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Locals Get Travelers' Diarrhea Too: Risk factors for diarrheal illness and pathogenic *E. coli* infection across an urban-rural gradient in Ecuador

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ABSTRACT

Objectives: Diarrhea is a common and well-studied cause of illness afflicting international travelers. However, traveler's diarrhea can also result from travel between high and low disease transmission regions within a country, which is the focus of this study.

Methods: We recruited participants for a case-control study of diarrhea at four sites along an urban-rural gradient in Northern Ecuador: Quito, Esmeraldas, Borbón and rural communities outside of Borbón. At each of these sites, approximately 100 subjects with diarrhea (cases) were recruited from Ministry of Health clinics and were age-matched with subjects visiting the same clinics for other complaints (controls).

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Results: Travelers to urban destinations had higher risk of diarrhea and diarrheagenic *E. coli* (DEC) infections. Travel to Quito was associated with diarrhea (aOR = 2.01, 95% CI = 1.10-3.68) and travel to Guayaquil (another urban center in Ecuador) was associated with Diffuse Adherent *E. coli* infection (OR = 2.09, 95% CI = 1.01-4.33). Compared to those not traveling, urban origins were also associated with greater risk of diarrhea in Esmeraldas (aOR = 2.28, 95% CI = 1.20-4.41), and with higher risk of diarrheagenic *E. coli* infections in Quito (aOR = 2.61, 95% CI = 1.16-5.86), with >50% of travel from Quito and Esmeraldas specified as to another urban destination.

Conclusions: This study suggests that individuals traveling from lower transmission regions (rural areas) to higher transmission regions (urban centers) within a single country are at a greater risk of acquiring a diarrhea-related illness. Investments to improve water, sanitation and hygiene conditions in urban areas could have impacts on outlying rural areas within a given country.

Keywords travel; diarrhea; diarrheagenic *E. coli*; diffusely adherent *E. coli*; urban; rural; Ecuador

INTRODUCTION

Diarrheal diseases are a leading cause of global morbidity and mortality, with an estimated 1.3 million deaths annually, and up to half a million deaths each year among children under five. [1-3] Diarrhea disproportionally affects people living in low- and middle- income countries (LMICs). [1, 2, 4-6] In Ecuador, diarrheal disease rates ranged from 2.0 to 2.7 cases per 100,000 inhabitants from 2000 to 2005. While disability-adjusted life years (DALYs) have reduced by 82% since 1990, [7, 8] diarrheal diseases in Ecuador remain one of the top five communicable diseases causing premature death annually. [7] Repeat episodes and the most severe cases of diarrhea are often present among children less than five years old and can result in cognitive and physical growth deficiencies later in life. [4, 9-11] These latent consequences of diarrhea contribute to morbidity, therefore targeted interventions are still needed to reduce the transmission of diarrheal pathogens.

Diarrhea is caused by a wide range of etiological agents, including viruses, bacteria and parasites. Diarrheagenic *E. coli* (DEC) are among the most common etiologic agents of diarrhea and can be subdivided into distinct pathotypes based on the presence of specific virulence factors: enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), Shiga-toxin producing *E. coli* (STEC), typical enteropathogenic *E. coli* (EPECt), atypical

enteropathogenic *E. coli* (EPECa), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). [6, 12] Two recent large studies of etiologic agents of diarrhea, the Global Enteric Multicenter Study (GEMS) and the Malnutrition and Enteric Disease study (Mal-ED), found ETEC and Shigellae (considered an *E. coli* pathotype similar to EIEC) to be associated with more severe diarrheal disease, and EPECt and ETEC to be associated with increased risk of mortality in infants. [13, 14] The predominant transmission route for these enteric pathogens is fecal-oral (foodborne, waterborne and person-to-person), and the majority of diarrheal cases are due to a lack of access to clean water supply, poor sanitation, and inadequate personal hygiene behaviors, especially in LMICs. [15-19]

While water, sanitation, and hygiene (WASH)-related conditions are the most salient factors in determining diarrhea rates, and the target of most intervention programs, diarrheal diseases are complex due to the variety of etiological agents and the dynamic interaction between personal socio-economic status, environment and behavior contributing to disease transmission. One factor not traditionally considered in WASH studies is human travel, which can facilitate movement of enteric pathogens from one region to another. [20] Human movement is often considered for other types of infectious diseases, [21-23] but for enteric diseases consideration of human travel as a risk factor has mostly been limited to introduction of new strains of cholera to naïve populations, [24-26] and to "traveler's diarrhea," i.e., the acquisition of diarrhea by people traveling internationally from low-transmission to high-transmission countries.

International travel is an established risk factor for diarrhea, the most common illness afflicting travelers, [27, 28] afflicting approximately 50% of travelers after a two-week travel period. [29, 30] "Traveler's diarrhea" is often attributed to bacterial infections, particularly ETEC or EAEC. [31-35] Most studies focus on travelers from high-income countries who tend to be more immunologically naïve. [31-36] However, “traveler’s diarrhea” can also feasibly occur within LMIC countries, as a result of within-country travel between areas of high versus low transmission. [20] This is important because pathogens can have different prevalence even within small regions. [37, 38] Urban areas with higher transmission may be a source of pathogens for more rural regions.

This study was designed to explore the importance of within-country traveler's diarrhea and to understand how risk factors differ in urban versus rural areas within a given country for diarrhea and specifically DEC.

METHODS

Study Design & Setting

The EcoZUR (E. coli *en Zonas Urbanas y Rurales*) study was an age-matched case-control study of diarrhea conducted in four sites along an urban-rural gradient in northern Ecuador. Figure 1 shows the four study locations: Quito (0°10'50.35"S, 78°28'4.20"W), Esmeraldas (0°57'33.12"N, 79°39'14.29"W), Borbón (1°05'21.13"N, 78°59'23.86"W), and rural villages near the Borbón region. Quito is the capital of Ecuador (population ~1.6 million); [39] Esmeraldas is the capital of Esmeraldas Province in northwest Ecuador (population ~162,000); [39] Borbón is a town in Esmeraldas Province located at the junction of Cayapas, Santiago, and Onzole rivers (population ~7,000); and the rural villages comprise about 125 villages along the three rivers (populations ~50 to 500). The more urban centers (Quito and Esmeraldas) are more densely populated with greater access to water, sanitation, roads, and medical infrastructure; whereas the more rural regions (Borbón and rural villages) are less densely populated, with minimal infrastructure. This study was designed to understand how factors associated with living conditions (e.g., population density, human travel, WASH conditions, animal contact) affect diarrhea and the enteric pathogens in circulation along this urban-rural gradient. This case-control study design allowed for the capture of both symptomatic and asymptomatic enteric infections.

Participant Recruitment

Individuals of all ages were recruited from Ecuadorian Ministry of Public Health facilities at each study site between April 2014-September 2015. Recruitment centers included Centro de Salud N°4 Chimbacalle in Quito, Hospital Delfina Torres de Concha in Esmeraldas, and Hospital Básico in Borbón. Participants from the rural villages were recruited either through scheduled Ministry of Public Health clinical visits on site or at Borbón Hospital if they presented there for medical attention. Through this recruitment strategy we were able to capture subjects from 58 of the 125 villages in the region. At each study location, our goal was to recruit 100 cases of patients presenting with diarrhea – defined as three or more loose stools within the previous 24 hours. Controls were individuals presenting with a non-diarrheal complaint at the same facility, without diarrhea or vomiting in the prior seven days. A one-to-one age-match of cases and controls was carried out in real-time, based on the following age-matching criteria: 0-24 months (+/- 6 months), 25-60 months (+/- 12 months), 61-180 months (+/- 24 months) and >181 months. Rural village control subjects were matched by the location of recruitment (i.e., through Ministry of Health visits to their village or

at Borbón Hospital). Participants provided written consent unless they could not read or write, in which case they provided oral consent with a witness. An assent form was used for participants <18 years old, in conjunction with parental consent. Additionally, participants (cases and controls) were required to be a resident of the study location for at least six months in order to be included in the study. Any participants that reported usage of antibiotics in the prior week were also excluded.

All consenting participants and/or their guardians completed an electronic survey about demographics, socioeconomic status, medical history, WASH practices, and travel history. The surveys were adapted from instruments previously developed for and implemented in this region (e.g.,[37, 40]). Our trained study staff conducted these surveys using the Open Data Kit platform (http://opendatakit.org). Additionally, both cases and controls were asked to provide a stool sample for enteric pathogen analysis. Study staff provided the participant with a plastic stool collection container and instructions to return a stool sample within 24 hours. If a participant was in diapers, the team member provided additional instructions to assist the parent or guardian in properly handling the stool specimen. A total of 907 subjects enrolled successfully completed the survey and 85% of those provided a stool sample.

E. coli *Pathotype Determination*

Fresh stool samples were cultured locally upon receipt and tested for DEC pathotypes based on the presence of specific virulence factors. At the Esmeraldas, Borbón, and rural village sites samples were streaked for isolation of *E. coli* in a field laboratory on MacConkey Lactose agar media (MKL), and re-isolated for pure cultures upon arrival at the Universidad San Francisco de Quito (USFQ) laboratory. At the Quito site, Cary-Blair transport media swabs (BD, Franklin Lakes, NJ) were inoculated with fecal material, maintained at 4ºC for a maximum of 24 hours, and streaked for isolation upon arrival at USFQ on MKL agar. After 24 hours of incubation, up to five lactose-positives and one non-lactose fermenting isolate were cultured on Chromocult agar media (Merck, Darmsladt, Germany) and tested for β–glucoronidase activity. Non-lactose-fermenting colonies were further characterized by biochemical tests to detect Shigellae or *E. coli* (some EIEC are non-fermenters) using the API 20E test (BioMérieux, Marcy l'Etoile, France). The 5-6 colonies were pooled, resuspended in 300 µl of sterile distilled water, boiled for 10 minutes to release the DNA, and the resulting supernatant was used for PCR testing. Nine singleplex PCR assays were used to detect the presence of virulence genes associated with each diarrheagenic *E. coli* pathotype: *lt* and *sta* for ETEC [41]; *ipaH* for EIEC and Shigella [41]; *aggR* for EAEC [42]; *afa* for DAEC [43]; *bfp* for typical EPEC [41];

and *eaeA* for atypical EPEC. [44] Positive pools for *eaeA* were subsequently tested for *stx1* and *stx2* genes for the differentiation of potential EHEC infections. [44] If a pooled sample tested positive for any virulence factor then each of the five isolates were re-tested individually to identify the positive isolate.

Water and Sanitation Behaviors

Survey responses regarding water and sanitation behaviors were converted into "improved" versus "unimproved" categories according to the WHO Joint Monitoring Programme (JMP) for Water Supply and Sanitation guidelines, with the exception of purchased bottled water which was considered improved based on the culture and practices of this region. [45]

Defining Domestic Travel

Domestic travel was considered as any event in which the participant left his or her city/village of origin to visit another destination within Ecuador. Common options for destinations on all forms included Guayaquil, Quito, Santo Domingo, Esmeraldas, San Lorenzo, Borbón, and rural villages surrounding the study site. Participants could also input other destinations of travel.

Data Analysis

All analysis was completed using R Studio Statistical Software (http://www.rstudio.org/). Unadjusted odds ratios and corresponding *p*-values were computed using the Pearson's Chi Square Test. If expected cell counts were less than five, the Fisher's Exact Test was used.

Multivariate mixed-effect and general logistic regression models were used to compute adjusted odds ratios of the risk of diarrheal disease associated with WASH conditions, animal contact and travel. Models were adjusted for other extraneous factors including sex, age, race, government welfare status, and study site was included as a random effect for multi-site models. The DEC cross-sectional models were also adjusted for diarrhea case status. The lme4 R package was used to run all mixed-effect models that included all study participants. [46] Mixed-effect models were used to assess risk among all participants across the study whereas general logistic regression models were used to assess risks within individual study locations. General logistic regression was also used to examine demographic characteristics associated with travel; variables included in this model were

age, sex, government welfare status, education, family employment status, and study location (urban vs. rural).

Ethics

Permission for the study and approval of human subjects was obtained from the Ecuadorian Ministry of Public Health (MSP-DIS-2014-0055-O), Emory Institutional Review Board (IRB) (IRB00065781) and the USFQ Ethical Committee (2013-145M).

RESULTS

The study included a total of 907 participants age 0 to 85 years old from Quito (*n*=253, 28% of subjects), Esmeraldas (*n*=209, 23% of subjects), Borbón (*n*=243, 27% of subjects), and rural villages (*n*=202, 22% of subjects). These four study sites successfully captured both an urban-rural socio-economic gradient and a water and sanitation accessibility gradient, with the urban sites having higher education, employment, improved sanitation and improved drinking water sources (Table 1).

There were no significant socio-economic or demographic differences between cases and controls within any given site, indicating robust case-control matching (Table 1). Across all sites, there were slightly more males among cases, but this difference was not statistically significant within any single site. Among all participants who provided stool samples (*n*=771), 27% (*n*=208) were positive for DEC infection, with 43% (*n*=90) of these infections present in asymptomatic controls.

Water and sanitation risk factors

Most participants used an improved household drinking water source, with a higher prevalence among urban participants (Quito 99.6%; Esmeraldas 99.5%) than rural participants (Borbón 90.9%; rural villages 82.7%). Subjects from urban sites were more likely to report treating their drinking water (Quito 64.4%; Esmeraldas 40.9%) than subjects in rural sites (Borbón 16.1%; rural villages 21.8%). The prevalence of reported use of improved household sanitation was lower than of reported improved drinking water. Rural participants were less likely to have improved sanitation (61% in Borbón, 41% in rural villages) than urban participants (78% in Quito, 71% in Esmeraldas).

Multivariable logistic regression exploring associations between WASH practices and diarrhea case status and DEC infections are shown in Table 2. The use of improved household drinking water sources was not significantly associated with diarrhea case status or DEC infections, either collectively or within any individual site. However, household drinking water treatment was protective against diarrhea (adjusted Odds Ratio [aOR] = 0.72, 95% CI = 0.54-0.96, p = 0.03). The coefficients were higher in urban regions (Quito aOR = 0.64, 95% CI = 0.37-1.10, p = 0.11; Esmeraldas aOR = 0.34, 95% CI = 0.17-0.64, p = 0.001), where >40% of participants reported these practices. Of those reporting treatment of household drinking water, nearly all (89%) reported boiling as their form of treatment, with a few reporting use of chlorine (8%) or filtration techniques (2%). Drinking water treatment was not associated with DEC infections. Improved household sanitation was associated with increased risk of diarrhea in Borbón (aOR = 2.23, 95% CI = 1.14-4.50, p=0.02) and increased risk of DEC infections in Quito (aOR = 3.67, 95% = 1.17-13.3, p=0.05), but reduced the risk of DEC infection in Esmeraldas (aOR = 0.39, 95% = 0.15-0.97, p=0.05).

Animal exposures risk factors

Forty-four percent of all participants reported contact with animals, including cows, chickens, pigs, dogs, cats, and others. A higher proportion of urban study participants reported animal contact, defined as contact with any animal(s) in the past week, (55% in Quito; 45% in Esmeraldas) than rural study participants (41% in Borbón; 32% in rural villages). Urban participants most commonly reported contact with domestic animals, including dogs and cats. Conversely, rural participants reported a more diverse array of animal contacts, including dogs, cats, chickens, pigs, cows and wild game. No significant association was found between reported animal contacts and diarrhea among the urban participants. However, in Borbón, cases were at least twice as likely to report contact with animals (aOR = 2.08, 95% CI = 1.19-3.67, p = 0.01). No significant association was found between reported animal contact and the presence of DEC at any site.

Domestic travel within Ecuador

Forty-six percent of all participants reported some form of domestic travel in the past year, with a median of four trips. Rural participants reported higher rates of travel (67% in Borbón and 63% in the rural villages) than urban participants (22% in Quito and 33% in Esmeraldas). Figure 2 illustrates the prevalence and distribution of domestic travel in the past year. Urban participants reported more travel to other urban centers (i.e. Guayaquil, Quito, and Santo Domingo) and rural participants

reported travel to the more local rural areas (other rural villages, Borbón, and San Lorenzo) (Figure S1). Duration of stay varied based on destination. 62% of participants traveling to urban destinations reported a duration of stay up to one week, with those traveling to Guayaquil and Quito staying for up to two weeks. Rural destinations on the other hand had shorter visits, with 73% of travelers to rural destinations reporting a visit of just one day. The reasons reported for travel are depicted in Figure 3**,** again with variable differences by destination. There were no significant differences in travel reasons between cases and controls. Across all sites, the most common reason for traveling was for "family" reasons (31% across all sites) followed by “medical” reasons (20% across all sites) and “shopping” (15% across all sites). Few participants (6%) reported traveling within Ecuador in the past week, so we were unable to carry out a robust analysis of these data.

Common characteristics of Ecuadorian travelers

Travel was significantly more common among rural participants than urban participants, with rural participants having eight times higher odds of travel in the past year compared to their urban counterparts (aOR = 8.10, 95% CI = 5.77-11.52), $p<0.001$) (Figure 2). Other demographic factors were analyzed to highlight characteristics associated with travel in this population (Table 3). Participants over the age of 5 were twice as likely to have traveled in the past year compared to those <5 (aOR = 2.04, 95% CI = 1.51-2.76, $p<0.001$). Additionally, individuals who report receiving government subsidized welfare were less likely to report travel in the past year (aOR=0.51, 95% CI = 0.35-0.75, $p<0.001$)

Domestic travel risk factors

To assess the potential risk that domestic travel presents to diarrheal disease transmission, we analyzed reported travel data to any destination in Ecuador in the past year. These data were analyzed both by travel origin and destination of travel. All analyses were adjusted for potential confounding by sex, age, race, economic status, study site (random effect for “all sites” models only), and known risk factors for diarrhea disease transmission, i.e., sanitation and water treatment. DAEC was the most commonly encountered *E. coli* pathotype, and the only pathotype with sufficient sample size for this analysis. Figure 4 displays the results by origin of travel. Any reported travel in the past year trended toward a positive association with diarrhea case status across all participants, although this result was not statistically significant (aOR = 1.30, 95% CI = 0.99-1.72, $p = 0.06$). However, participants traveling from Esmeraldas had more than double the risk of diarrhea as those

not traveling (aOR = 2.28, 95% CI = 1.20-4.41, p = 0.01). Additionally, participants from Quito (but not other sites or all sites combined) reporting travel in the past year had a higher risk of DEC infections (aOR = 2.61, 95% CI = 1.16-8.86, p = 0.02). No associations were observed between domestic travel in the past year and DAEC infections.

Figure 5 shows travel risks by destination of travel. Travel destinations included in this analysis, from most populous to least populous are as follows: Guayaquil, Quito, Santo Domingo, Esmeraldas, San Lorenzo, Borbón and rural villages. Participants from each of the four study sites reported destinations visited in the past year, which allowed us to observe trends in which destinations were frequented most by urban versus rural travelers and assess risks between expected high and low transmission regions (Figure S2). Participants reporting travel to Quito (primarily from Esmeraldas) were more likely to be a diarrhea case than those who did not report travel to Quito (aOR = 2.01, 95% CI = 1.10-3.68, p = 0.02). Participants reporting travel to San Lorenzo (primarily from Borbón) were nearly twice as likely to be infected with DEC than those who did not travel to this destination (aOR = 1.96, 95% CI = 1.09-3.47, p = 0.02). Participants reporting travel to Guayaquil (primarily from Esmeraldas and Quito) were twice as likely to be infected with DAEC (aOR = 2.09, 95% CI = 1.01-4.33, p = 0.04). However, those reporting travel to Esmeraldas (solely participants from Borbón and rural villages) were significantly less likely to present with DEC infections (aOR = 0.56, 95% CI = 0.33-0.96, p = 0.03), and specifically DAEC infections (aOR = 0.37, 95% CI = 0.17-0.78, p = 0.007), than those who did not travel to Esmeraldas.

DISCUSSION

"Travelers' diarrhea" is typically associated with visitors from low-transmission countries visiting high-transmission countries, but this study suggests that this phenomenon can also occur as a result of travel between higher- and lower-transmission locations within a given country. Global urbanization in recent decades has brought about infrastructure changes that provide increased accessibility to roads and transportation networks, facilitating travel and increasing contact with neighboring communities. This interconnectivity can be beneficial for rural communities' access to jobs and medical care. However, it can also facilitate transmission of enteric and other pathogens from high-transmission urban areas, especially when new or more virulent pathogens are introduced to previously naïve populations.

Results from our case-control study across a rural-urban gradient in northern Ecuador suggest that travel in the past year, and particularly travel to urban areas, was associated with an increased risk of illness and/or infection. We found elevated risk of diarrhea for subjects reporting domestic travel in the past year, and in particular travel from Esmeraldas, 69% of whom listed the more urban sites of Quito and Guayaquil as their destinations. (Figure 4a). Reported travel in the past year from Quito was associated with elevated risk of DEC infection (Figure 4b); 50% of these travelers listed Guayaquil, the only city in Ecuador larger than Quito, as their destination. The models assessing destination provide further insights. The elevated diarrhea risk associated with travel to Quito (Figure 5a) was primarily driven by travelers from Esmeraldas, who comprised 46% of travelers to Quito. The elevated diarrhea risk associated with travel to Borbón (Figure 5a) was primarily driven by travelers from rural villages, who comprised 98% of travelers to Borbón; however this elevated risk was not significant. DEC infection was higher in people reporting travel to San Lorenzo, 90% of whom were from Borbón or the rural villages (Figure 5b). Travel to Quito and Guayaquil, the most urban sites, also trended toward elevated risk of DAEC infection (Figure 5c). Interestingly, this pattern did not hold up for travelers reporting visits to Esmeraldas, 100% of whom were from the two rural study sites. Traveling to Esmeraldas was even protective against DEC and DAEC infections. The destination models were adjusted for study site, so the patterns cannot be attributed to specific pathogens circulating within a given site.

In general, we found that significantly more rural than urban participants travel and that the movement is towards more urban destinations. This makes sense as people living in rural areas need to travel for basic needs not found locally such as medical purposes, shopping, and paperwork (Figure 3). Most of the urban participants reporting travel also went to more urban destinations (Figure S1). Furthermore, travel to urban destinations such as Quito and Guayaquil was often associated with longer durations of stay, which increases the probability of contracting an enteric infection.

Previous studies in Northwestern Ecuador have shown that more rural communities have lower diarrhea rates, potentially due to reduced contact with outside individuals. [38, 37] Our study has identified a similar pattern but as it relates to travel, with rural participants from lower disease transmission environments being exposed to outside environments with higher disease transmission within urban centers. Urban, more populous regions may act as a hub for transmission of pathogens because of the higher density of people living and moving through the region, increasing intensity of pathogen transmission. Pathogen virulence has been shown to be related to transmission intensity in theoretical, natural, and experimental systems. [47, 48] In particular, high transmission rates,

denser host populations, and greater host mobility tend to select for highly virulent pathogens, whereas the reverse conditions select for less-virulent pathogens. This well-described evolutionary relationship may have consequences for diarrheal disease management over large scales. If urban-derived pathogen strains cause increased morbidity and mortality amongst rural populations as a function of travel, a cost-effective country-wide strategy may be to focus on large-scale infrastructural water provision or vaccination efforts in urban areas. In order to better target these interventions, further investigation into regional and even local pathogen-specific disease prevalence is needed to better understand the potential in-country travel risks between areas of higher and lower disease transmission.

In addition to travel, a variety of environmental factors was assessed. We found few and even contradicting associations between improved water and sanitation conditions and diarrhea case status as well as DEC-specific infections. Nearly all participants in this study had an improved drinking water source, even in rural areas, so we might not have had sufficient variability in this exposure to detect a difference. However, despite the high prevalence of improved drinking water, treatment of household drinking water was protective against diarrhea, especially in urban areas where this behavior was more heavily practiced. This finding emphasizes that "improved" drinking water sources may not necessarily be safe [49, 50], and that the practice and encouragement of water treatment is justified to reduce the transmission of diarrheal illness via water consumption.

We also found that recent contact with animals was associated with diarrheal disease. While we did not have sufficient data to explore these associations in depth, our results are suggestive that contact with animals in and around the home may increase the risk of diarrheal disease. Animals are proving to be important in the transmission of some enteric pathogens and further research is needed [51-53].

This study had several limitations. We focused on reported travel in the past year due to a lack of individuals reporting travel in the past week, limiting our ability to attribute risk to recent travel. We adjusted our models to account for a number of potential confounding factors associated with travel in the past year (e.g., socioeconomic status) but there may be other unmeasured factors influencing rates of travel. Travel itself is variable, with individual differences in destinations, reasons for travel, duration of stay, and behavioral practices of which we incorporated into these findings but not all factors could be included in our models. Travelers to urban locations reported longer durations of stay, so the increased risk of disease associated with urban destinations may simply be attributable to increased probability of acquisition of a pathogen over longer time periods. Our data unfortunately does not have the resolution to account for this possibility. However, even if length of

stay accounts for the increased risk, the overall finding of increased risk associated with travel to urban area still holds. Additionally, subject recruitment was based on diarrhea case or control status, so the DEC results were based on a cross-sectional analysis within our case-control study. However, we adjusted the DEC models for diarrhea case status to account for potential confounding based on disease status. In addition, due to logistical considerations, sampling in rural communities was somewhat different than in the urban communities. In the rural communities, we recruited subjects via two strategies: First, we recruited participants from rural communities who presented at the Borbón Hospital (31% of rural participants). This strategy was limited by the number of diarrhea cases that presented, and also by the ability to obtain samples from age-matched controls, as visitors to Borbón were eager to return home. To supplement this strategy our staff accompanied Ministry of Health medical teams on their regular field visits to communities and enrolled cases and controls during these community clinics (69% of rural participants). Thus, these individuals may have had less severe diarrhea than those presenting at the hospitals or clinics in the other sites. However, the severity of these participants' diarrhea for rural visitors to the Borbón Hospital was likely greater than participants at the other sites, as their illness had to be severe enough to warrant a trip to Borbón. Furthermore, these findings many not be applicable to other regions with more or less interconnectivity than found in Ecuador.

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Table 1. Characteristics of study participants by site and disease status in the EcoZUR case-control study of diarrhea in four sites across an urban-rural gradient in northern Ecuador. This table highlights the expected differences in sociodemographic factors as well as water/sanitation practices along an urban-rural gradient with fewer urban participants receiving welfare and more receiving higher education, sustaining employment, and overall improved water and sanitation compared to rural participants. However, within each study site there were no significant differences in demographic or sociodemographic characteristics between cases and controls with the expectation of water, sanitation and animal contacts as referenced in the text.

	Quito (*N* = 253)			Esmeraldas (*N* = 209)			Borbón (*N* = 243)			Rural Villages (*N* = 202)			Overall differences across study sites
	Overall *N* (%)	Cases *n* (%)	Controls *n* (%)	**Overall *N* (%)**	Cases *n* (%)	Controls *n* (%)	**Overall *N* (%)**	Cases *n* (%)	Controls *n* (%)	**Overall *N* (%)**	Cases *n* (%)	Controls *n* (%)	*p*-value
Demographics													
Age													**0.01**
0-24 months	**92 (36.4)**	45 (34.6)	47 (38.2)	**73 (34.9)**	35 (35.0)	38 (34.9)	**85 (35.0)**	49 (36.8)	36 (32.7)	**58 (28.7)**	37 (30.8)	21 (25.6)	
25-60 months	**37 (14.6)**	20 (15.4)	17 (13.8)	**36 (17.2)**	16 (16.0)	20 (18.3)	**33 (13.6)**	20 (15.0)	13 (11.8)	**54 (26.7)**	32 (26.7)	22 (26.8)	
61-180 months	**43 (17.0)**	20 (15.4)	23 (18.7)	**47 (22.5)**	23 (23.0)	24 (22.0)	**45 (18.5)**	24 (18.0)	21 (19.1)	**41 (20.3)**	22 (18.3)	19 (23.2)	
181+ months	**81 (32.0)**	45 (34.6)	36 (29.3)	**53 (25.4)**	26 (26.0)	27 (24.8)	**80 (32.9)**	40 (30.1)	40 (36.4)	**49 (24.3)**	29 (24.2)	20 (24.4)	
Male	**124 (49.0)**	68 (52.3)	56 (45.5)	**113 (54.1)**	59 (59.0)	54 (49.5)	**132 (54.3)**	78 (58.6)	54 (49.1)	**108 (53.5)**	65 (54.2)	43 (52.4)	**0.61**
Race													**<0.001**
White	**8 (3.2)**	5 (3.8)	3 (2.4)	**1 (0.5)**	0 (0.0)	1 (0.9)	**0 (0.0)**	0 (0.0)	0 (0.0)	**0 (0.0)**	0 (0.0)	0 (0.0)	
Black	**3 (1.2)**	2 (1.5)	1 (0.8)	**87 (41.8)**	38 (38.0)	49 (45.4)	**133 (54.7)**	72 (54.1)	61 (55.5)	**108 (53.5)**	58 (48.3)	50 (61.0)	
Indigenous	**5 (2.0)**	3 (2.3)	2 (1.6)	**1 (0.5)**	1 (1.0)	0 (0.0)	**9 (3.7)**	6 (4.5)	3 (2.7)	**37 (18.3)**	29 (24.2)	8 (9.8)	
Mixed	**237 (93.7)**	120 (92.3)	117 (95.1)	**119 (57.2)**	61 (61.0)	58 (53.7)	**101 (41.6)**	55 (41.4)	46 (41.8)	**57 (28.2)**	33 (27.5)	24 (29.3)	
Sociodemographics													
Family receives welfare	**15 (5.9)**	5 (3.8)	10 (8.1)	**42 (20.1)**	20 (20.0)	22 (20.2)	**54 (22.2)**	27 (20.3)	27 (24.5)	**97 (48.0)**	58 (48.3)	39 (47.6)	**<0.001**
Family member employed	**196 (77.5)**	104 (80.0)	92 (74.8)	**94 (45.0)**	46 (46.0)	48 (44.0)	**105 (43.2)**	52 (39.1)	53 (48.2)	**39 (19.3)**	29 (24.2)	10 (12.2)	**<0.001**
Highest level household education													**<0.001**
None	**1 (0.4)**	1 (0.8)	0 (0.0)	**3 (1.4)**	0 (0.0)	3 (2.8)	**1 (0.4)**	0 (0.0)	1 (0.9)	**6 (3.0)**	3 (2.5)	3 (3.7)	
Elementary	**26 (10.3)**	11 (8.5)	15 (12.2)	**14 (6.7)**	9 (9.1)	5 (4.6)	**14 (5.8)**	10 (7.6)	4 (3.6)	**62 (30.7)**	36 (30.0)	26 (31.7)	
High School	**124 (49.0)**	69 (53.1)	55 (44.7)	**112 (53.8)**	56 (56.6)	56 (51.4)	**178 (73.6)**	101 (76.5)	77 (70.0)	**120 (59.4)**	69 (57.5)	51 (62.2)	
University	**102 (40.3)**	49 (37.7)	53 (43.1)	**79 (38.0)**	34 (34.3)	45 (41.3)	**49 (20.2)**	21 (15.9)	28 (25.5)	**14 (6.9)**	12 (10.0)	2 (2.4)	
Water, Sanitation, and Animal Contact													
Improved household sanitation[a]	**197 (77.9)**	103 (79.2)	94 (76.4)	**145 (71.1)**	70 (73.7)	75 (68.8)	**148 (60.9)**	86 (64.7)	62 (56.4)	**82 (40.6)**	45 (37.5)	37 (45.1)	**<0.001**
Improved drinking water source[b]	**252 (99.6)**	130 (100.0)	122 (99.2)	**207 (99.5)**	99 (99.0)	108 (99.1)	**221 (90.9)**	122 (91.7)	99 (90.0)	**167 (82.7)**	102 (85.0)	65 (79.3)	**<0.001**
Drinking water treatment[c]	**163 (64.4)**	77 (59.2)	86 (69.9)	**85 (40.9)**	32 (32.0)	53 (48.6)	**39 (16.1)**	20 (15.0)	19 (17.3)	**44 (21.8)**	30 (25.0)	14 (17.1)	**<0.001**
Reported animal contact[d]	**140 (55.3)**	70 (53.8)	70 (56.9)	**94 (45.0)**	49 (49.0)	45 (41.3)	**100 (41.2)**	64 (48.1)	36 (32.7)	**64 (31.7)**	34 (28.3)	30 (36.6)	**<0.001**

[a]Improved = Flush toilets, personal latrine, and/or septic system. [b]Improved = Household tap, reclaimed rainwater, and/or purchased bottled water. [c]Options included boiling, chlorine usage, filtration, UV irradiation, larvicide treatment and/or settling techniques. However the majority (89.6%) across all sites was boiling. [d]Animal contact was defined as reported contact with any animal(s) in the past week

Table 2. Multivariate Logistic Regression Models for Risk of (A) Diarrheal Disease and (B) Diarrheagenic *E. coli* infections across an urban-rural gradient: Effects of household water and sanitation practices on disease outcomes. Variables adjusted for in the models are given in the text. Total number of participants reporting diarrhea = 483. Total number of participants with diarrheagenic *E. coli* infections = 208. *=p<0.05, **=p<0.01, ***=p<0.001.

	Reported use of improved household sanitation facilities			Reported use of improved household drinking water source			Reported treatment of household drinking water		
Study Participants	*n*(%)	aOR (95%CI)	*p*-value	*n*(%)	aOR (95%CI)	*p*-value	*n*(%)	aOR (95%CI)	*p*-value
A. Association with diarrhea case status (*n*=483)									
All Participants	572 (63.4)	1.23 (0.88, 1.70)	0.22	847 (93.5)	1.15 (0.67, 1.98)	0.62	331 (36.6)	0.72 (0.54, 0.96)	0.03*
Quito	197 (77.9)	1.12 (0.54, 2.33)	0.76	252 (99.6)	–	1.00	163 (64.4)	0.64 (0.37, 1.10)	0.11
Esmeraldas	145 (71.1)	1.05 (0.46, 2.40)	0.91	207 (99.5)	–	0.99	85 (40.9)	0.34 (0.17, 0.64)	0.001***
Borbón	148 (60.9)	2.23 (1.14, 4.50)	0.02*	221 (90.9)	0.90 (0.31, 2.54)	0.84	39 (16.1)	0.77 (0.36, 1.63)	0.49
Rural River Communities	82 (40.6)	0.96 (0.50, 1.85)	0.91	167 (82.7)	1.57 (0.72, 3.43)	0.25	44 (21.8)	2.05 (0.98, 4.45)	0.06
B. Association with diarrheagenic *E. coli* (*n*=208)									
All Participants	489 (63.8)	0.90 (0.60, 1.33)	0.59	770 (93.0)	1.00 (0.50, 1.97)	0.99	266 (34.5)	0.79 (0.55, 1.14)	0.21
Quito	152 (82.2)	3.67 (1.17, 13.3)	0.03*	184 (99.5)	–	1.00	116 (62.7)	1.19 (0.55, 2.65)	0.66
Esmeraldas	134 (71.7)	0.39 (0.15, 0.97)	0.05*	190 (99.5)	–	1.00	80 (41.9)	1.18 (0.58, 2.42)	0.64
Borbón	128 (59.5)	0.83 (0.37, 1.86)	0.64	196 (91.2)	1.60 (0.47, 6.17)	0.47	32 (14.9)	0.79 (0.26, 2.08)	0.64
Rural River Communities	75 (41.9)	0.52 (0.22, 1.18)	0.12	146 (81.6)	1.31 (0.51, 3.75)	0.59	38 (21.2)	0.55 (0.18, 1.50)	0.27

aOR = adjusted odds ratio; CI = confidence interval.

Table 3. Multivariate Logistic Regression Model for Characterizing Ecuadorian Travelers: Demographic Characteristics Associated with Domestic Travel in the Past Year among Study Participants. All variables listed were included together in the model. *=p<0.05, **=p<0.01, ***=p<0.001.

		Reported Domestic Travel in Past Year	No reported domestic travel in the past year		
Demographics	***n* (%)**	***n* (%)**	***n* (%)**	**aOR (95% CI)**	**p-value**
Study Site					
Urban (Quito + Esmeraldas)	462 (50.9)	125 (30.0)	337 (68.6)	Ref.	Ref.
Rural (Borbón + Rural Villages)	445 (49.1)	291 (70.0)	154 (31.4)	8.10 (5.77, 11.52)	<0.001***
Age					
0 – 5 yrs old	468 (51.6)	185 (44.5)	283 (57.6)	Ref.	Ref.
>5 yrs old	439 (48.4)	231 (55.5)	208 (42.4)	2.04 (1.51, 2.76)	<0.001***
Male	477 (52.6)	230 (55.3)	247 (50.3)	1.30 (0.96, 1.75)	0.09
Receives government welfare	208 (22.9)	88 (21.1)	120 (24.4)	0.51 (0.35, 0.75)	<0.001***
Member of family employed	434 (48.0)	192 (46.2)	242 (49.4)	1.11 (0.80, 1.56)	0.53
Highest level of household education					
University	244 (27.0)	109 (26.3)	135 (27.6)	Ref.	Ref.
High School	534 (59.0)	262 (63.1)	272 (55.5)	0.70 (0.48, 1.01)	0.06
Elementary	116 (12.8)	40 (9.6)	76 (15.5)	0.34 (0.19, 0.59)	<0.001***
None	11 (1.2)	4 (1.0)	7 (1.4)	0.30 (0.07, 1.16)	0.09

Figure 1. Region of Study. This map highlights the region (red dashed circle) and four study sites (yellow stars) where the EcoZUR study was conducted. These four sites comprise an urban-rural gradient in northern Ecuador: including Quito (pop. ~1.62 M), Esmeraldas (pop. ~162,000), Borbón (pop. ~5,000) and the rural villages (pop. ~50-500).

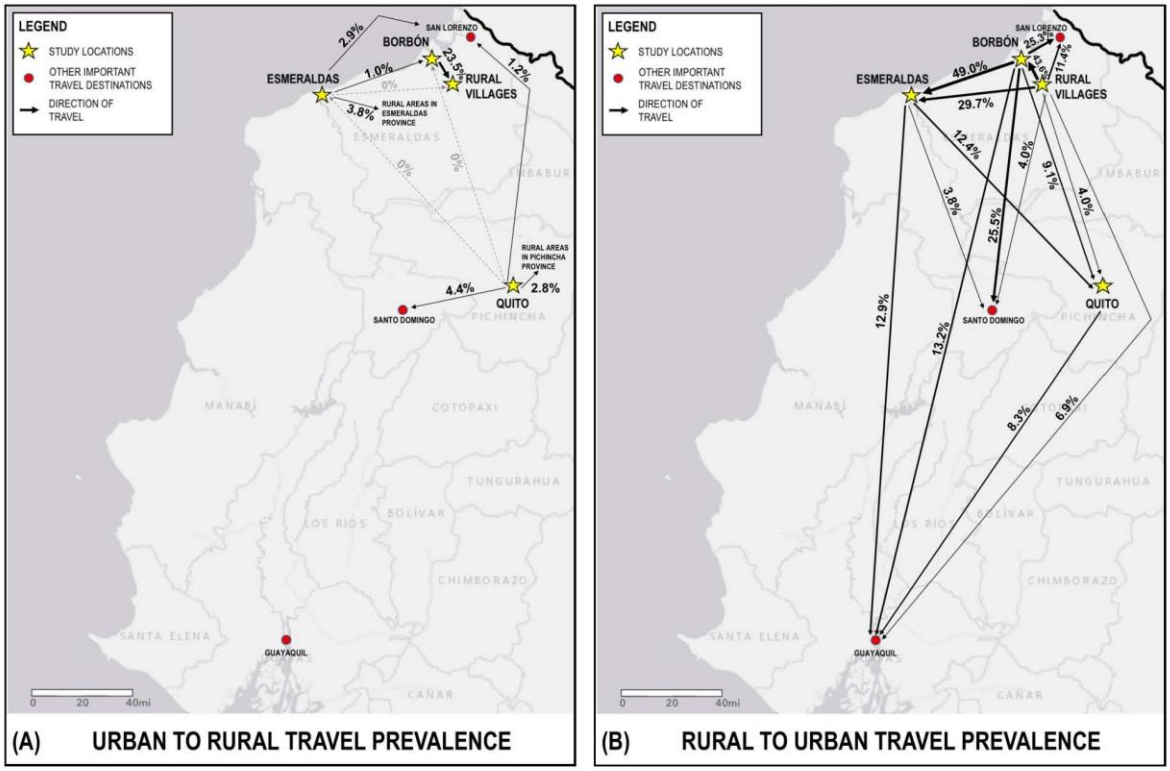

Figure 2. Prevalence of reported travel between study sites in the past year. Travel destination is denoted by the arrow, with the thickness of the arrow weighted by prevalence of reported travel to this destination. (A) Travel from urban/more populated cities to more rural/less populous communities. (B) Travel from rural/less populous communities to urban/more populated cities. It is important to note that not all travel is represented in these figures since data was only collected from participants at the four study locations (yellow stars). Individuals residing in Guayaquil, Santo Domingo and San Lorenzo may engage in more urban to rural travel or rural to urban travel that is not illustrated above.

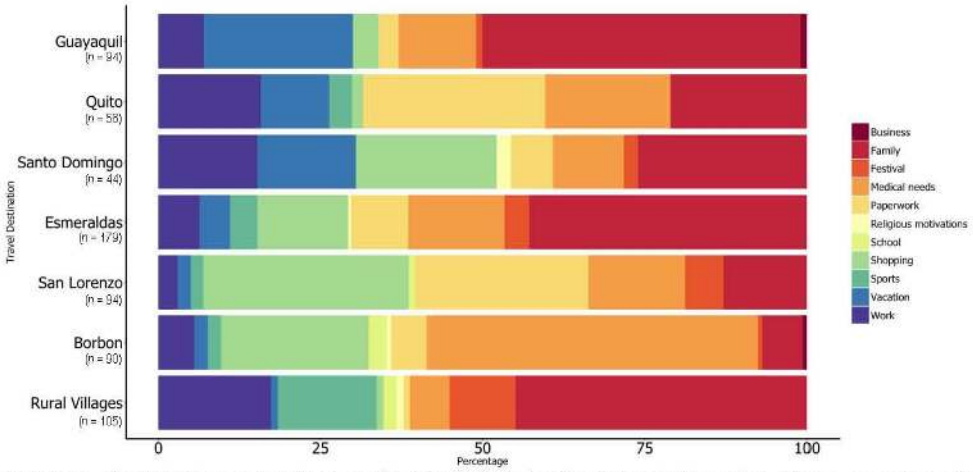

Figure 3. Reported reasons for traveling to listed destinations in Ecuador. These responses include those of all participants reporting travel in the past year (*N*=434), with varying proportions of individuals traveling to each destination.

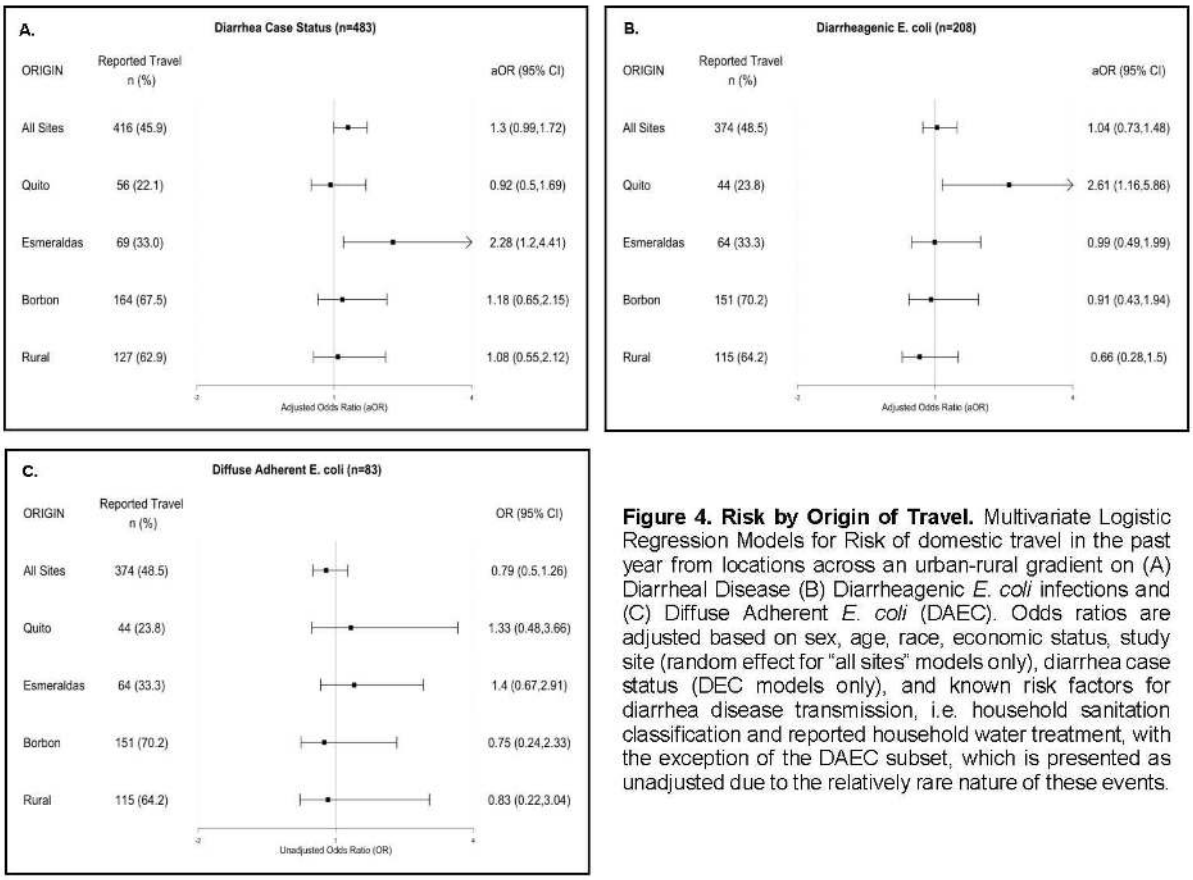

Figure 4. Risk by Origin of Travel. Multivariate Logistic Regression Models for Risk of domestic travel in the past year from locations across an urban-rural gradient on (A) Diarrheal Disease (B) Diarrheagenic *E. coli* infections and (C) Diffuse Adherent *E. coli* (DAEC). Odds ratios are adjusted based on sex, age, race, economic status, study site (random effect for "all sites" models only), diarrhea case status (DEC models only), and known risk factors for diarrhea disease transmission, i.e. household sanitation classification and reported household water treatment, with the exception of the DAEC subset, which is presented as unadjusted due to the relatively rare nature of these events.

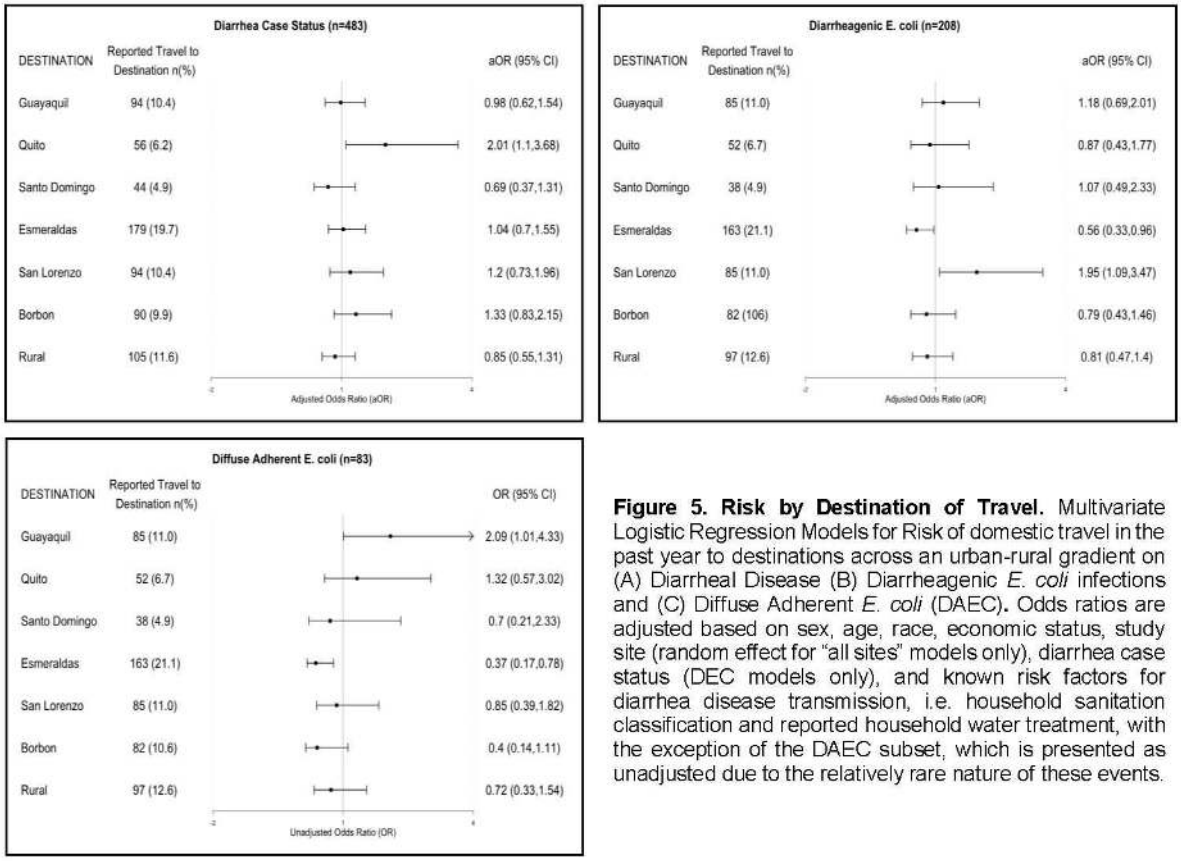

Figure 5. Risk by Destination of Travel. Multivariate Logistic Regression Models for Risk of domestic travel in the past year to destinations across an urban-rural gradient on (A) Diarrheal Disease (B) Diarrheagenic *E. coli* infections and (C) Diffuse Adherent *E. coli* (DAEC)**.** Odds ratios are adjusted based on sex, age, race, economic status, study site (random effect for "all sites" models only), diarrhea case status (DEC models only), and known risk factors for diarrhea disease transmission, i.e. household sanitation classification and reported household water treatment, with the exception of the DAEC subset, which is presented as unadjusted due to the relatively rare nature of these events.