimates that for every *Salmonella* spp. infection reported, there are 29 cases that are not diagnosed (31). In sub-Saharan Africa, non-Typhi *Salmonella* is estimated to be responsible for 120 cases per 100,000 person-years, and comprises one of the three leading causes of bacteremia in adults and children (39).

Yersinia enterocolitica and *Y. pseudotuberculosis.* The EU notification rate of yersiniosis was 1.92 cases per 100,000 population in 2013, and 2 fatal cases due to infections with *Y. pseudotuberculosis* (26). America, Asia and Africa also report outbreaks (40). In 2013, the USA rate was 0.36 per 100,000 and the CDC estimates that for every *Yersinia* case reported, there are 123 cases that are not diagnosed (31). The most important source for outbreaks were pork, beef and unpasteurized cow milk. The principal reservoirs of *Y. enterocolitica* are usually pigs. While *Y. pseudotuberculosis* is often associated with rodents, lagomorphs (e.g., rabbits) and birds (26). Both *Yersinia* species could be present in wildlife animals, cattle, sheep, goats, dogs, cats, solipeds and others (26).

Giardia spp. *Giardia* is considered the most common parasitic infection in humans worldwide. It contributes to an estimated 280 million symptomatic human infections per year (41) and has been included as part of the WHO Neglected Disease Initiative since 2004 (42). It is estimated that the prevalence of giardiasis in temperate climates is 2-10% in adults and 25% in children whereas in tropical countries 50-80% of people are carriers (43). CDC reports approximately 20,000 cases per year in the USA (31). Drinking untreated

water is a common source of infection and can result in community wide epidemics (44). Mammals, such as dogs, cats, cattle, sheep, pigs, rodents, beavers and bears may carry these parasites (43).

Cryptosporidium spp. Cryptosporidiosis prevalence varies based on climate and level of development, accounting for an estimated 0.1-2% of diarrheal illness in cooler and developed areas and 5-10% in warmer and developing countries (45). *Cryptosporidium* spp. is associated with morbidity and mortality in young children in LMICs (32, 46).Outbreaks have been associated with contaminated food, drinking and recreational water. There are 26 valid species of *Cryptosporidium* in mammals, fish, amphibians, reptiles, and birds, 20 of them were reported in humans (47).

In Ecuador, diarrhea and gastroenteritis of infectious origin is the second leading cause of morbidity (48). Diarrhea is the eleventh cause of death in children less than 5 years old (49), and has been shown to impact the development of children who experience chronic infections (50-52). In 2012, a case-control study carried out in low-income communities of Ecuador, determined the presence of enteric pathogens in both cases and controls; nevertheless, co-infections seemed to be more symptomatic than single infection especially between Rotavirus and *E. coli* Shigellae with *Giardia* (46). *Campylobacter* were present in a high proportion of cases and controls, while non-Typhi *Salmonella* and enteropathogenic *Escherichia coli* were absent (46).

Campylobacter

Campylobacter spp. belongs to the epsilon class of proteobacteria, in the order *Campylobacter*iales; this order includes two other genera, *Helicobacter* and *Wolinella*. To date there are 34 species of *Campylobacter* and 14 subspecies (53). The most common

species recovered from human diarrhea cases are *C. jejuni* and *C. coli* (30). Emerging *Campylobacter* species recovered from sporadic human cases are *concisus, ureolyticus, upsaliensis* and *hyointestinalis* (30).

This motile, Gram-negative, non-spore forming, S-shaped, microaerophilic organisms have a fastidious nature. Both *C. jejuni* and *C. coli* are thermophilic, and the laboratory growth conditions include microaerophilic (O_2 3-15% and, CO_2 3-5%), 42ºC incubation and usually cephalosporin to reduce contaminating flora (54).

Campylobacter spp. are sensitive to many environmental stresses including: desiccation, heat, ultra-violet radiation, atmospheric oxygen and high salinity. However, it has been shown to survive in moist conditions for prolonged periods, between 3 to 10 months (55, 56). Free-living protozoa may assist transmission and survival of *Campylobacter*, through similar mechanisms of bacterial survival within macrophages (57, 58).

Campylobacter spp. are naturally competent, and horizontal gene transference (HGT) events occur at twice the rate of de novo mutation (59). The index of recombination or point mutation (r/m) of *C. jejuni* is ~47, similar to *Streptococcus pneumoniae* (~50 r/m) or *Neisseria meningitidis* (100 r/m) (60). Since *C. jejuni* and *C. coli* have large population sizes and high rates of recombination have the potential to evolve rapidly, by maintaining large amounts of information (genes) (61). Horizontal gene transfer enable the acquisition of antibiotic resistance genes and the ability to colonize multiple hosts (62).

C. jejuni do not have homologues of the many *E. coli* DNA-repair genes and shows hypervariable sequences that consist of homopolymeric tracts (63). These tracts are usually found in regions that encode proteins implicated in the biosynthesis or

modification of surface-accessible carbohydrate structures, such as the capsule, lipooligosaccharide (LOS) and flagellum (63). These structures allow for phase variation, by means of changes in reading frames of genes, which are important for host adaptation (64). Some genes required for transformation, invasion and pathogenicity are encoded by the plasmid *pVir*, for instance: type IV secretion system, *N*-linked glycosylation, LOS biosynthesis and a DNA-processing enzyme homologue of *H. pylori* DprA (65).

In developed countries, *C. jejuni/coli* infections can cause acute enteritis with bloody diarrhea, mucus and abdominal pain lasting for 7 days or more. Although such infections are generally self-limiting, complications can arise and may include bacteremia, Guillain–Barré syndrome (1 in every 1,000 cases), Miller–Fisher syndrome reactive arthritis, and abortion (30, 54). In addition, this species has been associated with immunoproliferative small intestinal disease (30). In LMICs, children are more likely to experience symptomatic cases and may present with watery diarrhea. Possibly, early infection may have a role in providing natural immunity, protecting against adult infections (30, 66).

Even antimicrobials are infrequently prescribed for Campylobacteriosis, there is a high rate of resistance to fluoroquinolones and tetracyclines worldwide (67); however, resistance to erythromycin and gentamicin has been reported to be low (67).

The primary source of *C. jejuni/coli* infections is handling and/or consumption of contaminated meat, especially poultry (26). Neverthless, contact with pets and livestock, the consumption of contaminated water or raw milk and travelling in high prevalence areas are also considered risks factors (26, 30).

In order to understand the sources of infection in epidemiological studies it is crucial to characterize *Campylobacter*. The high phenotypic and genotypic variation of *Campylobacter* spp. make typing them more complex. Newer methods for typing *Campylobacter* spp. have been developed, since serotyping and phage typing. *Fla*-typing, ribotyping, Pulsed Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP), Repetitive Extragenic Palindromic Polymerase Chain Reaction (REP-PCR), Random Amplified Polymorphic DNA (RAPD) and Clustered Regularly Interspaced Short Palindromic Repeat typing (CRISPR) are all methods that have allowed the differentiation and phylogenetic analysis among isolates (68-70).

In 2001, Dingle and colleagues developed a multilocus sequence typing (MLST) for *Campylobacter jejuni* (71). This method is the most accurate and preferred system for studying the relationships between *Campylobacter* strains. Together MLST and whole genome sequencing (WGS) have been instructive for understanding the epidemiology and evolution of *Campylobacter*, however WGS is not yet accessible for many laboratories (72).

MLST allows for the characterization of genetic variation at several chromosomal *loci*-encoding housekeeping genes through the analysis of their sequences (~400 bp) (59, 73). Generally, seven *loci* are used (corresponding to ~3300 bp or ~0.2% of the *C. jejuni* genome of 1.6–2.0 megabases) (71). An arbitrary allele number is assigned to each unique sequence of each locus. Allelic profiles or "sequence types" (STs) comprise the unique combinations of these allelic variants and are assigned unique arbitrary numbers too (73). This system uses a web-accessible database (http://pubmlst.org/*Campylobacter*/), where the allele and ST numbers are assigned, and epidemiological information worldwide is available (74). STs are grouped into "clonal complexes" that can be pragmatically defined

as groups of isolates with STs that share identical alleles at four or more MLST *loci* with a definable "central genotype" (75, 76). WGS have confirmed the common ancestral relationship of members of clonal complexes and, therefore, frequently also share phenotypic properties. Interestingly, analyses of *Campylobacter* MLST alleles and STs have shown that the large number of STs (genotypes) are mostly generated by the reassortment of existing alleles by continuous HGT and not by progressive mutation (59).

Atypical Enteropathogenic *Escherichia coli* (aEPEC)

Atypical *Escherichia coli* may or may not belong to the classical EPEC serogroups and produce the histopathologic lesion known as attaching and effacing (A/E) on intestinal cells by the adherence factor plasmid (pEAF), but do not express the bundle-forming pilus (BFP) and lack Shiga-toxin genes (77, 78).

The genes necessary for the establishment of A/E lesions are located on the LEE pathogenicity island (PAI) (79). LEE encodes type III secretion system (T3SS), regulators, translocators, chaperones, effector molecules that alter diverse cell signaling processes, membrane adhesive protein intimin and its translocated receptor Tir (translocated intimin receptor) (80, 81). EPEC strains have variable location on the LEE, possibly due to the acquisition of LEE from different ancestors at various times, or by LEE mobilization and reintegration within individual *E. coli* strains (82).

Atypical EPEC have been typed through phenotyping and genotyping methods (78). Commonly Polymerase Chain Reaction (PCR) is used to detect pEAF (EAF probe) and/or *eae* (or other conserved LEE-genes) and to confirm the absence of *bfpA* (encoding the major pilin subunit of BFP), and *stx* (Shiga-like toxins) genes (78). To further confirm the potential

pathogenicity of an aEPEC strain, it is necessary to demonstrate its ability to produce A/ E lesions on epithelial cells.

In 2008, Afset and colleagues serotyped and analyzed aEPEC by MLST and pulsed-field gel electrophoresis (PFGE) (83). These authors concluded that aEPEC strains are heterogeneous both phylogenetically and by virulence profile. In general, aEPEC strains may carry genes encoding virulence factors of other pathotypes more often than tEPEC strains (77). Interesting genetic combinations were observed, as extraintestinal pathogenic *E. coli*, EAEC, tEPEC (bfpA) and EHEC (ehly) genes (84). Indeed, some aEPEC strains showed a much closer relationship to EHEC strains than to tEPEC strains (85, 86).

The epidemiological association of aEPEC with diarrhea is still controversial. The high prevalence worldwide and the involvement of some strains with diarrheal outbreaks support the concept that some aEPEC strains with certain genetic combinations are diarrheagenic (87). Some studies related aEPEC with persistent diarrhea presumably because they can act as invasive organisms avoiding the immune system and some antibiotics (35). A case-control study in Iranian children found differences between atypical EPEC serotypes from children with diarrhea (88). While, other studies showed no significant differences among aEPEC strains isolated from patients and healthy controls (89).

There is no clear evidence of direct transmission of aEPEC from animals to humans. Serotyping analyses found that aEPEC serogroups that were identified in animals, were implicated in human diseases, suggesting their role as reservoirs (78). Furthermore, virulence markers and clonal similarity by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) of aEPEC of different serotypes isolated from humans,

domestic and wild animals showed that atypical EPEC strains isolated from animals have the potential to cause diarrhea in humans and have a close clonal relationship with human isolates (36). In 2013, Wang et al. studied the role of domestic animals as the source of atypical enteropathogenic *E. coli* (aEPEC) classifying them by phylogroup, virulence profile, and intimin typing. They found strains from diarrheal patients similar to bovine strains, while the aEPEC strains in healthy humans were different, and some of these were present in porcine samples (37). A study of aEPEC from dogs (with and without diarrhea) showed that phenotypic and genotypic markers of aEPEC were similar to those found in isolates recovered from human disease (38).

As previously mentioned, the characterization of *E. coli* using various methods has provided relevant phylogenetic and/or epidemiological information (86). Furthermore, MLST is a useful tool in epidemiological studies of *E. coli*, nevertheless it is important to take in account temporal and geographic scales (90). Three *E. coli* MLST schemes can be used. The most widely used scheme is Mark Achtman's scheme, which uses 7 housekeeping genes (91). The other two schemes are EcMLST for pathogenic *E. coli* (92) and MLST scheme developed at Institute Pasteur (93), analyzing 15 and 8 genes respectively. High-resolution in genomic epidemiology can be reached with the whole genome sequence (WGS), but it remains expensive and limited for use in research.

Justification

Given the strong association between zoonoses, animal husbandry, poverty and malnutrition in developing countries (7), their control and prevention should be a public health priority. Ecuador possesses the highest population density in South America, with

56.5 inhabitants per square kilometer, and the rural population is estimated at 38% (94). In rural and semi-rural communities, commercial and backyard animal productions have been intensified due to increased demand for animal products. The geographic proximity as well as commercial and social interactions between rural and urban areas may result in the spread of zoonotic pathogens. In Ecuador, there are little information about zoonotic intestinal pathogens. It is important to determine which pathogens are circulating between animals and humans in specific space and time scales in order to understand their dynamics and transmission. Zoonotic enteric pathogens are both preventable and treatable; however, it requires multidisciplinary collaboration embodied in the 'One Health' initiative, involving human, animal and environmental health.

Although mortality and morbidity from acute diarrhea are diminishing in Ecuador, under-diagnosis of disease and the presence of asymptomatic carriers are common (46, 48). It is essential to identify the chief sources of zoonotic diseases to more effectively prevent chronic infections that can affect the development of children.

Objectives

In this study, we determined the prevalence of zoonotic enteropathogens in children and animals in a semi-rural community of Ecuador, characterized by their proximity to poultry farms and agricultural activities, mainly strawberry production. Traditional and molecular microbiology techniques and immunological tests were used to analyze fecal samples for *Campylobacter* spp., non-Typhi *Salmonella*, Atypical Enteropathogenic *Escherichia coli* (aEPEC), Shiga Toxin-producing *E. coli* (STEC/VTEC), *Yersinia enterocolitica, Giardia lamblia* and *Cryptosporidium* sp.

In order to characterize the transmission of enteric pathogens between animals and humans, Multilocus Sequence Typing (MLST) was carried out for the most prevalent pathogens found in the study area: *Campylobacter jejuni* and Atypical Enteropathogenic *E. coli*. Additionally, mapping analyses were performed to characterize the distribution of animal and human pathogens.

References

1. Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, Aldrich S, Harrington T, Formenty P, Loh EH, Machalaba CC, Thomas MJ, Heymann DL. 2012. Ecology of zoonoses: natural and unnatural histories. Lancet 380:1936-1945.
2. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. 2008. Global trends in emerging infectious diseases. Nature 451:990-993.
3. World Heatlh Organization. 2011. The control of neglected zoonotic diseases: community based interventions for NZDs prevention and control. Geneva, Switzerland: Author. Retrieved from http://apps.who.int/iris/bitstream/10665/44746/1/9789241502528_eng.pdf Accessed 20 October 2015.
4. World Heatlh Organization. 2015. World Health Statistics. Luxembourg: Author. Retrieved from http://apps.who.int/iris/bitstream/10665/170250/1/9789240694439_eng.pdf?ua=1. Accessed 20 October 2015.
5. Spickler AR, Roth JA, Gaylon J, Lofstedt J (eds). 2010. Emerging and exotic diseases of animals (4th edition). Ames IA: CFSPH Iowa State University.
6. Zambrano LD, Levy K, Menezes NP, Freeman MC. 2014. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg 108:313-325.
7. Grace D, Mutua F, Ochungo P, Kruska R, Jones K, Brierley L, Lapar L, Said M, Herrero M, Phuc P, Thao N, Akuky I, Ogutu F. 2012. Mapping of poverty and likely zoonoses hotspots. Geneva, Switzerland: Author. Retrieved from http://hdl.handle.net/10568/21161 Accessed 20 October 2015.

8. **Mackenzie JS, Jeggo M, Daszak P, Richt JA (eds).** 2013. One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases. The Concept and Examples of a One Health Approach. Springer Berlin Heidelberg. doi:10.1007/978-3-642-36889-9.

9. **World Health Organization.** 2013. Fact sheet on Diarrhoeal disease Nº330. http://www.who.int/mediacentre/factsheets/fs330/en/. Accessed 20 October 2015.

10. **Kosek M, Bern C, Guerrant RL.** 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bulletin of the World Health Organization **81:**197-204.

11. **Cohen J, Opal SM, Powderly WG.** 2010. Infectious Diseases (3rd edition). UK: Mosby/Elsevier.

12. **Scipioni A, Mauroy A, Vinje J, Thiry E.** 2008. Animal noroviruses. Vet J **178:**32-45.

13. **Kobayashi N, Ishino M, Wang Y, Chawla-Sarkar M, Krishnan T, Naik T.** 2007. Diversity of G-type and P-type of human and animal rotaviruses and its genetic background. Communicating Curr Res Educ Top Trends Appl Microbiol**:**847-858.

14. **Rajendran P, Kang G.** 2014. Molecular epidemiology of rotavirus in children and animals and characterization of an unusual G10P[15] strain associated with bovine diarrhea in south India. Vaccine **32 Suppl 1:**A89-94.

15. **Schlundt J, Toyofuku H, Jansen J, Herbst SA.** 2004. Emerging food-borne zoonoses. Rev Sci Tech **23:**513-533.

16. Iaquinto G, Marano A, Perna A, Rotondi Aufiero V, Mazzarella G, De Luca A. 2015. Mechanisms of Alteration of Epithelial Cell by Associated Adherent-Invasive *E. coli*. The FASEB Journal **29**.

17. Sears CL, Kaper JB. 1996. Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. Microbiol Rev **60:**167-215.

18. Andersen YS, Gillin FD, Eckmann L. 2006. Adaptive immunity-dependent intestinal hypermotility contributes to host defense against *Giardia* spp. Infect Immun **74:**2473-2476.

19. Hanevik K, Dizdar V, Langeland N, Hausken T. 2009. Development of functional gastrointestinal disorders after *Giardia lamblia* infection. BMC Gastroenterol **9:**27.

20. Ankarklev J, Jerlstrom-Hultqvist J, Ringqvist E, Troell K, Svard SG. 2010. Behind the smile: cell biology and disease mechanisms of *Giardia* species. Nat Rev Microbiol **8:**413-422.

21. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. 2013. *Cryptosporidium* pathogenicity and virulence. Clin Microbiol Rev **26:**115-134.

22. Caccio SM, Thompson RC, McLauchlin J, Smith HV. 2005. Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol **21:**430-437.

23. Xiao L, Feng Y. 2008. Zoonotic cryptosporidiosis. FEMS Immunol Med Microbiol **52:**309-323.

24. Ludington JG, Ward HD. 2015. Systemic and Mucosal Immune Responses to *Cryptosporidium*—Vaccine Development. Current tropical medicine reports **2:**171-180.

25. Woolhouse MEJ, Gowtage-Sequeria S. 2005.Host range and emerging and reemerging pathogens. Emerg Infect Dis 11(12):1842. http://doi.org/10.3201/eid1112.050997

26. European Food Safety Authority, European Center for Disease Prevention and Control. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal 13(1):3991 [165 pp.]. doi: 10.2903/j.efsa.2015.3991

27. Lal A, Hales S, French N, Baker MG. 2012. Seasonality in human zoonotic enteric diseases: a systematic review. PLoS One 7:e31883.

28. Abdel-Haq NM, Asmar BI, Abuhammour WM, Brown WJ. 2000. *Yersinia enterocolitica* infection in children. Pediatr Infect Dis J 19:954-958.

29. Long C, Jones TF, Vugia DJ, Scheftel J, Strockbine N, Ryan P, Shiferaw B, Tauxe RV, Gould LH. 2010. *Yersinia pseudotuberculosis* and *Y. enterocolitica* infections, FoodNet, 1996-2007. Emerg Infect Dis 16:566-567.

30. Kaakoush NO, Castano-Rodriguez N, Mitchell HM, Man SM. 2015. Global Epidemiology of *Campylobacter* Infection. Clin Microbiol Rev 28:687-720.

31. Centers for Disease Control and Prevention. 2014. Estimates of Foodborne Illness in the United States. Atlanta. Retrieved from http://www.cdc.gov/foodborneburden/. Accessed 20 October 2015.

32. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio

M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acacio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet **382:**209-222.

33. Araujo JM, Tabarelli GF, Aranda KR, Fabbricotti SH, Fagundes-Neto U, Mendes CM, Scaletsky IC. 2007. Typical enteroaggregative and atypical enteropathogenic types of *Escherichia coli* are the most prevalent diarrhea-associated pathotypes among Brazilian children. Journal of clinical microbiology **45:**3396-3399.

34. Estrada-Garcia T, Lopez-Saucedo C, Thompson-Bonilla R, Abonce M, Lopez-Hernandez D, Santos JI, Rosado JL, DuPont HL, Long KZ. 2009. Association of diarrheagenic *Escherichia coli* pathotypes with infection and diarrhea among Mexican children and association of atypical enteropathogenic *E. coli* with acute diarrhea. Journal of clinical microbiology **47:**93-98.

35. Nguyen RN, Taylor LS, Tauschek M, Robins-Browne RM. 2006. Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. Emerg Infect Dis **12:**597-603.

36. Moura RA, Sircili MP, Leomil L, Matte MH, Trabulsi LR, Elias WP, Irino K, Pestana de Castro AF. 2009. Clonal relationship among atypical enteropathogenic *Escherichia coli* strains isolated from different animal species and humans. Appl Environ Microbiol **75:**7399-7408.

37. Wang L, Wakushima M, Aota T, Yoshida Y, Kita T, Maehara T, Ogasawara J, Choi C, Kamata Y, Hara-Kudo Y, Nishikawa Y. 2013. Specific properties of enteropathogenic

Escherichia coli isolates from diarrheal patients and comparison to strains from foods and fecal specimens from cattle, swine, and healthy carriers in Osaka City, Japan. Appl Environ Microbiol **79:**1232-1240.

38. **de Almeida PM, Arais LR, Andrade JR, Prado EH, Irino K, Cerqueira Ade M.** 2012. Characterization of atypical Enteropathogenic *Escherichia coli* (aEPEC) isolated from dogs. Vet Microbiol **158:**420-424.

39. **Morpeth SC, Ramadhani HO, Crump JA.** 2009. Invasive non-Typhi *Salmonella* disease in Africa. Clin Infect Dis **49:**606-611.

40. **Berger S.** 2015. Yersiniosis: Global Status. California: GIDEON Informatics Inc.

41. **Lane S, Lloyd D.** 2002. Current trends in research into the waterborne parasite *Giardia*. Critical reviews in microbiology **28:**123-147.

42. **World Health Organization.** 2010. The control of neglected zoonotic diseases: Community-based interventions. Geneva: Author. Retrieved from http://apps.who.int/iris/bitstream/10665/44746/1/9789241502528_eng.pdf Accessed 20 October 2015.

43. **Krauss H, Weber A, Appel M, Enders B, Isenberg HD, Schiefer HG, Slenczka W, von Graevenitz A, Zahner H.** 2003. Parasitic Zoonoses, Zoonoses doi:doi:10.1128/9781555817787.ch4. American Society of Microbiology.

44. **Huang DB, White AC.** 2006. An updated review on *Cryptosporidium* and *Giardia*. Gastroenterol Clin North Am **35:**291-314, viii.

45. **Checkley W, White AC, Jr., Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD, Kotloff KL, Kang G, Mead JR, Miller M, Petri WA, Jr., Priest JW, Roos DS, Striepen B, Thompson RC, Ward HD, Van**

Voorhis WA, Xiao L, Zhu G, Houpt ER. 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for C*ryptosporidium*. Lancet Infect Dis 15:85-94.

46. Vasco G, Trueba G, Atherton R, Calvopina M, Cevallos W, Andrade T, Eguiguren M, Eisenberg JN. 2014. Identifying etiological agents causing diarrhea in low income Ecuadorian communities. Am J Trop Med Hyg 91:563-569.

47. Ryan U, Fayer R, Xiao L. 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141:1667-1685.

48. Ministerio de Salud Pública, Instituto Nacional de Estadísticas y Censos, Organización Panamericana de la Salud. 2010. Indicadores Básicos de Salud Ecuador 2010. Retrieved from: http://www.paho.org/ecu/index.php?option=com_content&view=article&id=25:situacion-salud. Accessed 20 October 2015.

49. Instituto Nacional de Estadísticas y Censos. 2013. Anuario de Nacimientos y Defunciones. Retrieved from: http://www.ecuadorencifras.gob.ec/documentos/web-inec/Poblacion_y_Demografia/Nacimientos_Defunciones/Publicaciones/Anuario_Nacimientos_y_Defunciones_2013.pdf. Accessed 20 October 2015.

50. Lee G, Pan W, Penataro Yori P, Paredes Olortegui M, Tilley D, Gregory M, Oberhelman R, Burga R, Chavez CB, Kosek M. 2013. Symptomatic and asymptomatic *Campylobacter* infections associated with reduced growth in Peruvian children. PLoS Negl Trop Dis 7:e2036.

51. Checkley W, Gilman RH, Epstein LD, Suarez M, Diaz JF, Cabrera L, Black RE, Sterling CR. 1997. Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. American journal of epidemiology 145:156-163.

52. Guerrant RL, Oria RB, Moore SR, Oria MO, Lima AA. 2008. Malnutrition as an enteric infectious disease with long-term effects on child development. Nutr Rev 66:487-505.

53. Euzéby J, Parte A. 2015. List of prokaryotic names with standing in nomenclature. Retrieved from: http://www.bacterio.net/*Campylobacter*.html. Accessed 20 October 2015.

54. Wagenaar JA, Newell DG, Kalupahana RS, Mughini-Gras L. 2015. *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control, p 159-177. *In* Sing A (ed), Zoonoses - Infections Affecting Humans and Animals doi:10.1007/978-94-017-9457-2_6. Springer Netherlands.

55. Nicholson FA, Groves SJ, Chambers BJ. 2005. Pathogen survival during livestock manure storage and following land application. Bioresour Technol 96:135-143.

56. Inglis GD, McAllister TA, Larney FJ, Topp E. 2010. Prolonged survival of *Campylobacter* species in bovine manure compost. Appl Environ Microbiol 76:1110-1119.

57. Vieira A, Seddon AM, Karlyshev AV. 2015. *Campylobacter-Acanthamoeba* interactions. Microbiology 161:933-947.

58. Snelling WJ, McKenna JP, Lecky DM, Dooley JS. 2005. Survival of *Campylobacter jejuni* in waterborne protozoa. Appl Environ Microbiol 71:5560-5571.

59. Sheppard SK, Maiden MC. 2015. The Evolution of *Campylobacter jejuni* and *Campylobacter coli*. Cold Spring Harb Perspect Biol 7:a018119.

60. Hensel M, Schmidt H. 2008. Horizontal gene transfer in the evolution of pathogenesis, vol 16. Cambridge University Press.

61. Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Hart CA, Diggle PJ, Fearnhead P. 2009. Rapid evolution and the importance of recombination to the gastroenteric pathogen *Campylobacter jejuni*. Mol Biol Evol 26:385-397.

62. Sheppard SK, Cheng L, Méric G, Haan C, Llarena AK, Marttinen P, Vidal A, Ridley A, Clifton-Hadley F, Connor TR. 2014. Cryptic ecology among host generalist *Campylobacter jejuni* in domestic animals. Molecular ecology 23:2442-2451.

63. Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S, Barrell BG. 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 403:665-668.

64. Bayliss CD, Bidmos FA, Anjum A, Manchev VT, Richards RL, Grossier J-P, Wooldridge KG, Ketley JM, Barrow PA, Jones MA. 2012. Phase variable genes of *Campylobacter jejuni* exhibit high mutation rates and specific mutational patterns but mutability is not the major determinant of population structure during host colonization. Nucleic acids research:gks246.

65. Young KT, Davis LM, Dirita VJ. 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol 5:665-679.

66. Havelaar AH, van Pelt W, Ang CW, Wagenaar JA, van Putten JP, Gross U, Newell DG. 2009. Immunity to *Campylobacter*: its role in risk assessment and epidemiology. Crit Rev Microbiol **35:**1-22.

67. Ge B, Wang F, Sjolund-Karlsson M, McDermott PF. 2013. Antimicrobial resistance in *Campylobacter*: susceptibility testing methods and resistance trends. J Microbiol Methods **95:**57-67.

68. Schouls LM, Reulen S, Duim B, Wagenaar JA, Willems RJ, Dingle KE, Colles FM, Van Embden JD. 2003. Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. J Clin Microbiol **41:**15-26.

69. Nachamkin I, Bohachick K, Patton CM. 1993. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. J Clin Microbiol **31:**1531-1536.

70. Gibson JR, Fitzgerald C, Owen RJ. 1995. Comparison of PFGE, ribotyping and phage-typing in the epidemiological analysis of *Campylobacter jejuni* serotype HS2 infections. Epidemiol Infect **115:**215-225.

71. Dingle K, Colles F, Wareing D, Ure R, Fox A, Bolton F, Bootsma H, Willems R, Urwin R, Maiden M. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. Journal of clinical microbiology **39:**14-23.

72. Didelot X, Bowden R, Wilson DJ, Peto TE, Crook DW. 2012. Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet **13:**601-612.

73. Maiden MC. 2006. Multilocus sequence typing of bacteria. Annu Rev Microbiol 60:561-588.

74. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA. 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Sciences 95:3140-3145.

75. Dingle KE, McCarthy ND, Cody AJ, Peto TE, Maiden MC. 2008. Extended sequence typing of *Campylobacter* spp., United Kingdom. Emerg Infect Dis 14:1620-1622.

76. Colles FM, Maiden MC. 2012. *Campylobacter* sequence typing databases: applications and future prospects. Microbiology 158:2695-2709.

77. Trabulsi LR, Keller R, Tardelli Gomes TA. 2002. Typical and atypical enteropathogenic *Escherichia coli*. Emerg Infect Dis 8:508-513.

78. Hernandes RT, Elias WP, Vieira MA, Gomes TA. 2009. An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiol Lett 297:137-149.

79. McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proceedings of the National Academy of Sciences 92:1664-1668.

80. Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB. 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell 91:511-520.

81. Garmendia J, Frankel G, Crepin VF. 2005. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. Infect Immun 73:2573-2585.

82. Donnenberg MS, Whittam TS. 2001. Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic *Escherichia coli*. J Clin Invest **107:**539-548.

83. Afset JE, Anderssen E, Bruant G, Harel J, Wieler L, Bergh K. 2008. Phylogenetic backgrounds and virulence profiles of atypical enteropathogenic *Escherichia coli* strains from a case-control study using multilocus sequence typing and DNA microarray analysis. Journal of clinical microbiology **46:**2280-2290.

84. Bando SY, Andrade FB, Guth BE, Elias WP, Moreira-Filho CA, Pestana de Castro AF. 2009. Atypical enteropathogenic *Escherichia coli* genomic background allows the acquisition of non-EPEC virulence factors. FEMS Microbiol Lett **299:**22-30.

85. Moreira FC, Vieira MA, Ferreira AJ, Girao DM, Vaz TM, Rosa AC, Knobl T, Irino K, Freymuller E, Gomes TA. 2008. *Escherichia coli* strains of serotype O51:H40 comprise typical and atypical enteropathogenic *E. coli* strains and are potentially diarrheagenic. J Clin Microbiol **46:**1462-1465.

86. Bielaszewska M, Middendorf B, Kock R, Friedrich AW, Fruth A, Karch H, Schmidt MA, Mellmann A. 2008. Shiga toxin-negative attaching and effacing *Escherichia coli*: distinct clinical associations with bacterial phylogeny and virulence traits and inferred in-host pathogen evolution. Clin Infect Dis **47:**208-217.

87. Mohammadzadeh M, Oloomi M, Bouzari S. 2013. Genetic evaluation of Locus of enterocyte effacement pathogenicity island (LEE) in Enteropathogenic *Escherichia coli* isolates (EPEC). Iran J Microbiol **5:**345-349.

88. **Alikhani MY, Mirsalehian A, Aslani MM.** 2006. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. J Med Microbiol **55:**1159-1163.

89. **Jenkins C, Tembo M, Chart H, Cheasty T, Willshaw GA, Phillips AD, Tompkins D, Smith H.** 2006. Detection of enteroaggregative *Escherichia coli* in faecal samples from patients in the community with diarrhoea. J Med Microbiol **55:**1493-1497.

90. **Sabat AJ, Budimir A, Nashev D, Sa-Leao R, van Dijl J, Laurent F, Grundmann H, Friedrich AW, Markers ESGoE.** 2013. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill **18:**20380.

91. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol **60:**1136-1151.

92. **Weihong Q, Lacher DW, Bumbaugh AC, Hyma KE, Ouellette LM, Large TM, Tarr CL, Whittam TS.** EcMLST: an online database for multi locus sequence typing of pathogenic *Escherichia coli*, p 520-521. *In* (ed). Retrieved from http://shigatox.net/ecmlst/EcMLST.pdf Accessed 20 October 2015.

93. **Jaureguy F, Landraud L, Passet V, Diancourt L, Frapy E, Guigon G, Carbonnelle E, Lortholary O, Clermont O, Denamur E, Picard B, Nassif X, Brisse S.** 2008. Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains. BMC Genomics **9:**560.

94. **Instituto Nacional de Estadísticas y Censos.** 2010. Censo de Población y Vivienda 2010. Retrieved from http://www.ecuadorencifras.gob.ec/ Accessed 20 October 2015.

PART II

SCIENTIFIC PAPER

"Zoonotic transmission of *Campylobacter jejuni* and Atypical Enteropathogenic *Escherichia coli* in peri-urban Quito, Ecuador".

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Introduction

Diarrheal diseases are the major cause of death and disability in low- and middle-income countries (LMICs), especially in rural areas where children are in high risk of severe diarrhea due to poverty, malnutrition, lack of sanitary infrastructure and contact with animal carriers (1). Zoonotic enteropathogens are often overlooked, however, the contribution of different zoonotic pathogens to diarrheal disease is significant (2), although, their detection may be hindered by patterns of seasonality (2, 3).

Among zoonotic enteropathogens *Salmonella* spp. and enterohemorrhagic *Escherichia coli* have reached more notoriety probably due to large outbreaks or disease severity (4, 5). *Campylobacter* spp., however, are the most frequent gastrointestinal bacterial pathogens in humans (90%) and their prevalence is increasing dramatically worldwide (6, 7). Campylobacteriosis is mainly associated with the ingestion of chicken meat, beef, eggs, water and milk or contact with domestic animals (5). Infections are generally self-limiting, although complications can arise and may include bacteremia, Guillain–Barré and Miller–Fisher syndromes, reactive arthritis and immunoproliferative small intestinal disease (6, 7). Furthermore, an emerging potentially zoonotic enteric pathogen is atypical enteropathogenic *Escherichia coli* (aEPEC), which causes attaching and effacing (A/E) lesions in enterocytes (8-10). aEPEC are prevalent in both developed and developing countries, but its pathogenicity and zoonotic potential are still unclear (11-13).

We investigated the prevalence of seven zoonotic enteropathogens (bacteria and protozoa) in children and domestic animals in a three-month longitudinal study in a semi-rural community of Ecuador. In addition, Multilocus Sequence Typing (MLST) was used to

assess the zoonotic transmission of *Campylobacter jejuni* and aEPEC occurring in this region.

Materials and methods

Study location. The study was conducted in Otón de Vélez-Yaruquí, a low-income semi-rural community east of Quito, at an altitude of 2,527 meters 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).

Ethical considerations. The study protocol was approved by the Institutional Animal Care and Use Committee at the George Washington University (IACUC#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#101355).

Sample collection. Sixty-five households were recruited randomly, between June to August 2014 during the dry season. Fifty-nine of them had domestic animals (1 to 8 animal species), 3 did not have animals, and 3 households did not provide samples. We collected 64 stool samples from children (47% female and 53% male; ages: 11 of 3-12 months old, 31 of 1-3 years old, 18 of 3-5 years old and 4 of 6 years old) (Table S7), and 203 samples from 12 animal species (Table 1; Fig. S1). The stool samples were placed in a cooler for transportation to the laboratory. All bacteria culturing and sample preservation began less than 8 hours 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 *Escherichia coli* were obtained by culturing samples on MacConkey Lactose agar (Difco, Sparks, Maryland) (at 37°C for 18 h), lactose fermenting colonies were plated in Chromocult® Coliform agar (Merck KGaA, Darmstadt, Germany) to identify the ß-D-glucuronidase activity. The DNA from *E. coli* isolates was amplified by polymerase chain reaction (PCR) to identify *eae* and *bfpA* genes for aEPEC (14, 15), and *stx-1* and *stx-2* for STEC, as previously described (16) (Table S1). To recover *Salmonella* spp., samples were pre-enriched in Selenite Broth (at 37°C for 18 h) and cultured in Xylose-lysine-deoxycholate agar (Difco, Sparks, Maryland) (at 37°C for 18 h). Suggestive colonies were subjected to RapiD-20E tests (bio Merieux, Marcy l'Etolie, France). To isolate *Yersinia* spp. the samples were pre-enriched in PBS 1X by 21 days at 4°C and cultured in Cefsulodin Irgasan Novobiocin agar (at 28°C for 24 and 48 hours) (Oxoid Ltd, Basingstoke, Hampshire, England). Suggestive colonies were confirmed with oxidase (Bactident Oxidase, Merck) and RapiD-20E tests.

To investigate thermophilic *Campylobacter* spp., samples were cultured 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 hours in 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/coli* were confirmed by PCR of hippuricase and aspartokinase genes according to the protocol developed by Persson & Olsen in 2005 (17). *Campylobacter* species not belonging to *C. jejuni/coli* were identified through 16S RNAr

gene sequencing in FunctionalBiosciences (Madison, WI) (http://functionalbio.com/web), and sequences were uploaded to genBank.

Giardia lamblia and *Cryptosporidium parvum* were detected using Enzyme-Linked Immunosorbent Assay (Ridascreen®Giardia, Ridascreen®Cryptosporidium, r-Biopharm, Darmstadt, Germany).

Water samples and analyses for *Campylobacter*. Seven water samples were collected from irrigation channels that transect the study area; the samples were collected from different points equally distributed across the community. Five hundred ml of each sample was filtered in 0.45 µm pore–size nitrocellulose membrane, in duplicate. The membrane was placed on Campylobacter Agar (Oxoid Ltd, Basingstoke, Hampshire, England) within 30 minutes and then was removed and placed on a different plate of Campylobacter Agar. Both petri dishes were incubated at 42°C during 48 hours in microaerobic conditions and identified in the same manner as the stool samples. The membranes with bacterial growth were subjected to DNA extraction with the PowerFecal DNA isolation kit (MO BIO Laboratories, CA, USA) and *Campylobacter jejuni/coli* were confirmed by PCR analysis as previously described (17).

Multilocus Sequence Typing (MLST). The clonal relationship of aEPEC and *C. jejuni*, the most common bacterial enteropathogens in both children and animals, was analyzed using MLST. DNA extraction of *C. jejuni* and aEPEC isolates was performed using Dnazol® Reagent (Invitrogen Carlsbad, CA, USA) following the manufacturer's protocol. To detect potential clonal relationships, we screened isolates by amplifying and sequencing the phosphoglucomutase (*pgm*) and fumarate hydratase (*fumC*) genes of *C. jejuni* and aEPEC, respectively. Isolates with identical sequences were subjected to full MLST analysis. Seven

loci were amplified and sequenced from 27 isolates of *C. jejuni* and 14 isolates of aEPEC using the primers and conditions previously described (Tables S2, S4) (18, 19). The PCR products were sequenced in FunctionalBiosciences (Madison, WI) (http://functionalbio.com/web), 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 profile.

Data analyses. Evolutionary relationships of MLST sequence types were inferred using eBURST V3 (http://eburst.mlst.net/). 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/).

Results

Prevalence of zoonotic enteropathogens. *Campylobacter* spp. were the most common bacteria genera found in human and domestic animals (46.1 % of fecal samples) where 65% of them were identified as *C. jejuni*, 25% as *C. coli* and 10% as *Campylobacter* non *jejuni/coli*. *Campylobacter jejuni* was the most prevalent specie (30.7%) identified in both children and animals, followed by *Giardia lamblia* (13.1%), *C. coli* (11.6%), aEPEC (10.5%), *Campylobacter* non *jejuni/coli* (5.2%), STEC (2.6%), *Salmonella* sp. (1.9%), *Cryptosporidium parvum* (1.1%) and *Yersinia enterocolitica* (0.4%) (Table 1, Fig. S2).

Campylobacter spp. was found in 17.2% of children (7 samples for *C. jejuni*, 3 for *C. coli* and 1 for *C. hyointestinalis*) and 57.1% of samples were positive in domestic animals. A high percentage of guinea pigs (77.5%) and chickens (76%) were positive for *Campylobacter* spp. (mostly *C. jejuni*). Also seventy-five percent of pigs were positive for *Campylobacter* spp.; however, *C. coli* (38.9%) and *C. hyointestinalis* (27.8%) were the main

Campylobacter species identified in the samples. Dogs were also commonly found with *Campylobacter* (30%; n= 12), mainly *C. jejuni*. In addition, *Campylobacter* was also present in rabbits, cows, cats, ducks and quails, but with 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). *Campylobacter* was detected by PCR in irrigation water: *C. jejuni* in 1 out of 7 samples and *C. coli* in 2 out of 7 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), duck (20.0%; n=1) and sheep (50.0%; n=1) (Table 1).

Seven fecal samples out of 267 total were positive for STEC; 4 were present in cattle, while the other 3 samples belonged to 1 child, 1 guinea pig and 1 chicken. *Giardia lamblia* was present mainly in children (n=22), and was detected in 13 animal fecal samples of guinea pigs, dogs, pigs, rabbits and a sheep. *Salmonella* spp. was detected in 5 samples from dogs (*S. enterica* serovar Infantis). While, three samples from 2 children and 1 sheep were positive for *Cryptosporidium parvum* and 1 fecal sample from a pig was positive for *Yersinia enterocolitica* (Table 1).

There were twenty types of coinfections in 9 children, 7 pigs, 4 guinea pigs, 5 dogs, 2 cattle, 3 chicken, 1 sheep, 1 cat and 1 duck (Table S6). The most important coinfection 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 1-3 years old (56.4% of 47 positive samples; 27 of 31 children of this age group); followed by children aged 3-5 years old

(31.9% of 47 positive samples; 15 of 18 children of this category) (Table S7). The youngest children infected were one baby 3 months old with aEPEC, a baby 3 months old with *G. lamblia,* an infant 10 months old with *C. coli*, an infant 13 months old with co-infection of *C. parvum* and *C. jejuni*, an infant 4 years old with *C. hyointestinalis* and a child 5 years old with STEC (Table S6, Fig. S3). Interestingly none of the samples showed evidence of acute diarrhea.

Campylobacter jejuni MLST. We identified 12 *C. jejuni* Sequence-types (STs), of which five were novel: ST-7643, ST-7662, ST-7669, ST-7671 and ST-7672 (Table 2). Four of the STs detected in children were also found in domestic animals: ST-137, ST-1233, ST-3515, and ST-7671; none of the humans or domestic animals carrying the same ST belonged to same household (Table 2; Fig. 1). Ten STs belonged to 5 Clonal Complexes (CC): the most common was CC-353, which comprised 8 isolates (2 children and 6 animals); followed by CC-607, which comprised 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; 1 dog and 1 quail). The rest of STs and CCs were randomly distributed in the community (Table 2; Table S3, Fig. 1).

Atypical EPEC MLST. Fourteen aEPEC isolates found in the study belonged to 9 STs. Four STs: ST-20, ST-137, ST-517 and ST-4550, were present in both children and animals. Isolates from a sheep and a duck belonged to ST-317. 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,

Table S5). The ST-4550 was present in a child and a chicken living in close proximity (Fig. 2) and was genetically related to ST-29 identified in a child (identical at 6 of 7 loci).

Discussion

We studied the prevalence of seven zoonotic intestinal pathogens in children and domestic animals living in a semi-rural community during a 3-month period. We found evidence that two of the most frequently found bacterial pathogens (*Campylobacter jejuni* and atypical EPEC) were likely transmitted from domestic animals such as poultry, guinea pigs, rabbits, pigs and dogs (Tables 2 and 3; Figs. 1 and 2).

Chickens had high prevalence *C. jejuni* (n=25/42; 59,5%) and MLST found that *C. jejuni* STs from chickens were present in humans and other animal species, which may suggest that chickens were primary source of *C. jejuni* (Table 2; Fig. 1). Conversely, guinea pigs had also high prevalence of *C. jejuni* (n=29/40; 72,5%), however, *C. jejuni* STs found in guinea pigs were not found in other animal species including humans (20). These results are consistent with previous studies which identified *C. jejuni* from chickens as the major source of *C. jejuni* in human infections (21-24); other *C. jejuni* strains may be more adapted to one animal species such as guinea pigs or cattle and may be less likely to infect humans (25, 26). Furthermore, the association of *C. jejuni* STs to domestic animals was consistent with previous reports: *C. jejuni* ST-137 in rabbits (34), ST-354 in birds; ST-464 in poultry and rabbits (24); ST-607 in chickens and dogs; and ST-1212 in chickens (22). Three STs of the CC-353 (ST-1233, ST-3515 and ST-7643) found in chickens, quail, dogs and guinea pigs in our study have not been reported in these animal hosts.

Similarly, aEPEC infections appeared to be also associated with animal-human transmission in this location. MLST analysis revealed that pigs, chickens and dogs were

potential sources of the aEPEC found in children (Table 3, Fig. 2). Other animal species that may act as aEPEC reservoirs were guinea pigs, cattle, ducks and sheep (Table 3). Previous studies showed the presence of aEPEC in animals like cattle (27, 28), sheep (29), goats (30), avian species (31), dogs, rabbits, and monkeys (10, 32). *MLST of aEPEC (using database at the University of Warwick) showed that two STs were previously reported in similar hosts: ST-4550 in chickens and ST-327 in ruminants; the rest of STs in the present study did not coincide with animal hosts in the data base.*

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 equally possible that domestic animals became colonized by aEPEC from humans (9, 10, 33).

Most households in this community, however, have improved drinking water and sanitation facilities, which prompt us to suggest that the main route of infection for humans of these zoonotic pathogens was contact with animals or contaminated environment. In fact, we detected *C. jejuni and C. coli* in water from the irrigation channels. Additionally, the spread 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 community, which corresponds to the second largest conglomerate of poultry farms in Ecuador (151 poultry farms) (35). Additionally, *Escherichia coli* (and probably pathogenic members of Enterobacteriaciae) grow massively in fresh fecal matter in the presence of oxygen (36, 37). A high prevalence of zoonotic enteric pathogens in the environment may increase the possibility of crop contamination or the high presence of these pathogens in animal products. This area possibly represents

a hotspot 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 close proximity to Quito International airport.

Giardia lamblia was the most prevalent enteric pathogen among children (34.4%). This prevalence was higher than previous studies in Ecuador (11-24%) (38, 39). Dogs, rabbits, pigs, guinea pigs and sheep also carried *Giardia lamblia* in this location, which may be 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–G), humans are susceptible to genotypes A and B, which zoonotic transmission is mainly related to companion animals, such as dogs and cats, while livestock and contaminated water appear to be uncommon sources (40).

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 (41). *Cryptosporidium* spp. are associated with morbidity and mortality in young children in developing countries (42), and may be an important cause of diarrhea in Ecuadorian rural villages (43). Although *Cryptosporidium* spp. are highly prevalent in livestock (44), several studies in developing countries suggest that zoonotic transmission of *Cryptosporidium* spp. is uncommon (45). Meanwhile, STEC is considered a foodborne disease with ruminants as the main reservoir (5), however symptomatic diesease in humans seems to be uncommon in Ecuador (43, 46).

Despite their proximity to poultry industrial operations, *Salmonella* was not detected in children, although it was isolated from five dogs (*S. enterica* serovar Infantis). *Yersinia enterocolitica* was only present in one pig fecal sample (pigs are known as the main reservoir for *Y. enterocolitica*) (5).

The elevated presence of these pathogens in domestic animals (77% of the birds and 59% of the mammals) may contribute to environmental contamination and subsequent human infection. Most children under five years of age (59.4%) carried intestinal pathogens, but were asymptomatic (non-diarrheic stool), a phenomenon also observed with non-zoonotic human enteric pathogens in LMICs (47-51) and may be due to herd immunity resulting from permanent exposure to these pathogens (52). Another factor protecting people from symptomatic infection may be the composition of their microbiota (53). Low symptomatology may be also associated to breastfeeding, which is a crucial behavior to inhibit the pathogenicity of some microorganisms through immunoglobulins and other compounds, such as human milk oligosaccharides (HMOs) that may inhibit attaching/effacing pathogens like EPEC (54). Despite the absence of diarrhea, asymptomatic infections, such as *Campylobacter*ioris and cryptosporidiosis, may reduce growth in children (48, 49).

The control of the dissemination of these pathogens calls for a comprehensive and multidisciplinary approach (55). It is necessary to have a complete analysis of the spatial, ecological, evolutionary, social, economic and epidemiological aspects in order to reduce the prevalence of these pathogens. This coordinated, collaborative and cross-sectoral approach has resulted in the 'One Health' initiative, which aims to link human, animal and

environmental health (55). This study complements the information presented in previous reports focusing in non-zoonotic pathogens in Ecuador (43).

Future pespectives

This study reveals the need to study deeper the role of zoonotic enteric pathogens. Case-control studies are necessary to assess the impact of these pathogens in Ecuadorian communities. The control of zoonotic infections requires interventions to improve agricultural management practices, sanitization facilities, education, malnutrition, poverty and access to medical care in rural and semirural areas. Additionally, the understanding of immune response in asymptomatic children and their microbiotas could clarify this phenomenon in children highly exposed to zoonotic pathogens.

Conclusion

In summary, this study found evidence of *Campylobacter jejuni* and atypical EPEC transmission between children and domestic animals. Transmission was likely due to direct contact or close proximity with animals or by environmental contamination with animal feces. Other pathogens detected in the area were *Giardia lamblia, Cryptosporidium parvum,* STEC, *Salmonella* spp. and *Yersinia enterocolitica*.

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References

1. Grace D, Mutua F, Ochungo P, Kruska R, Jones K, Brierley L, Lapar L, Said M, Herrero M, Phuc P. 2012. Mapping of poverty and likely zoonoses hotspots. Report of the third conference organized with ICONZ, DFID-RIU, Gates Foundation, SOS, EU, TDR and FAO with the participation of ILRI and OIE. 23-24 November 2010, WHO Headquarters, Geneva, Switzerland.
2. Lal A, Hales S, French N, Baker MG. 2012. Seasonality in human zoonotic enteric diseases: a systematic review. PLoS One 7:e31883.
3. Zambrano LD, Levy K, Menezes NP, Freeman MC. 2014. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg 108:313-325.
4. Centers for Disease Control and Prevention. 2014. Estimates of Foodborne Illness in the United States. Atlanta. Retrieved from http://www.cdc.gov/foodborneburden/. Accessed 20 October 2015.
5. European Food Safety Authority, European Center for Disease Prevention and Control. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal 13(1):3991 [165 pp.]. doi: 10.2903/j.efsa.2015.3991
6. Wagenaar JA, Newell DG, Kalupahana RS, Mughini-Gras L. 2015. *Campylobacter*: Animal Reservoirs, Human Infections, and Options for Control, p 159-177. *In* Sing A (ed), Zoonoses - Infections Affecting Humans and Animals doi:10.1007/978-94-017-9457-2_6. Springer Netherlands.

7. Kaakoush NO, Castano-Rodriguez N, Mitchell HM, Man SM. 2015. Global Epidemiology of *Campylobacter* Infection. Clin Microbiol Rev 28:687-720.

8. Hernandes RT, Elias WP, Vieira MA, Gomes TA. 2009. An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiol Lett 297:137-149.

9. Wang L, Wakushima M, Aota T, Yoshida Y, Kita T, Maehara T, Ogasawara J, Choi C, Kamata Y, Hara-Kudo Y, Nishikawa Y. 2013. Specific properties of enteropathogenic *Escherichia coli* isolates from diarrheal patients and comparison to strains from foods and fecal specimens from cattle, swine, and healthy carriers in Osaka City, Japan. Appl Environ Microbiol 79:1232-1240.

10. Moura RA, Sircili MP, Leomil L, Matte MH, Trabulsi LR, Elias WP, Irino K, Pestana de Castro AF. 2009. Clonal relationship among atypical enteropathogenic *Escherichia coli* strains isolated from different animal species and humans. Appl Environ Microbiol 75:7399-7408.

11. Nguyen RN, Taylor LS, Tauschek M, Robins-Browne RM. 2006. Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. Emerg Infect Dis 12:597-603.

12. Estrada-Garcia T, Lopez-Saucedo C, Thompson-Bonilla R, Abonce M, Lopez-Hernandez D, Santos JI, Rosado JL, DuPont HL, Long KZ. 2009. Association of diarrheagenic *Escherichia coli* pathotypes with infection and diarrhea among Mexican children and association of atypical enteropathogenic *E. coli* with acute diarrhea. Journal of clinical microbiology 47:93-98.

13. Mohammadzadeh M, Oloomi M, Bouzari S. 2013. Genetic evaluation of Locus of enterocyte effacement pathogenicity island (LEE) in Enteropathogenic *Escherichia coli* isolates (EPEC). Iran J Microbiol **5:**345-349.

14. Karns J, Van Kessel J, McClusky B, Perdue M. 2007. Incidence of *Escherichia coli* O157: H7 and *E. coli* virulence factors in US bulk tank milk as determined by polymerase chain reaction. Journal of dairy science **90:**3212-3219.

15. Tornieporth NG, John J, Salgado K, de Jesus P, Latham E, Melo MC, Gunzburg ST, Riley LW. 1995. Differentiation of pathogenic *Escherichia coli* strains in Brazilian children by PCR. J Clin Microbiol **33:**1371-1374.

16. Pollard D, Johnson W, Lior H, Tyler S, Rozee K. 1990. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. Journal of Clinical Microbiology **28:**540-545.

17. Persson S, Olsen KE. 2005. Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. J Med Microbiol **54:**1043-1047.

18. Dingle K, Colles F, Wareing D, Ure R, Fox A, Bolton F, Bootsma H, Willems R, Urwin R, Maiden M. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. Journal of clinical microbiology **39:**14-23.

19. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol **60:**1136-1151.

20. Graham J, Vasco K, Trueba G. 2015. Hyperendemic *Campylobacter jejuni* in guinea pigs (*Cavia porcellus*) raised for food in Ecuador. Submitted to Environmental Microbiology.

21. Mullner P, Shadbolt T, Collins-Emerson JM, Midwinter AC, Spencer SE, Marshall J, Carter PE, Campbell DM, Wilson DJ, Hathaway S, Pirie R, French NP. 2010. Molecular and spatial epidemiology of human Campylobacteriosis: source association and genotype-related risk factors. Epidemiol Infect 138:1372-1383.

22. Levesque S, Fournier E, Carrier N, Frost E, Arbeit RD, Michaud S. 2013. Campylobacteriosis in urban versus rural areas: a case-case study integrated with molecular typing to validate risk factors and to attribute sources of infection. PLoS One 8:e83731.

23. Strachan NJ, Gormley FJ, Rotariu O, Ogden ID, Miller G, Dunn GM, Sheppard SK, Dallas JF, Reid TM, Howie H, Maiden MC, Forbes KJ. 2009. Attribution of *Campylobacter* infections in northeast Scotland to specific sources by use of multilocus sequence typing. J Infect Dis 199:1205-1208.

24. Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA, Diggle PJ. 2008. Tracing the source of Campylobacteriosis. PLoS Genet 4:e1000203.

25. Sheppard SK, Didelot X, Jolley KA, Darling AE, Pascoe B, Meric G, Kelly DJ, Cody A, Colles FM, Strachan NJ, Ogden ID, Forbes K, French NP, Carter P, Miller WG, McCarthy ND, Owen R, Litrup E, Egholm M, Affourtit JP, Bentley SD, Parkhill J, Maiden MC, Falush D. 2013. Progressive genome-wide introgression in agricultural *Campylobacter coli.* Mol Ecol 22:1051-1064.

26. Sheppard SK, Maiden MC. 2015. The Evolution of *Campylobacter jejuni* and *Campylobacter coli*. Cold Spring Harb Perspect Biol 7:a018119.

27. Hornitzky MA, Mercieca K, Bettelheim KA, Djordjevic SP. 2005. Bovine feces from animals with gastrointestinal infections are a source of serologically diverse atypical enteropathogenic *Escherichia coli* and Shiga toxin-producing *E. coli* strains that commonly possess intimin. Appl Environ Microbiol 71:3405-3412.

28. Blanco M, Schumacher S, Tasara T, Zweifel C, Blanco JE, Dahbi G, Blanco J, Stephan R. 2005. Serotypes, intimin variants and other virulence factors of eae positive *Escherichia coli* strains isolated from healthy cattle in Switzerland. Identification of a new intimin variant gene (eae-η2). BMC microbiology 5:23.

29. Otero V, Rodriguez-Calleja JM, Otero A, Garcia-Lopez ML, Santos JA. 2013. Genetic characterization of atypical enteropathogenic *Escherichia coli* isolates from ewes' milk, sheep farm environments, and humans by multilocus sequence typing and pulsed-field gel electrophoresis. Appl Environ Microbiol 79:5864-5869.

30. Cortes C, De la Fuente R, Blanco J, Blanco M, Blanco JE, Dhabi G, Mora A, Justel P, Contreras A, Sanchez A, Corrales JC, Orden JA. 2005. Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. Vet Microbiol 110:67-76.

31. Farooq S, Hussain I, Mir MA, Bhat MA, Wani SA. 2009. Isolation of atypical enteropathogenic *Escherichia coli* and Shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India. Lett Appl Microbiol 48:692-697.

32. de Almeida PM, Arais LR, Andrade JR, Prado EH, Irino K, Cerqueira Ade M. 2012. Characterization of atypical Enteropathogenic *Escherichia coli* (aEPEC) isolated from dogs. Vet Microbiol **158:**420-424.

33. Krause G, Zimmermann S, Beutin L. 2005. Investigation of domestic animals and pets as a reservoir for intimin- (eae) gene positive *Escherichia coli* types. Vet Microbiol **106:**87-95.

34. Kwan PS, Barrigas M, Bolton FJ, French NP, Gowland P, Kemp R, Leatherbarrow H, Upton M, Fox AJ. 2008. Molecular epidemiology of *Campylobacter jejuni* populations in dairy cattle, wildlife, and the environment in a farmland area. Appl Environ Microbiol **74:**5130-5138.

35. MAGAP, SESA, CONAVE. 2006. Censo Nacional Avícola del Ecuador. Ecuador. Retrived from http://www.conave.org/informacionlistall.php?pagina=2. Accessed 20 October 2015.

36. Russell JB, Jarvis GN. 2001. Practical mechanisms for interrupting the oral-fecal lifecycle of *Escherichia coli*. J Mol Microbiol Biotechnol **3:**265-272.

37. Vasco G, Spindel T, Carrera S, Grigg A, Trueba G. 2015. The role of aerobic respiration in the life cycle of *Escherichia coli*: Public health implications. Avances en Ciencias e Ingenierías **7:**B7-B9.

38. Jacobsen KH, Ribeiro PS, Quist BK, Rydbeck BV. 2007. Prevalence of intestinal parasites in young Quichua children in the highlands of rural Ecuador. J Health Popul Nutr **25:**399-405.

39. Berger S. 2015. Yersiniosis: Global Status. GIDEON Informatics Inc.

40. Thompson RC. 2004. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. Vet Parasitol 126:15-35.

41. Beutin L, Geier D, Steinrück H, Zimmermann S, Scheutz F. 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. Journal of Clinical Microbiology 31:2483-2488.

42. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acacio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 382:209-222.

43. Vasco G, Trueba G, Atherton R, Calvopina M, Cevallos W, Andrade T, Eguiguren M, Eisenberg JN. 2014. Identifying etiological agents causing diarrhea in low income Ecuadorian communities. Am J Trop Med Hyg 91:563-569.

44. Palacios Ordóñez TE. 2014. Prevalencia de *Cryptosporidium* spp. y *Giardia* spp. en terneros, como contaminante de los recursos hídricos y su efecto en la población infantil en el cantón San Fernando. Master. Universidad Estatal de Cuenca, Ecuador.

45. Xiao L, Feng Y. 2008. Zoonotic cryptosporidiosis. FEMS Immunology & Medical Microbiology 52:309-323.

46. Trueba G, Garcés V, Colman RE, Seymour M, Vogler AJ, Keim P. 2013. *Escherichia coli* O157: H7 in Ecuador: animal reservoirs, yet no human disease. Vector-Borne and Zoonotic Diseases 13:295-298.

47. Alikhani MY, Mirsalehian A, Aslani MM. 2006. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. J Med Microbiol 55:1159-1163.

48. Checkley W, Gilman RH, Epstein LD, Suarez M, Diaz JF, Cabrera L, Black RE, Sterling CR. 1997. Asymptomatic and Symptomatic Cryptosporidiosis: Their Acute Effect on Weight Gain in Peruvian Children. American Journal of Epidemiology 145:156-163.

49. Lee G, Pan W, Penataro Yori P, Paredes Olortegui M, Tilley D, Gregory M, Oberhelman R, Burga R, Chavez CB, Kosek M. 2013. Symptomatic and asymptomatic *Campylobacter* infections associated with reduced growth in Peruvian children. PLoS Negl Trop Dis 7:e2036.

50. Quiroga M, Oviedo P, Chinen I, Pegels E, Husulak E, Binztein N, Rivas M, Schiavoni L, Vergara M. 2000. Asymptomatic infections by diarrheagenic *Escherichia coli* in children from Misiones, Argentina, during the first twenty months of their lives. Revista do Instituto de Medicina Tropical de São Paulo 42:9-15.

51. Ramirez JD, Heredia RD, Hernandez C, Leon CM, Moncada LI, Reyes P, Pinilla AE, Lopez MC. 2015. Molecular diagnosis and genotype analysis of *Giardia* duodenalis in asymptomatic children from a rural area in central Colombia. Infect Genet Evol 32:208-213.

52. **Belongia EA, Chyou PH, Greenlee RT, Perez-Perez G, Bibb WF, DeVries EO.** 2003. Diarrhea incidence and farm-related risk factors for *Escherichia coli* O157:H7 and *Campylobacter jejuni* antibodies among rural children. J Infect Dis **187:**1460-1468.

53. **Singh Y, Ahmad J, Musarrat J, Ehtesham NZ, Hasnain SE.** 2013. Emerging importance of holobionts in evolution and in probiotics. Gut Pathog **5:**12.

54. **Manthey CF, Autran CA, Eckmann L, Bode L.** 2014. Human milk oligosaccharides protect against enteropathogenic *Escherichia coli* attachment in vitro and EPEC colonization in suckling mice. J Pediatr Gastroenterol Nutr **58:**165-168.

55. **Mackenzie JS, Jeggo M, Daszak P, Richt JA (eds).** 2013. One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases. The Concept and Examples of a One Health Approach. Springer, Berlin, Heidelberg. doi:10.1007/978-3-642-36889-9.

PART III

TABLES AND FIGURES

Table 1. Frequency of zoonotic enteropathogens identified in children and animals.

Source	# Samples	*C. jejuni* (%)	*C. coli* (%)	aEPEC (%)	*Campylobacter* sp.* (%)	STEC (%)	*Salmonella* sp. (%)	*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)
Chicken	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 (28.6)	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 (20.0)	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 (50.0)	0 -	0 -	0 -	0 -	1 (50.0)	1 (50.0)
Goose	1	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -
Horse	1	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -
Total	267	82 (30.7)	31 (11.6)	28 (10.5)	13 (4.9)	7 (2.6)	5 (1.9)	1 (0.4)	35 (13.1)	3 (1.1)

**Campylobacter* non *jejuni/coli*, include: *C. hyointestinalis* (pigs and child), *C. lanienae* (pig) and *C. canadensis* (ducks).

Table 2. Number of isolates of *C. jejuni*, by sequence types (STs), recovered from children 0-3 years of age and from each animal source.

CC	ST	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

The STs marked in bold correspond to new STs.
CC indicates clonal complex

Table 3. Number of isolates of atypical EPEC, by sequence types (STs), recovered from children 0-5 years of age and from each animal source.

CC	ST	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 CC	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

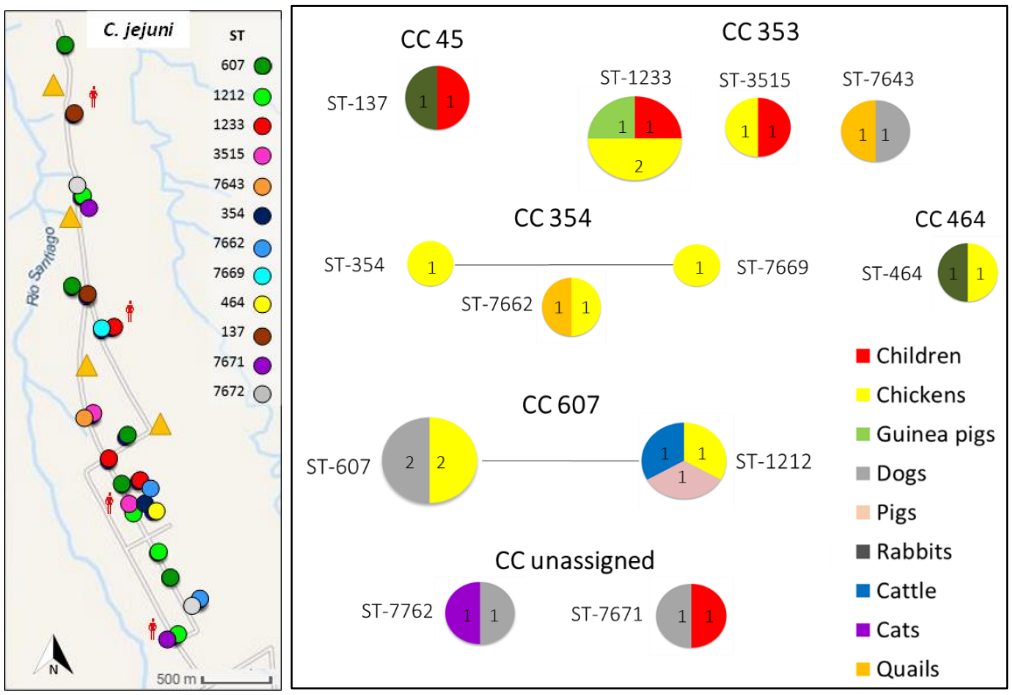

Figure 1. *Left:* Map of the community showing the *Campylobacter jejuni* STs distribution; red figure indicate an isolate from child. *Right:* eBURST diagram depicting 12 C. *jejuni* STs identified in children and animals.*CC: Clonal complex

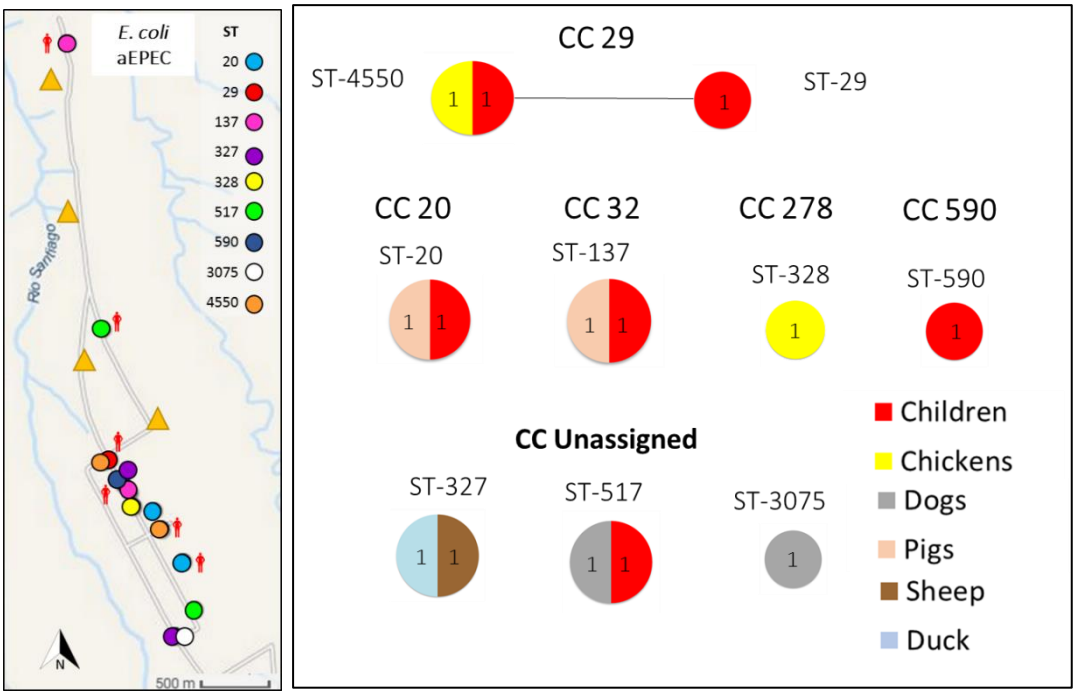

Figure 2. *Left:* Map of the community showing the aEPEC STs distribution; red figure indicate an isolate from child. *Right:* eBURST diagram depicting 9 aEPEC STs identified in children and animals.*CC: Clonal complex

PART IV

ANNEXES

Table S1. PCR primers and conditions used for EPEC, STEC and *Campylobacter jejuni/coli*

Gene (function)	Primers (a)	Amplicon size (bp)	PCR reaction	PCR conditions	References
bfp (bundle-forming pili)	F 5'-CAATGGTGCTTGCGCTTGCT-3' R 5'-GCCGCTTTATCCAACCTGGT-3'	324	Reaction 25 ul: PCR Buffer 1X, MgCl2 1,5 mM, dNTPs 200 uM, Go Taq promega 0,02 U/µl, forward 0,2 µM, reverse, 0,2 µM, 10 ng DNA.	94 °C for 5 min, followed by 30 cycles of 94 °C for 1 m, 56 °C for 2 m and 72 °C for 1 m, and finally 72 °C for 5 min.	(Tornieporth, N., et. al. 1995)
eae (intimin)	JKP11 5′-GGCGATTACGCGAAAGATACC-3' JKP12 5′-CCAGTGAACTACCGTCAAAGTTATTACC-3'	110	Reaction 25 ul: PCR Buffer 1X, MgCl2 1,5 mM, dNTPs 200 uM, Go Taq promega 0,02 U/µl, forward 0,3 µM, reverse, 0,3 µM, 10 ng DNA.	95 °C for 2 min, followed by 30 cycles of 95 °C for 1 m, 59 °C for 1 m and 72 °C for 1 m, and finally 72 °C for 5 min.	(Karns, J., et. al. 2007)
stx1 (Shiga-like toxin 1)	VT1a 5′-GAAGAGTCCGTGGGATTACG-3' VT1b 5′-AGCGATGCAGCTATTAATAA-3'	130	Reaction 25 ul: PCR Buffer 1X, MgCl2 1,5 mM, dNTPs 200 uM, Go Taq promega 0,02 U/µl, forward 1 µM, reverse, 1 µM, 10 ng DNA.	94 °C for 5 min, followed by 30 cycles of 94 °C for 2 m, 55 °C for 1 m and 72 °C for 1 m, and finally 72 °C for 7 min.	(Pollard, D. et. al., 1990)
stx2 gene (Shiga-like toxin 2)	VT2a 5′-TTAACCACACCCACGGCAGT-3' VT2b 5′-GCTCTGGATGCATCTCTGGT-3'	346			
hipO (hippurate hydrolase, *C. jejuni*) *asp* (aspartokinase , *C. coli*) *16S* (16S ribosomal RNA)	hipO-F 5′-GACTTCGTGCAGATATGGATGCTT-3' hipO-R 5′-GCTATAACTATCCGAAGAAGCCATCA-3' CC18F 5′-GGTATGATTTCTACAAAGCGAG-3′ CC519R 5′-ATAAAAGACTATCGTCGCGTG-3′ 16S-F 5′-GGAGGCAGCAGTAGGGAATA 16S-R 5′-TGACGGGCG GTGAGTACAAG	344 500 1062	Reaction 25 ul: PCR Buffer 1X, MgCl2 2,6 mM, dNTPs 260 uM, Go Taq promega 0,02 U/µl, hipOf 0,2 µM, hipOr 0,2 µM, cc18F 0,4 µM, cc519r 0,4 µM, 16Sf 0,2 µM, 16Sr 0,2 µM 10 ng DNA.	94 °C for 6 min, followed by 35 cycles of 94 °C for 50 s, 57 °C for 40 s and 72 °C for 50 s, and finally 72 °C for 3 min.	(Persson, S., & Olsen, K. E. 2005).

Table S2. PCR primers for *Campylobacter jejuni* MLST

Gene (function)	Primers amplification*	Amplicon size (bp)	Primers sequence*
asp (aspartase A)	asp-A9 5'-AGTACTAATGATGCTTATCC-3' asp-A10 5'-ATTTCATCAATTTGTTCTTTGC-3'	899	aspA-S3 5'-CCAACTGCAAGATGCTGTACC-3' aspA-S6 5'-TTCATTTGCGGTAATACCATC-3'
gln (glutamine synthetase)	gln-A1 5'-TAGGAACTTGGCATCATATTACC-3' gln-A2 5'-TTGGACGAGCTTCTACTGGC-3'	1262	glnA-S3 5'-CATGCAATCAATGAAGAAAC-3' glnA-S6 5'-TTCCATAAGCTCATATGAAC-3'
glt (citrate synthase)	glt-A1 5'-GGGCTTGACTTCTACAGCTACTTG-3' glt-A2 5'-CCAAATAAAGTTGTCTTGGACGG-3'	1012	gltA-S3 5'-CTTATATTGATGGAGAAAATGG-3' gltA-S6 5'-CCAAAGCGCACCAATACCTG-3'
gly (serine hydroxymethyltransferase)	gly-A1 5'-GAGTTAGAGCGTCAATGTGAAGG-3' gly-A2 5'-AAACCTCTGGCAGTAAGGGC-3'	816	glyA-S5 5'-GCTAATCAAGGTGTTTATAT-3' glyA-S4 5'-AGGTGATTATCCGTTCCATCGC-3'
tkt (transketolase)	tkt-A3 5'-GCAAACTCAGGACACCCAGG-3' tkt-A6 5'-AAAGCATTGTTAATGGCTGC-3'	1150	tkt-S5 5'-GCTTAGCAGATATTTTAAGTG-3' tkt-S6 5'-AAGCCTGCTTGTTCTTTGGC-3'
pgm (phosphoglucomutase)	pgm-A7 5'-TACTAATAATATCTTAGTAGG-3' pgm-A8 5'- ACAACATTTTTCATTTCTTTTTC-3'	1102	pgm-S5 5'-GGTTTTAGATGTGGCTCATG-3' pgm-S2 5'-TCCAGAATAGCGAAATAAGG-3'
unc (ATP synthase a subunit)	unc-A7 5'-ATGGACTTAAGAATATTATGGC-3' unc-A8 5'-ATAAATTCCATCTTCAAATTCC-3'	1120	uncA-S3 5'-AAAGTACAGTGGCACAAGTGG-3' uncA-S4 5'-TGCCTCATCTAAATCACTAGC-3'

*Primers are maintained at http://pubmlst.org/*Campylobacter*/info/primers.shtml

Table S3. Sequence type (ST) and ST complex of *C. jejuni*

	HH #	Specie	ST	Allelic Profile							ST Complex
				aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*	
1	04	Child	137	4	7	10	4	42	7	1	ST-45 complex
2	14	Rabbit	137	4	7	10	4	42	7	1	ST-45 complex
3	19	Child	1233	7	17	5	10	10	177	6	ST-353 complex
4	27	Chicken	1233	7	17	5	10	10	177	6	ST-353 complex
5	29	Guinea pig	1233	7	17	5	10	10	177	6	ST-353 complex
6	29	Chicken	1233	7	17	5	10	10	177	6	ST-353 complex
7	22	Chicken	3515	7	17	2	2	10	3	6	ST-353 complex
8	22	Quail	**7643**	7	17	5	2	10	3	54	ST-353 complex
9	22	Dog	**7643**	7	17	5	2	10	3	54	ST-353 complex
10	33	Child	3515	7	17	2	2	10	3	6	ST-353 complex
11	34	Chicken	354	8	10	2	2	11	12	6	ST-354 complex
12	31	Quail	**7662**	**390**	2	2	2	11	5	6	ST-354 complex
13	56	Chicken	**7662**	**390**	2	2	2	11	5	6	ST-354 complex
14	18	Chicken	**7669**	8	10	95	2	11	12	6	ST-354 complex
15	36	Rabbit	464	24	2	2	2	10	3	1	ST-464 complex
16	36	Chicken	464	24	2	2	2	10	3	1	ST-464 complex
17	21	Chicken	607	8	2	5	53	11	3	1	ST-607 complex
18	34	Dog	607	8	2	5	53	11	3	1	ST-607 complex
19	51	Chicken	607	8	2	5	53	11	3	1	ST-607 complex
20	54	Dog	607	8	2	5	53	11	3	1	ST-607 complex
21	13	Pig	1212	8	2	5	53	11	3	105	ST-607 complex
22	42	Chicken	1212	8	2	5	53	11	3	105	ST-607 complex
23	62	Cattle	1212	8	2	5	53	11	3	105	ST-607 complex
24	07	Dog	**7671**	8	113	5	121	11	25	6	****
25	62	Child	**7671**	8	113	5	121	11	25	6	****
26	06	Dog	**7672**	2	114	5	298	13	61	**460**	****
27	56	Cat	**7672**	2	114	5	298	13	61	**460**	****

The STs and alleles marked in bold correspond to new ones.

Table S4. PCR primers for *Escherichia coli* MLST

Gene (function)	Primer amplification*	Amplicon size (bp)
adk (adenylate kinase)	F 5'-ATTCTGCTTGGCGCTCCGGG-3' R 5'-CCGTCAACTTTCGCGTATTT-3'	583
fumC (fumarate hydratase)	F 5'-TCACAGGTCGCCAGCGCTTC-3' R 5'-GTACGCAGCGAAAAAGATTC-3'	806
gyrB (DNA gyrase)	F 5'-TCGGCGACACGGATGACGGC-3' R 5'-ATCAGGCCTTCACGCGCATC-3'	911
icd (isocitrate/ isopropylmalate dehydrogenase)	F 5'-ATGGAAAGTAAAGTAGTTGTTCCGGCACA-3' R 5'-GGACGCAGCAGGATCTGTT-3'	878
mdh (malate dehydrogenase)	F 5'-ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG-3' R 5'-TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT-3'	932
purA (adenylosuccinate dehydrogenase)	F 5'-CGCGCTGATGAAAGAGATGA-3' R 5'-CATACGGTAAGCCACGCAGA-3'	816
ecA (ATP/GTP binding motif)	F 5'-CGCATTCGCTTTACCCTGACC-3' R 5'-TCGTCGAAATCTACGGACCGGA-3'	780

*F, forward; R, reverse

Primers are maintained at http://mlst.warwick.ac.uk

Table S5. Sequence type (ST) and ST complex of atypical enteropathogenic *E. coli*

	HH #	Specie	ST	Allelic profile							ST complex
				adk	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*	
1	36	Pig	20	6	4	3	18	7	7	6	ST-20 complex
2	47	Child	20	6	4	3	18	7	7	6	ST-20 complex
3	27	Child	29	6	4	12	16	9	7	7	ST-29 complex
4	32	Pig	137	19	23	51	24	21	2	16	ST-32 complex
5	55	Child	137	19	23	51	24	21	2	16	ST-32 complex
6	34	Chicken	328	9	23	81	18	11	8	6	ST-278 complex
7	29	Child	590	6	4	12	10	24	18	7	ST-590 complex
8	18	Child	517	109	65	5	1	9	13	14	****
9	29	Sheep	327	6	4	4	85	43	12	7	****
10	39	Child	4550	6	4	12	476	9	7	7	****
11	58	Dog	517	109	65	5	1	9	13	14	****
12	62	Duck	327	6	4	4	85	43	12	7	****
13	62	Dog	3075	10	23	109	8	270	8	2	****
14	63	Chicken	4550	6	4	12	476	9	7	7	****

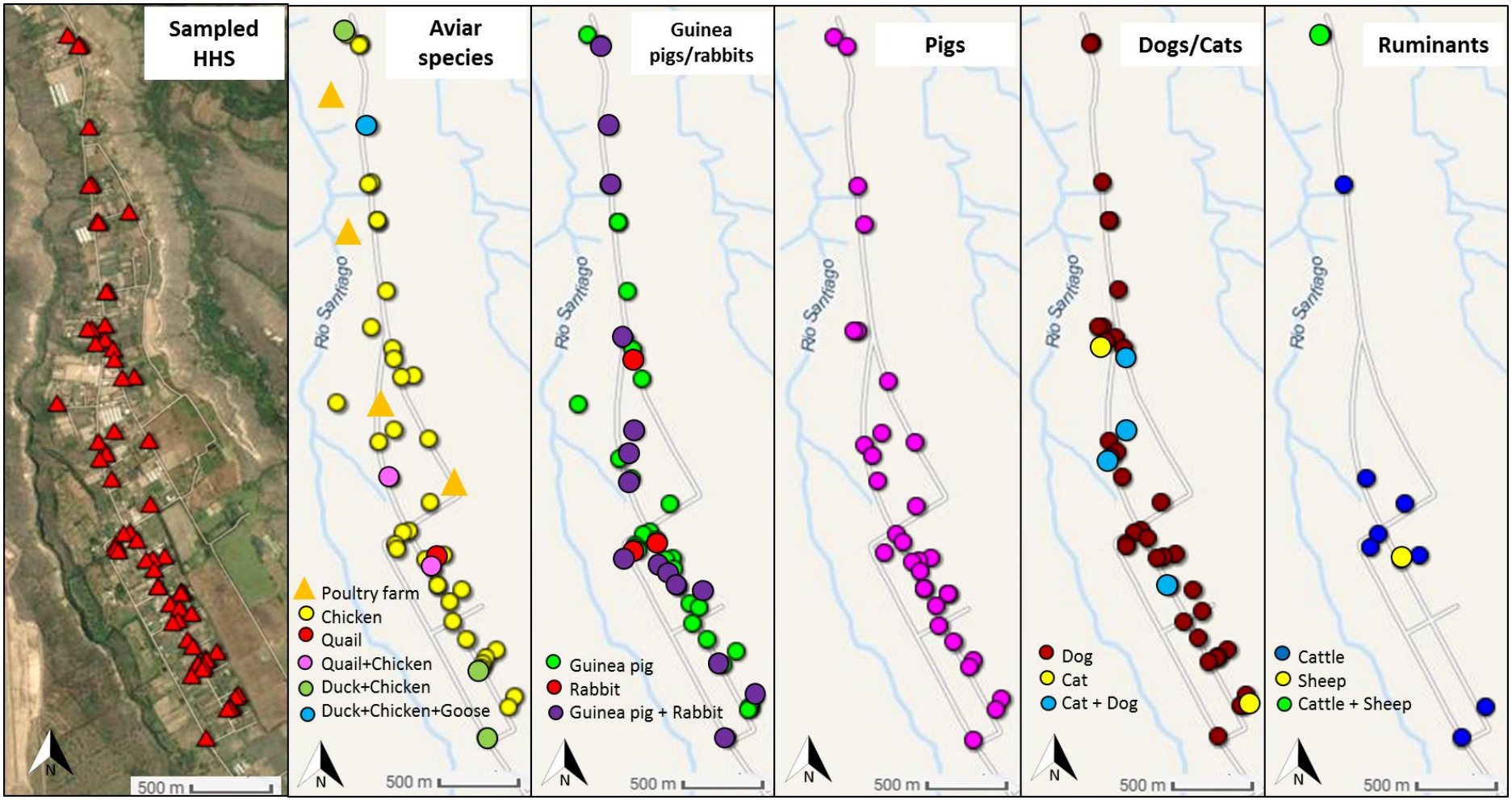

Figure S1. Maps of the community depicting the distribution of animal species.

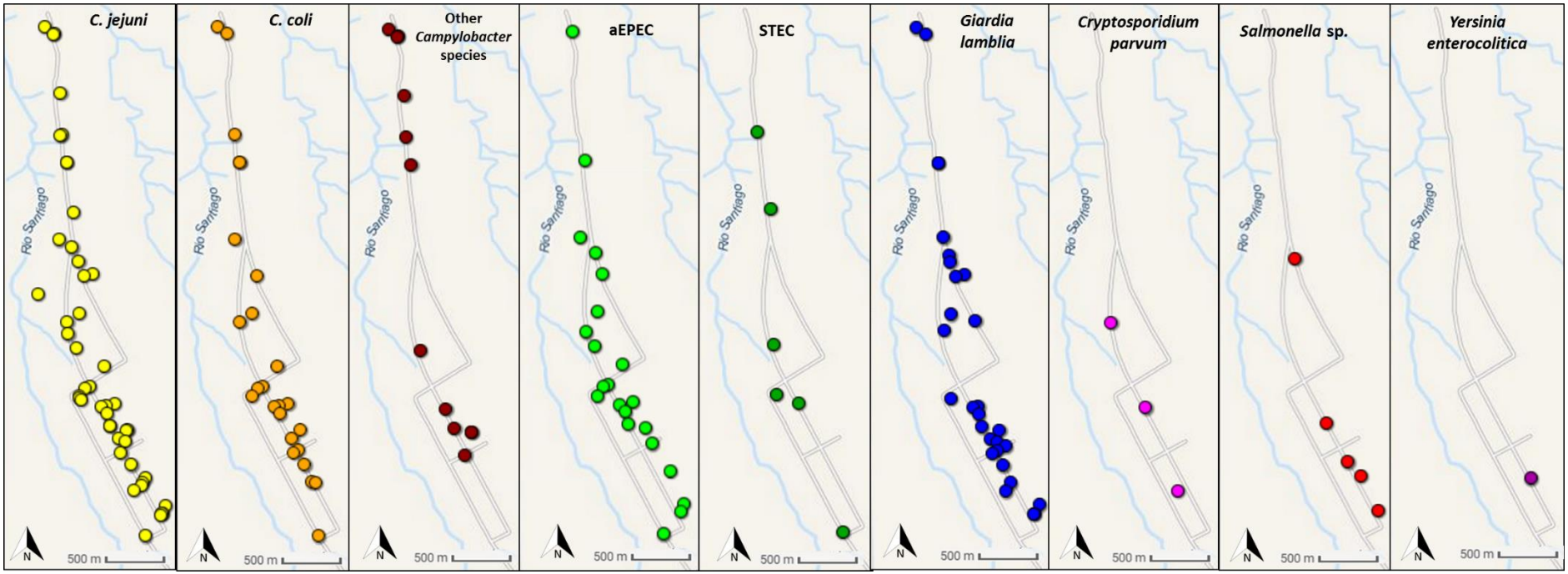

Figure S2. Maps of the community depicting the distribution of zoonotic enteric pathogens in the households in children and/or animals.

Table S6. Coinfections with zoonotic enteropathogens found in fecal samples.

#	# HH	Specie	Zoonotic enteric pathogens	*Campylobacter* spp., *aEPEC*	*Campylobacter* spp., *Giardia*	aEPEC, *Giardia*	aEPEC, *C. coli*	*C. jejuni, Giardia*	aEPEC, *C. jejuni*	*Salmonella, Giardia*	STEC, *C. jejuni*	*C. coli, Giardia*	*C. jejuni, Campylobacter* sp.	*C. coli, Salmonella*	*C. jejuni, C. coli*	*C. jejuni, Cryptosporidium, Giardia*	*Campylobacter* sp, *Giardia*	*C. coli, Yersinia*	aEPEC, *C. jejuni, Giardia*	aEPEC, *Campylobacter* sp.	aEPEC, STEC
1	56	Cat	*C. jejuni, C. coli*	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
2	22	Cattle	aEPEC, STEC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3	62	Cattle	STEC, *C. jejuni*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
4	29	Chicken	STEC, *C. jejuni*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
5	34	Chicken	aEPEC, *C. jejuni*	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
6	63	Chicken	aEPEC, *C. coli*	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	18	Child	aEPEC, *C. jejuni, Giardia*	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0
8	19	Child	*C. jejuni, Giardia*	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
9	26	Child	aEPEC, *Giardia*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	39	Child	aEPEC, *Giardia*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	50	Child	*C. jejuni, Cryptosporidium, Giardia*	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
12	55	Child	aEPEC, *Giardia*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	29	Child	aEPEC, *Giardia*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	62	Child	aEPEC, *C. coli*	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	64	Child	aEPEC, *Giardia*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	07	Dog	*C. jejuni, Giardia*	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
17	17	Dog	*Salmonella, Giardia*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
18	34	Dog	*C. jejuni, Salmonella, Giardia*	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
19	42	Dog	*C. coli, Salmonella*	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
20	59	Dog	*Salmonella, Giardia*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
21	04	Duck	*C. jejuni, Campylobacter canadensis*	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
22	18	Guinea pig	aEPEC, *C. jejuni*	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
23	48	Guinea pig	*C. coli, Giardia*	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
24	56	Guinea pig	aEPEC, *C. jejuni*	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
25	64	Guinea pig	*C. jejuni*, STEC	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
26	07	Pig	*C. jejuni, C. hyointestinalis*	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
27	07	Pig	*C. jejuni, C. coli*	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	21	Pig	aEPEC, *C. coli*	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	32	Pig	aEPEC, *C. coli*	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	36	Pig	aEPEC, *C. hyointestinalis*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
31	41	Pig	*Campylobacter lanienae, Giardia*	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
32	45	Pig	*C. coli, Yersinia enterocolitica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
33	29	Sheep	*C. coli, Giardia, Cryptosporidium,* aEPEC	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
			TOTAL	11	8	7	6	5	4	3	3	2	2	1	1	1	1	1	1	1	1

Table S7. Number of cases of zoonotic enteropathogens found in children by age group

Age	# children	STEC	aEPEC	*C. jejuni*	*C. coli*	*C. hyointestinalis*	*Giardia lamblia*	*Cryptosporidium parvum*	Total pathogens	% by age	% Total pathogens
3-12 months	11	0	1	0	1	0	1	0	3	27.3	6.4
1-3 years	31	0	5	7	2	0	12	1	27	87.1	57.4
3-5 years	18	1	5	0	0	1	7	1	15	83.3	31.9
6 years	4	0	0	0	0	0	2	0	2	50.0	4.3
Total	**64**	**1**	**11**	**7**	**3**	**1**	**22**	**2**	47	73.4*	100.0

*There were 9 children with coinfections. The real proportion of children infected was 59.4%.

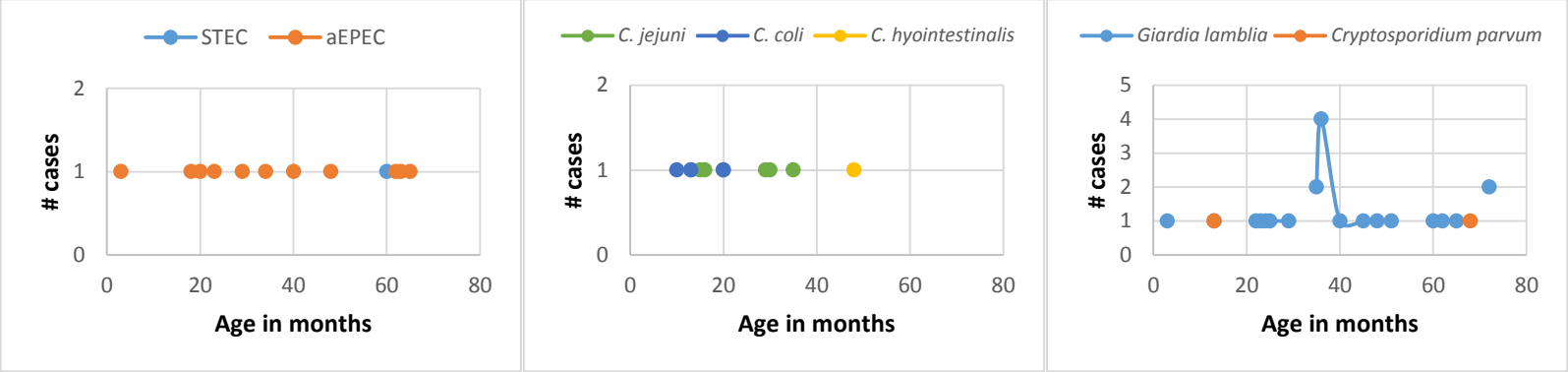

Figure S3. Cases found in children by age.