Whose Feces Matter? Household Fecal Contamination and Drug-Resistant Campylobacteriosis in the Peruvian Amazon

by
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Abstract

Household fecal contamination is regularly targeted outcome in community-wide interventions, yet the origin of fecal contamination remains unexplored. This research gap is critical given the high attributable fraction of zoonotic enteric pathogens related to enteric disease among pediatric populations living in resource constraint settings. Of these, *Campylobacter* spp., a poultry-associated bacterium, is the most prevalent among epidemiologic and geographic contexts. As a result, this body of work 1) validated a molecular tool to attribute household surface fecal contamination to a specific source, 2) measured specie-specific fecal contamination on household surfaces and 3) characterized the population structure and antimicrobial resistance of *Campylobacter* spp. isolated from industrially raise and household-raised poultry. Of the eight microbial source tracking markers validated, avian fecal markers *Av4143*, and swine fecal marker *Pig2Bac* showed excellent performance parameters, while human fecal markers *BacHum* and *HF183*-Taqman, as well as avian exposure markers *CytB* and *ND5* showed moderate performance. Household surfaces were more frequently contaminated with animal fecal material, specifically avian fecal material), in comparison to human fecal material. Floors were more contaminated than tables, and unfinished floors and wooden tables were more contaminated than their counterparts. A higher burden of avian fecal contamination was associated with lower age of the primary caregiver and shorter household tenancy. Detecting *Campylobacter* spp. in household surfaces was highly associated with the presence of the avian fecal marker *Av4143*. *Campylobacter* spp. isolates from industrially raised chickens had a distinct population structure form its household-raised counterparts, as determined by three independent genomic assessments. Moreover, higher prevalence

of antimicrobial resistance genes associated with macrolides, fluoroquinolones and aminoglycosides antibiotics were found among *Campylobacter* spp. isolates from industrially raised poultry. However, *Campylobacter* spp. from household-raised poultry had a lipooligosacharide class associated with post-infection neuropathies such as Guillan-Barre Syndrome. Based on this research, we propose that future interventions that seek to reduce household fecal contamination should target both human and animal sources of feces, as well as household infrastructure characteristics. Finally, household prevention measures to reduce *Campylobacter* spp. transmission events at the human-poultry interphase should be prioritized, as well as the implementation of legislative measures that aim to reduce sub-therapeutic use of antimicrobials at the industry level for growth promotion of prophylactic measures.

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List of Terms and Abbreviations

AB Prisma	Asociación Benéfica Prisma
AMP	Ampicillin
AMX	Amoxicillin + Clavulanic Acid
AZM	Azithromycin
C	Chloramphenicol
CC	Clonal Complex
CFU	Colony Forming Units
CIP	Ciprofloxacin
CRO	Ceftriaxone
EE	Environmental Enteropathy
EED	Environmental Enteric Dysfunction
ERY	Erythromycin
ETEC	Enterotoxigenic E. coli
FAO	Food and Agricultural Organization of the United Nations
GBD	Global Burden of Disease
GBS	Guillain Barre Syndrome
GEMS	Global Enteric Multicenter Study
GM	Gentamicin
HAZ	Height for Age Z-Score
LOS	Lipopolysaccharides
MALED	Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health
MLST	Multi Locus Sequence Typing
MST	Microbial Source Tracking
NAL	Nalidixic Acid
TE	Tetracycline
TFIB	Traditional Fecal Indicator Bacteria
TMS	Trimethoprim + Sulfamethoxazole
WAZ	Weight for Age Z-Score
WHZ	Weight for Height Z-Score

Chapter 1. Introduction

1.1 Study objectives

Poor access to sanitation and environmental fecal contamination continue to be pressing human health issues in low-resource, tropical communities. The human health consequences include the continuous exposure to pathogenic bacteria and resulting environmental enteropathy (EE). Environmental Enteropathy is a subclinical pathology that is an important cause of undernutrition and stunting among children (1-3). In the semi-rural communities of Santa Clara de Nanay, Iquitos, Peru, the burden of enteric diseases is among the highest reported in the world (4-8). In this setting, fecal contamination is common and ubiquitous, yet the relative source attribution of contamination and associated health impacts remain largely unexplored (9).

Recent studies indicate that *Campylobacter* spp., a zoonotic bacterial pathogen, is one of the leading contributors to EE in children (4, 7, 10-12). *Campylobacter* spp. is often transmitted via consumption of undercooked poultry meat and by-products, with water-borne transmission being a secondary, related pathway (13). *Campylobacter* spp. transmission may also be influenced by climatic and environmental factors and may have, in many locations, a springtime peak driven directly by changes in temperature, or indirectly by seasonal changes in animal husbandry activity increasing contact between human and animal hosts (14, 15). However, in highly endemic areas the relative importance of these interrelated pathways are as yet poorly characterized. Additionally, the emergence of fluoroquinolone and macrolide resistance of *Campylobacter* species is of global concern due to a number of factors: the lack of alternative therapeutics; the

hypothesized association with antibiotic use in the industrial production of poultry; and the transferability of antibiotic resistance (ABR) genes, such as the rRNA *erm(B)* gene conferring macrolide resistance, the *tet(O)* and *tet(A)* plasmid mediated genes conferring tetracycline resistance, the *aacA4* gene conferring aminoglycoside resistance, and the multidrug *cmeABC* efflux pump which confers high level resistance to fluoroquinolones, macrolides, tetracycline and ampicillin (16-24).

In the Peruvian Amazon, 5.6% of diarrhea cases in children under 11 months and 10% in children 12 to 24 months of age are attributable to *Campylobacter* spp.(4, 5) Further, our preliminary data suggests that atypical *Campylobacter* species (i.e. non *Campylobacter jejuni* or *Campylobacter coli*) are responsible for a high proportion of infections indicating an additional, unknown source of infection that is not derived from poultry(25). Dogs, a known reservoir of atypical *Campylobacter* species, may also be important in this setting (26-28). Additionally, our own investigations have demonstrated that 79% of over 900 *Campylobacter* spp. isolates in children show phenotypic resistance to ciprofloxacin and 25.0% of *C. coli* isolates to azithromycin, suggesting the presence of high antibiotic pressure from undetermined sources, a finding that has important consequence as these are the two principal oral antibiotics recommended for the treatment of Campylobacteriosis (29). In this same setting, poultry production takes place both at the industrial and at the household level, providing a source of income and a stable source of animal protein in the form of both flesh and eggs associated with a largely underestimated risk of enteric disease (30). Antibiotics are freely used at all production levels, and it is unclear whether households or industrial producers are the priority to target in order to diminish human AMR *Campylobacter* infections.

In order to develop effective disease control interventions and mitigation strategies, there is a need for a systematic approach to identifying the species-specific source of fecal contamination within the household as well as to investigate the molecular epidemiology of drug-resistant Campylobacteriosis in this endemic setting. Specifically, there is a need to explore the phylogenetic relationship of poultry derived *Campylobacter* spp. isolates from distinct rearing and production sectors to determine force of antibiotic pressure from animal use, as well as to determine the relationship between human and poultry *Campylobacter* spp. isolates, As a result, this dissertation seeks ***to understand how interactions among humans, animals and environment contribute to household fecal exposure and human Campylobacteriosis, and to characterize the role of industry and backyard raised poultry in the transmission of multi-drug resistant Campylobacter spp.*** This will be achieved through the following specific aims:

Aim 1: **To validate eight microbial source tracking markers for the detection of avian, canine and human feces, as well as avian exposure, for future evaluation of household surfaces fecal contamination in the Peruvian Amazon.**

This dissertation aims to determine the burden of animal fecal contamination within household of peri-urban Iquitos, Peru. We have chosen to use microbial source tracking markers as the method of preference to attribute fecal contamination to a specific animal or human source. Before these markers are used in the field they need to be validated to determine their true performance in this setting. Aim1 feeds directly into the development of Aim 2.

- Hypothesis 1: Microbial source tracking markers slightly variable levels of sensitivity and specificity in comparison to validation studies done elsewhere around the world but are useful tools in delineating routes of exposure and informing mitigation strategies.

Aim 2: To determine the burden of avian exposure and fecal contamination of household surfaces using microbial source tracking markers, and associate the burden of fecal contamination to household sanitation and infrastructure.

Using the previously validated microbial source tracking markers, we will detect and quantify avian, canine, swine and human fecal contamination in floor and table surfaces of households in Iquitos, Peru. We will then determine the association of these markers with the materials of each surface, as well as other demographic and socio-economic characteristics of the households. Finally, we will determine the association between the presence of avian fecal markers and the probability of detecting *Campylobacter* spp. DNA in surface samples.

- Hypothesis 2: Animal fecal material will be detected in a greater number of surfaces and in greater quantities than human fecal material. The material of each surface will influence the quantities of animal fecal material or avian exposure detected. The detection of avian fecal material will be correlated with detecting *Campylobacter* spp. on household surfaces.

Aim 3: To assess the phylogenetic relatedness and compare the genetic antimicrobial resistance determinants between *Campylobacter* spp. isolated from free-range and farm raised poultry in Iquitos, Peru.

We will collect fecal samples from poultry raised in the backyards of community households, as well as from poultry raised in concentrated animal feeding operations (i.e. industrial farms). We will compare the phenotypic and genetic patterns of antimicrobial resistance from *Campylobacter* spp. isolates, and we will establish the phylogenetic relationship of our isolates.

- Hypothesis 3: *Campylobacter jejuni* and *Campylobacter coli* will be detected from poultry fecal samples. There will not be a clear population structure that differentiates *Campylobacter* spp. isolates from farm and backyard raised poultry. Higher number of antimicrobial resistance genes and mutation will be detected in industrially raised poultry in comparison to backyard raised poultry.

1.2 Organization of the dissertation

The first chapter of this dissertations reviews the current literature on the global burden of diarrhea and focuses on the importance of zoonotic enteric pathogens. It also seeks to propose a shift in paradigm when thinking about water, hygiene and sanitation interventions, arguing that animal fecal waste is as important than human fecal waste when designing and executing intervention studies. Further, it presents the use of microbial source tracking (MST) markers as a method of attributing and measuring animal fecal waste within household surfaces. Finally, it reviews the current literature on the clinical importance and burden of multi-drug resistant *Campylobacter* spp. in the developing world. The body of the dissertation is divided into three chapters. The first and second paper validate MST markers in Iquitos, Peru, and applies them to detect, attribute and quantify the burden of animal and human fecal waste on household floors and table surfaces. The second chapter also links the burden of source specific fecal contamination with household infrastructure characteristics, socio-economic characteristics, and the detection of *Campylobacter* spp. DNA in household surfaces. The third chapter of this dissertation presents a comparative analysis of *Campylobacter* spp. isolated from backyard and industrially-raised poultry, identifying industrially-raised poultry as the main contributor of multi-drug resistant *Campylobacter* spp. in Iquitos, Peru. The next chapter includes the overall strengths and limitations of the three research papers, as well as a broad set of conclusions. The final chapter provides the overall public health implications and policy recommendations. A curriculum vitae of the author, list of tables and figures as well as references used throughout this dissertation are provided at the end of this document.

Chapter 2. Literature review

2.1 Global Burden of Diarrhea and Zoonotic Enteric Disease

Diarrheal diseases continue to be a leading causes of death in adults and children of low income settings. The Global Burden of Disease study estimated that in 2015 diarrheal diseases were the 4th leading cause of death in children under 5 years of age, causing 499,000 deaths a year. These deaths correspond to 38% of the total number of deaths attributed to diarrhea worldwide (1.3 million) (31). Results from the Global Enteric Multicenter Study (GEMS) indicate that worldwide the most important pathogens responsible for moderate to severe diarrhea in children under 5 years old are *Shigella* sp. and Enterotoxigenic *Escherichia coli* (ETEC), while results from The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) multi-site cohort study report *Campylobacter* spp., Norovirus and Astrovirus as the pathogens with the highest attributable burden of diarrheal disease in children under 2 years of age, and *Campylobacter* spp. as a leading cause of moderate to severe diarrhea (4, 11).

Poor access to sanitation and environmental fecal contamination continue to be pressing human health issues in low-resource, tropical communities. The consequences of this reality include the well-described pervasive cycle of infection and malnutrition. Although this entity was first described in the 1960s, it continues to be widely studied but poorly understood and a pressing public health issue (32). The current thinking is that continuous exposure to pathogenic enteric pathogens causes consecutive insults to the

mucosal and epithelial lining of the human gastrointestinal tract, which in turn is associated with nutrient malabsorption, systemic inflammation, and altered nutrient utilization. As a consequence, the already malnourished child is unable to mount an innate immune response at the intestinal or systemic level, and subsequent infections are more prolonged and severe (1, 2).

In low resource tropical settings, where pathogen exposure starts at birth, it has been found that the loss of the normal histological architecture of the intestinal epithelium is positively correlated with age. Specifically, intestinal villi become flattened, cell surface area is reduced, and as a result, nutrient absorption is also reduced, increasing the risk of reduced growth or stunting. This condition was first recognized in expatriated healthy adults in South Asia and was named "tropical enteropathy" or "jejunitis" (33, 34). However, this same pathology (although generally irreversible) occurs in children living in tropical areas, in which the impairment of gut functions starts at an early age. As a result, it was renamed as "Environmental Enteric Dysfunction" or "Environmental Enteropathy" (1-3, 35). Environmental Enteropathy (EE) has been associated with a reduced absorptive capacity of the intestinal lining, increase permeability, and chronic intestinal and systemic inflammation, leading to undernutrition, impaired immunologic and cognitive responses, and overall loss of human potential (35-39). Many experts believe that this condition is one of the main reasons for poor outcomes in community-wide nutritional interventions as the gut does not uptake and the host does not utilize nutrients in a manner that supports optimal growth and development (40).

Results from the MAL-ED cohort study indicate that exposure to enteropathogens, particularly highly pathogenic bacterial pathogens such as *Campylobacter* spp., *Shigella* sp. are associated with a higher degree of inflammation, systemic inflammation and growth failure (according to the association of MPO, AAT and AGP with growth), in comparison to other less intrusive pathogens such as Rotavirus, Adenovirus and Enterototoxigenic *E. coli* (ETEC). However, these EE biomarkers are not able to explain the cumulative growth failure that is evidenced in children under 2 years of age in low resource settings (40).

Campylobacter spp, *Cryptosporidium* sp. and Shiga-Toxin producing *E. coli* (STEC) are three zoonotic enteropathogens with high attributable fraction of diarrhea, as determined by GEMS and the MAL-ED study (Table 1) (4, 11, 41). Although these studies have different underlying study designs and measures of association, they both report elevated attributable fractions of diarrhea associated with zoonotic enteropathogens. The impact in morbidity and mortality associated with these three zoonotic pathogens is enough to explore the causal associations between exposure to feces from domestic animal origin and human disease. That said, most of these studies assess human and child exposure to livestock, poultry or domestic animals, and few assess fecal contamination as the main exposure. Of the zoonotic enteric agents, *Campylobacter* spp. is the most prevalent across epidemiologic contexts.

2.2 A One Health Approach to Enteric Disease Prevention, Water, Sanitation and Hygiene

Animal fecal waste is ubiquitous. Fecal waste from poultry, cattle, sheep and pigs make up 85% of the worlds animal fecal waste, which translated to $2.62x10^3$ kg/year. Thus, it is not surprising that zoonotic enteric pathogens are responsible for a third of diarrhea attributed deaths, globally (31, 42). There is a large amount of human-animal interaction that takes place within the household domain, as well as human exposure to animal fecal waste resulting from community wide poor infrastructure and sanitation that exposes populations to animal fecal effluents. One of the reasons for this is that small scale animal production is a common source of income, and in many cultures, animals are important economic assets (43, 44). With such a high burden of animal fecal waste in most low income, tropical, settings, it is reasonable to hypothesize that trials using WASH (water, sanitation and hygiene) interventions to reduce diarrhea hygiene associated diseases have produced inconclusive results because the reduction of animal waste is seldom targeted. The association between diarrhea and domestic animal husbandry was evaluated by Zambrano and collaborators in a systematic review and meta-analysis (45). Overall, authors reviewed 23 studies that evaluated domestic livestock and poultry as risk factors for diarrhea among household members of all ages, and only included 7 studies evaluating the effect of *Campylobacter* spp. in a quantitative meta-analysis. This analysis yielded that the overall odds of human Campylobacteriosis increased by 2.73 (95% CI 1.90 – 3.93) with domestic exposure to poultry (45). An analysis of DHS data of 30 Sub-Saharan African countries evaluated the relationship between quantity of livestock owned by households and health outcomes including: 2-week prevalence of diarrhea, stunting

and all-cause mortality. Results were mixed and country dependent, especially for diarrhea as the main outcome. The reason for this would probably be the use of data with inherent response bias and not considering the effect of potential confounders such as geographic area and socioeconomic status (46).

Studies evaluating animal ownership and anthropometric outcomes are inconclusive, the reason for which can be hypothesized to be that animals, in addition to contributing to enteropathogen exposure, also contribute to nourishment and economic stability of the household (47-50). Multiple studies evaluating the effect of livestock or poultry ownership either in Asia or Sub-Saharan Africa have site specific associations, with both positive and negative relationships between anthropometric outcomes (such as height-for-age z-scores (HAZ)) and livestock ownership (51, 52). A cohort study in Kenya found no overall association between livestock ownership and child growth, yet they did determine that children under 2 years of age, in households with livestock with a history of gastrointestinal diseases gained less weight than children in household with healthy livestock (95% CI: -0.063, -0.003 kg/month) (53). A cohort study in Bangladesh reported higher adjusted odds of stunting among children sleeping near animal corrals 2.43 (95% CI 1.08, 5.43) (54). Finally, a cross-sectional survey done in rural Ethiopia found that poultry ownership was associated with a higher HAZ (0.291; 95%CI: 0.197,0.385), while children with lower HAZ (-0.250; 95%CI: -0.368, -0.132) were associated with households that corralled chickens within the house (52, 55).

Even fewer studies evaluate the EE and microbiome changes in children exposed to livestock or livestock feces. A study in Kenya investigated the shared fecal microbiome of children <5 years old, household surfaces and animals (N=156). Although the microbiome was not found to be shared between humans, animals and surfaces, the study found higher microbiome diversity among children responsible for animal husbandry chores, in comparison with children who did not handle animals (56). A cohort study in Bangladesh reported higher adjusted EE scores (composite score of AAT, NEO and MPO) among children who had an animal corral in their sleeping room (54).

2.3 Microbial Source Tracking (MST): Foundations and Applications

Fecal contamination and associated exposure to enteric pathogens within public and domestic domains is a well-known source infection and diarrheal disease, especially in settings were sanitation infrastructure is limited and human sanitation practices are lacking. Generally, intervention strategies intend to reduce human fecal contamination within these environments. This is attempted by improving sanitation practices or introducing technologies, such as improved latrines. A quantitative evaluation of these interventions is pursued by several methods, such as the quantification of traditional fecal indicator bacteria (TFIB), such as total coliforms and fecal coliforms (mainly *Escherichia coli*). However, traditional microbiologic methods are not able to discern human from non-human fecal contamination, a plausible situation in low income settings were human-animal interactions in the public and domestic domains are common (57). In order to detect a difference in environmental human fecal environmental contamination, diagnostic methods are needed to discern human vs. non-human sources of fecal matter.

Such is the case of Microbial Source Tracking (MST), an expanding field in which molecular methods intend to detect and quantify host specific 16S rRNA markers of *Bacteroidales* (order Bacteroidales, genera *Bacteroides* and *Prevotella*)(58, 59), and determine the relative contribution of host-specific fecal contamination within a specific sample (60). Bacteroidales are strictly anaerobic commensal bacteria of the human and animal gut, that account for a large percentage of the human gastrointestinal flora (61, 62). Additionally, *Bacteroidales* are specifically adapted to their host, allowing the development of genetic markers that can discriminate between species (63).

First and foremost, the MST markers described in this section have been developed and applied in water and sewage samples. To date, microbial source tracking markers have only been used to assess the presence of ruminant feces in floor surfaces from households in Bangladesh, yet this study did not compare the prevalence or quantities of human and other animal species within these surfaces (64).

However, the discriminatory power of these genetic markers is site specific, requiring a performance evaluation to determine the sensitivity and specificity of each marker in a particular setting (65). As shown in **Table 1**, performance assessment studies of human markers show variable results. Human markers validated in Australia, including *HF183*, *BacHum, BacH, HuBac*, and *Human Bac* report 95-100% sensitivity and a specificity that ranges between 79% and 99% (Table 2) (66-68). There are few validation studies in low resource settings, and those that have been published report variable sensitivity values (17 - 100%) and low to high specificity value (32 - 93%) (69, 70). This range could be

attributed to laboratory specific techniques, sample size, analysis and geographic (and hypothetically microbiome) differences in both humans and animals (71). For instance, the human markers *HF183* and *BacHum* have been validated in Eastern Africa, obtaining a sensitivity of 65% and 18%, respectively, and a specificity of 100% in both markers.(72) These same markers been implemented in Tanzania to evaluate fecal contamination in hands, soils and surfaces using validation data obtained from Kenya (73, 74). Human markers have also been validated in Nepal (70).

The most commonly validated non-human markers have been developed for livestock (mainly cows) and dogs. The *BacCan* marker has shown high sensitivity and specificity in both developing and developed settings, with a sensitivity of 90% in a validation study India (60, 69). The dog marker is specifically useful to discern positive human samples in which a false positive is suspected. This is because the *BacHum* markers has been shown to cross-react with dog fecal samples, yet *BacCan* shows no cross reactivity with human samples.(69) The most commonly reported livestock associated markers include *BacCow* and *CowM2*. *BacCow* was developed as a cow-specific marker, yet studies validation studies in India have determined that it is best used as a general livestock/domestic animal marker, with no cross-reaction with human fecal samples (60, 69). Interestingly, a study evaluating ruminant and human samples from 16 countries and 6 continents determined that MST markers used to detect ruminant fecal contamination (*BacCow*, *Bac R* and *BoBac*) are much more stable in comparison to human markers (*BacH* and *BacHum*). This means that the tested targets correspond to a universally shared intestinal marker and are thus suitable for MST studies (75).

Avian and/or poultry microbial source tracking markers have also been developed, yet they have not been validated in low-resource settings (**Table 2**). Lu and collaborators identified poultry specific markers associated with *Bacteroidales*, *Clostridium perfringes* and *Desulfitobacterium halniense*. yet, results did not exceeded the detection in more 40% of evaluated samples (76). Weidhaas and collaborators developed the LA35 marker for a species of *Brevibacterium* sp., specifically for a 16S rRNA gene segment, which demonstrated a sensitivity of 80% and a specificity of 93.1% (77). This same gene was also evaluated using poultry litter samples, obtaining a sensitivity of 68-76% and a specificity of 100% (78).

Avian mitochondrial markers of the NADH dehydrogenase subunit 5 (*ND5*) and cytochrome b (*cytb*) genes exhibited have demonstrated a sensitivity of 100%, and a specificity of 84.6% and 89.9% respectively in China (79). Finally, other microbes from the chicken microbiome have been evaluated with Lactobacillus, Gallinobacterium and Firmicutes 16S rRNA markers evaluated in Israel, showing a range of sensitivity of 70 – 91% and specificity of 85 – 99% (80).

No extraintestinal growth of *Brevibacterium* species associated with poultry litter has been reported (81). Finally, the *BacHum* human and dog markers have been shown to cross react with poultry fecal samples, thus, results should be interpreted with caution (75).

2.4 Campylobacteriosis: An Emerging Global Health Priority

Human Campylobacteriosis in tropical developing nations is an endemic disease (5, 12, 82). In such settings, the epidemiology and symptomatology of Campylobacteriosis changes in comparison to that of developed nations. Diarrheal episodes attributed to *Campylobacter* spp. occur mainly in children, with watery diarrhea and as the main symptom although dysentery can occur, especially in children under 6 months of life (83, 84).

The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) Study is a multi-site study funded by the Fogarty International Center (NIH) and the Foundation for the National Institutes of Health. This study aimed to explore the biologic and health associations between infections by enteric pathogens, undernutrition, intestinal inflammation and potentially associated outcomes related to reduced growth and cognitive development (85). Children were longitudinally followed from birth until 24 months of age. Diarrheal stool samples and periodic asymptomatic surveillance stool samples were tested for a diverse panel of 29 enteropathogens (85, 86). Among these, *Campylobacter* spp. was found to have one of the highest attributable burden of diarrhea within the first year of life (3·5%; 0.4–6.3), and within the second year of life (7·9%; 3.1–12.1) (4).

Amour and collaborators evaluated the epidemiology of *Campylobacter* spp. in symptomatic and asymptomatic colonization in the eight MAL-ED study sides, to explore risk factors for colonization and evaluate the association of infection with markers for

environmental enteropathy (12). The highest prevalence of *Campylobacter* spp. was found in Dhaka, Bangladesh, Naushero Feroze, Pakistan, and Haydom, Tanzania, with more than 50% positive surveillance and diarrhea stool samples. Additionally, for most study sites, there is an increasing positive trend with age. Overall, 84.9% of children had a positive stool sample by 12 months of age. The median proportion of surveillance stool samples positive for *Campylobacter* spp. between 0 and 24 months of age was 20% (IQR: 11-38%).

Across, all study sites, risk factors associated with a reduced proportion of positive asymptomatic fecal samples include exclusive breastfeeding, treatment of drinking water and access to an improved latrine. Household breeding of chickens was only marginally associated with positive surveillance stool samples.(12) Other studies have also found a protective effect of exclusive breastfeeding for *Campylobacter* infections. Specifically, studies done in Mexico City determined that exclusively breastfed infants had a lower rate of infections, in comparison with non-breastfed children (87, 88). Additionally, in a similar study performed in Guatemala, the breastmilk consumed by children who presented a higher rate of diarrhea attributed to *Campylobacter* spp. had no detectable secretory immunoglobulin A against flagellar antigens of *C. jejuni* (89). However, most of these studies were performed with a relatively low sample size and diagnostic techniques have been further developed.

Few studies have explored the association between *Campylobacter* infections and reduced linear growth in children. Longitudinal cohort analysis evaluating

Campylobacter spp. infections based on culture results and linear growth in the Peruvian Amazon show that children with symptomatic infections grew 0.059 cm less per *Campylobacter* episode over 9-month period, while children with severe *Campylobacter* episodes grew 0.169 cm less per episode over the subsequent 9-month period (5). Results from the entire MAL-ED study found that *Campylobacter* infections were significantly associated with a reduced LAZ score in infants under 2 years of age. More specifically, children with a high proportion of *Campylobacter* positive stool samples (top 90th percentile) had a decrease in LAZ score of 1.82 (95% CI -1.94, -1.70), even after adjusting for potential confounders. This association persisted for the subgroup of children that had a low prevalence of *Campylobacter* as well as within all study sites (12).

Additionally, the MAL-ED study also evaluated for the first time the association between markers of intestinal and systemic inflammation with *Campylobacter* positive stool samples. Specifically, higher concentrations myeloperoxidase (MPO), alpha-1-atitripsin (ATT) and alpha-1-acidglycoprotein (AGP) were associated with *Campylobacter* spp. positive stool samples, providing a possible pathway for the association between infection, alterations in the composition of the intestinal microbiota and decreased linear growth (12). The concentration of neopterin (NEO) was found to be inversely proportional to *Campylobacter* positivity in stool samples suggesting that inflammation in the intestinal environment may be downregulated by the pathogen.

Campylobacter in the Environment

Campylobacter spp. is a fastidious organism with specific atmospheric requirements. As a result, its isolation in environmental samples is cumbersome, requires specific enrichment conditions, and yields relative restricted rates of isolation when compared with molecular diagnostics (90). Studies have identified temperature and exposure to oxygen as the most important variables promoting *Campylobacter* inactivation in the environment, and that survival rates range between 8 to 32 days (90, 91). Soil samples are seldom queried for *Campylobacter* spp., yet it's been previously isolated in United Kingdom at rates that range between 0.1-0.4% (92). For a thorough review of *Campylobacter* isolation in other environmental samples, such as surface water and drinking water, see Whiley *et al* (93). Several survival mechanisms have been identified within *Campylobacter* spp. that allows this pathogen to survive in outside the intestinal environment of its host. These include the capacity to form biofilms, its mechanisms stand oxidative stress and its capacity to enter a viable but non-culturable state (94, 95). In the case of biofilms, the association between *Campylobacter jejuni* and *Pseudomona aeuriginosa* has been proven as an efficient mechanism of survival (96). Additionally, a recent study has provided robust evidence showing that fluoroquinolone resistance in *Campylobacter jejuni* increses its ability to form biofilms (97).

Antibiotic Resistance in *Campylobacter* species

The World Health Organization published in 2017 a list of the top 10 priority human microbial pathogens for which new antimicrobials are needed, in which fluoroquinolone resistant *Campylobacter* spp. holds an impressive fourth place in the high priority section.

Campylobacter spp. is intrinsically resistant to penicillins, vancomycin, rifampicin, trimethoprim, sulfamethoxazole, and most cephalosporins.(98) However, recently the prevalence of fluoroquinolone resistance (particularly to ciprofloxacin), has reached such staggering levels that macrolides are currently recommended as the first line of treatment for human Campylobacteriosis. However, increasing rates of *C. coli* resistance to macrolides are being reported and the next probable oral agent that remains is Ampicillin/sulbactam, an antibiotic that is scarce in developing world settings, expensive, and unlikely to be used empirically for diarrhea as it is not a good therapeutic option for other key bacterial enteropathogens causing moderate to severe diarrhea.

It is believed that antibiotic use in livestock (most importantly poultry) industry is one of the drivers of antibiotic resistance to clinically important human drugs. This is a regular practice that can be divided into three main categories: (1) antibiotic use for disease treatment, (2) antibiotic use for disease prevention (prophylaxis) and (3) antibiotic use for growth promotion (increase the feed conversion ratio). Antibiotic use as a prophylactic measure of disease as well as for growth promotion reasons entails sub-therapeutic uses of drugs, altering the environmental characteristics of commensal and pathogenic bacteria, and thus contributing to the emergence of drug resistance mechanisms. In developing areas of the tropics, poultry production is generally characterized by poor implementation of sanitary barriers and high density flocks, altering the immune sustem of the animal, making it more susceptible to infections and diminishing their feed-conversion ratio. As a result, the use of antibiotics is most likely crucial for most small or medium scale poultry producers. Thus, the use of readily available antibiotics, such as

enrofloxacin (fluoroquinolone) and tylosine (macrolide) is commonly observed in low-resource tropical areas were poultry is one of the main animal-sourced foods for the population.

Quinolone Resistance

Quinolone resistance is commonly reported in *Campylobacter* species. Current world-wide trends are alarming, with studies reporting more than 80% resistance in human *Campylobacter jejuni* and *Campylobacter coli* isolates. Results from the MAL-ED cohort study of Peru, a community-wide longitudinal cohort study in a pediatric population showed that 77% (N=588) of *Campylobacter jejuni* isolates were resistant to ciprofloxacin. This statistic is alarmingly high, with only few studies reaching such high rates, including a sample of 80 strains from children under 5 years of age with diarrhea (87% resistance) and a sample of 20 strains from Japanese children (90% resistance) (99, 100). Other studies in the UK, Korea, Israel and Peru have demonstrated lower levels of fluoroquinolone resistance in diarrhea associated strains, ranging from 24% to 65% (101-104). In *Campylobacter* isolates from retail food samples, most specifically poultry meat, equally high rates have been reported in the Asian continent (105). As a result, the increase in fluoroquinolone (and macrolide) resistance has been attributed to antibiotic administration for growth promotion in intensive poultry and hog production (106-108). A variety of quinolone resistance mechanisms have been reported in *Campylobacter*: (i) target mutations in the quinolone resistance determining region (QRDR) and (ii) a multi-drug efflux pump that decrease antibiotic intracellular accumulation.

(i) Target mutations in the QRDR

Quinolones inhibit the synthesis of bacterial DNA by targeting two enzymes responsible for bacterial replication, transcription, recombination and repair: DNA gyrase and topoisomerase IV. In *Campylobacter* species, the topoisomerase IV target appears to be absent. DNA gyrase is made of two subunits: GyrA and GyrB, encoded by the *gyrA* and *gyrB* genes respectively. Amino acid substitutions in the GyrA subunit infers quinolone resistance to *Campylobacter* strains (109). The most commonly reported AA substitution is the Thr86Ile, which confers high level ciprofloxacin resistance and is encoded by a C-257-T mutation. Other AA substitutions reported in *Campylobacter* species include the Asp90Asn and the The86Lys which convey moderate resistance (MIC 8-16 ug/mL), as well as the Thr86Val, The86Ala and Asp90Tyr substitutions. Up to date, there are no reported mutations in the gyrB that confer quinolone resistance (98).

(ii) Multi-drug efflux pump

The cmeABC efflux pump is composed of the periplasmic protein *CmeA*, the inner membrane efflux transporter *CmeB*, and the outer membrane protein *CmeC*. These proteins are encoded by the *cmeABC* operon. Many studies have shown that this efflux pump confers intrinsic resistance to fluoroquinolones, as well as many other antibiotics including ampicillin, tetracycline and chloramphenicol.(110-112) When both the *cmeABC* efflux pump and *gyrA* mutations are present within the same strain, a synergistic effect is triggered, causing high levels of quinolone resistance (98, 113).

Macrolide Resistance

Rates of macrolide resistance are wide-ranging and country dependent. Results from the MAL-ED cohort study of Peru report a prevalence of resistance of 25.2% for *C. coli* isolates. This is a considerably higher prevalence than what was reported previously in Iquitos (10.0%) (103). However, *C. jejuni* resistance to macrolides (5.27%) in this study was lower than what was previously reported in the same region (14.9%) (103). Worldwide, macrolide resistance in human *Campylobacter* isolates varies from very low rates, such as 0.8% in South Korea and 2.2% in the UK, to increasing rates such as 12.5% in Thailand, 21.8% in China and 22.2% in India (99-102, 114). Additionally, cases *Campylobacter* associated travelers' diarrhea in US military troops show as low as 2% non-susceptibility to azithromycin (115). Antibiotic resistance of *Campylobacter* spp. isolates from poultry also appear to be country-dependent. A study conducted in China reported that 73.2% of *C. coli* strains were erythromycin resistant, and a study from Spain similarly reports 73.0% resistance (116, 117). Reports from Latin America are limited. Sierra-Arguello *et al*. reported an overall 2% resistance to erythromycin, yet *Campylobacter* species were not specified (118). Given that azithromycin is the currently prescribed treatment for Campylobacteriosis, current trends of macrolide resistance are worrisome (119). Interestingly, the highest proportion of macrolide resistance is found in *C. coli*, and not in *C. jejuni*. Several hypothesis surrounding this phenomenon exist. The most commonly cited is that macrolide resistance determinants in *C. jejuni* are associated with fitness losses that hinder decrease its ability to colonize the chicken's gastrointestinal epithelial cells. However, only few in-vitro studies have explored this hypothesis, and for only some mechanisms of resistance (24, 111, 112, 120).

Reported mechanisms of macrolide resistance in *Campylobacter* species include (i) target mutations of the 23S rRNA genes, (ii) target mutations in ribosomal proteins and (iii) ribosomal methylation (16, 98). A brief overview of these is presented below. For a complete review of macrolide resistance in *Campylobacter* spp. consult Bolinger and Kathariou, 2017 (16).

(i) Target mutations in 23S rRNA

Point mutations in the position 2074 and 2075 have been reported. The most common of which is A2075G which confers high level resistance to erythromycin (MIC ≥ 512 ug/mL). High level resistance increases when the mutation is included in the three gene copies of the 23S rRNA (121, 122). Studies have also shown that when point mutations are not found in the three gene copies, resistance levels are reduced (16, 98, 123).

(ii) Target mutations in ribosomal proteins

Point mutations in the L4 and L22 ribosomal proteins encoded by the *rplD* and *rplV* genes have been reported. These mutations are associated with lower levels of resistance (MIC ≥ 32 ug/mL), yet when present with mutations associated with 23S rRNA, or with the cmeABC efflux pump, resistance levels increase exponentially (98, 124). The most commonly found point mutation is the A103V (98).

(iii) Ribosomal methylation

Enzyme mediated methylation of the macrolide ribosomal target is achieved by methylases encoded by the *erm* genes, of which the *erm(B)* gene is

associated with high-level resistance, mostly in *C. coli* (19, 125). *Erm(B*) is able to demethylate an adenine in the 23S rRNA, causing a decrease in macrolide-ribosomal binding (16). Additionally, whole genome sequencing explorations have found that this gene is located within a multidrug resistant genomic island (MDRGI) which contains antibiotic resistant determinants for other antibiotics, such as for tetracyclines and aminoglycosides (126). Eight MDRGI types have been described in *Campylobacter* species, with various numbers of open reading frames and antibiotic resistant genes. Interestingly, the *ermB* gene has only been found in *Campylobacter coli* and *Campylobacter jejuni* strains from China, the earliest of which was in 1994 (127). However, it was recently reported that a *C. coli* strain from Spain also contained the *ermB* gene within a MDRGI with 5 additional antibiotic resistance genes (16, 128).

(iv) Multidrug Efflux Pump

The cmeABC efflux pump, previously described as a quinolone resistance mechanism, also confers resistance to macrolides (110). The expression of the *cmeABC* operon is modulated by the *cmeR* gene. Mutation of the *cmeR* gene (reported as *RE-cmeABC*) has resulted in an overexpression of the cmeABC efflux pump, and thus an increase in the level of erythromycin resistance (16, 23, 98). Additionally, a synergistic effect with mutations in ribosomal proteins increases macrolide levels of resistance in *C. coli* (124). However, fitness impairments have been reported as an inability of *C. jejuni* to colonize chickens (24, 111, 112, 120).

Dissemination of all the reported macrolide resistance can occur through natural transformation (i.e. bacterial species that naturally carry the competence genes required for transformation of foreign DNA material, one of the three mechanisms of horizontal gene transfer), (124, 129-131) and through horizontal gene transfer of the *ermB* gene (132).

Resistance to other Antibiotics

Gentamicin resistance in detected in *Campylobacter* strains in the 1990s (133). However, increasing resistance in clinical isolates is a novel phenomenon (134). Human isolates from Iquitos, Peru show a prevalence of phenotypic gentamicin resistance of 16.0% for non-*C. jejuni* and almost negligible estimates for *C. jejuni*. These numbers are lower than the 28.8% reported by Pan and colleagues in China for human diarrheal samples, yet higher than reports form Korea (6.6%) (100, 102). In Belgium, gentamicin resistance in poultry isolates has increased from almost being nonexistent in 2004 to approximately 20% by 2009 (135). However, even higher rates have been recently reported in *C. coli* (79.9%) isolates from chicken and swine from China (136).

The molecular mechanisms of resistance to aminoglycosides in *Campylobacter* species are being increasingly studied. Multiple phosphotranspherase (*aph*) genes are commonly associated with aminoglycoside resistance and many of them have been identified as transferable (134, 137, 138). The chromosomally encoded aph(2")- is one of the most commonly reported resistance genes in China and the USA (134, 136-138), and it is suspected that it originated from poultry associated *Campylobacter* lineages (138).

Additionally, multiple aminoglycosides genes have been found within the MDGI associated with the *ermB* macrolide resistance gene in *C. coli* (126). These determinants have been found to be largely mobile, embedded plasmids that are capable of being horizontally transferred (21, 139-141).

Tetracycline resistance is mainly mediated by the *tet(O)* gene, which has been found in transferable elements such as the MDGI and multi-drug resistant plasmids (21). Additionally, the cmeABC multi-drug efflux pump has been shown to confer resistant to tetracycline (110). A plasmid-encoded *cfr*-like gene denominated *cfrC* was recently discovered in *Campylobacter coli* strains isolated from cattle. This gene is known to confer resistance to a number of antibiotics, a complex known as $PhLOPS_A$: phenicols (e.g. florfenicol), pleuromutilins (e.g. tiamuline), lincosamides (e.g. clindamycin), oxazolidones (e.g. linezolid) and streptogramin A (*Campylobacter* spp. is naturally resistant to this antibiotic) (142).

In Iquitos, Peru, multidrug resistance (defined as an isolates expressing phenotypic non-susceptibility to three or more classes of antibiotics), was observed in 56.8% (335/590) of *C. jejuni* isolates and 59.1 % (176/298) of non-*C. jejuni* isolates (143). This is striking given that the only previous evidence of such higher rates came from poultry isolates in China, were 81.1% of *C. jejuni* isolates and 47.7% of *C. coli* isolates were resistant to 3 or more antibiotics (116). However, it is important to consider the definition of multidrug resistance when comparing overall susceptibility trends across the globe.

Epidemiology of Campylobacteriosis in the USA and New Zealand

In the developed world, human *Campylobacter* spp. infections are a common cause of gastroenteritis in adult populations. Epidemiologic studies attribute the majority of human cases to poultry, followed by ruminants, and an almost negligible amount to environmental and wild animal sources (144, 145). In the USA, *Campylobacter* spp. is the fourth leading food-borne pathogen, causing 845,024 cases a year and costing 1.747 M $US (cost of illness). In combination with poultry, *Campylobacter* spp. is the pathogen with the highest food-born risk, costing 9541 QALYs per year (146). Also in the USA and similar settings, Campylobacteriosis by *C. jejuni* and *C. coli* is characterized by a self-limiting episode of dysentery, abdominal pain, fever and vomiting. Symptoms can last up to two weeks, yet the disease is generally self-resolved within 1 week (147, 148). The most recent outbreak (Jan 2016 – Jan 2018) of multi-drug resistant Campylobacteriosis in the USA involved 113 individuals (1-86 years old, mean 27 years old) from 17 states with laboratory confirmed infections. Of these 103 individuals, 22% were hospitalized. The main source of *Campylobacter* spp. was attributed to juvenile canines sold via a multistate pet-store chain. Other outbreaks in the USA have been associated with unpasteurized milk in Utah in 2014, and with undercooked chicken livers in Northeastern USA (2012).

One of the most commonly reported consequences of Campylobacteriosis is the Guillain Barre Syndrome (GBS) and the Miller-Fisher Syndrome (MSF) syndrome (149). These are two immune mediated neurological disorders characterized by an acute paralytic neuropathy (149). Although the incidence of GBS varies by country, rates of GBS in

North America and Europe among the general population range between 0.8-1.9 cases per 100,000 people per year, of which a higher proportion of cases are male than female (149). However, among people with Campylobacteriosis, rates are considerably higher. Results from a Swedish cohort estimate that the Incidence of GBS following *C. jejuni* infections was of 30.4 per 100,000 person-years (13.9-57.8 per 100,000 person-years; 9 cases of GBS out of 29,563 cases of *C. jejuni* infection),(150), while in the UK the rate reached 117/100,000 person-years (95% CI 38–363 per 100,000 person-years) (151). A review of GBS cases done by Robert Black and collaborators determined that 31.5% of 2502 GBS cases were attributable to *Campylobacter* spp. infections (152).The main hypothesis that explains GBS in Campylobacteriosis cases is that the N-acetyl neuraminic acid in the LOS mimics that of gangliosides, causing antibodies against LOS structures to cross react with gangliosides of human peripheral nerves (153).

By 2006 it was widely recognized that Campylobacteriosis was the leading infectious disease reported in New Zealand, with approximately 379 cases per 100,000 people and costing approximately 75 million USD (154). The geographic and environmental characteristics are unique in that it is an isolated island with a domestic agricultural and livestock industry, with strict border controls for livestock and agricultural products, and thus a unique bacterial population. Through various source attribution models, Müllner and collaborators established that retail poultry was the primary source of human infection, being responsible for between 34 – 76% of human cases, depending on the quantitative source attribution model used (155). Additionally, different epidemiological patterns of Campylobacteriosis between rural and urban areas were detected, such that

older individuals and people living in rural areas were most likely infected with a poultry attributed strain, in comparison with a strain attributed to another animal source (156). The most frequent sequence types detected in the region is ST474, a poultry associated strain rarely found outside New Zealand, and mainly associated with a particular commercial poultry company (145, 156). As a control measure, a robust and integrated surveillance system was established that encompassed a human and animal bacteriologic surveillance system, as well as a rigorous food safety measures within the poultry processing process. Specifically, a New Zealand Food Safety Authority launched a risk management strategy for poultry associated Campylobacteriosis which encompassed multiple interventions that monitored contaminated poultry flocks and carcass contamination during primary processing. As a result, Campylobacteriosis cases dropped from 353.8/100,000 cases between 2002-2006, to 161.5/100,000 cases in 2008, a decline that included 74.0% decline in poultry attributed cases, as well a drop in hospitalizations and Guillain-Barre cases (from 2.6 cases/ 100,000 person-years in 2002-2006, to 2.2 cases/100,000 person/years in 2008-2010) (157-159). Although this success can be attributed to several interventions, it is likely that these strict on-farm biosecurity measures during primary processing was not only the most efficient method, but also the most cost-effective one (158, 160).

Epidemiology of Campylobacteriosis in Peru

Campylobacter spp. has been described as an endemic infection in peri-urban areas of the coast and jungle of Peru, yet the molecular epidemiology (including source attribution modelling) of this pathogen has not been explored. Studies in the coastland of Peru,

specifically in Lima and Ica evidenced that among children between 2 and 5 years of age, between 10.0 – 49.3% of water diarrhea cases were attributed to *Campylobacter* spp. infections (161-163).

In the Peruvian Amazon, two longitudinal cohort studies in a pediatric population estimated that 5.6% of diarrhea cases in children under 11 months and 11.7% in 12 to 24 months are attributable to *Campylobacter* spp. (4, 5). The incidence of infection among children under 5 years of age remains moderate at 0.37 episodes per year per child, while among children under 12 months of age the incidence of infection increases exponentially to 0.89 episodes per year per child (5, 164). Additionally, population based studies have characterized a high rate of asymptomatic carriers, typical of a highly endemic setting (4, 5, 163). In this same geographic area, Lee and collaborators established an association between linear growth, weight gain and *Campylobacter* infections. Specifically, multilevel linear models established a reduced linear growth over a 9-month period, in both symptomatic (-0.059 cm per episode) and severely symptomatic (-0.169 cm per episode) children, as well as reduced weight gain over a 3-month period in symptomatic (-65.5 grams) and asymptomatic (-43.9 grams) children (5).

The epidemiology of *Campylobacter* spp. in animal hosts within an endemic transmission setting has been poorly characterized. Most studies include cross-sectional evaluations of animal fecal samples in which *Campylobacter jejuni* and *Campylobacter coli* have been most commonly identified. The isolation rate of thermotolerant *Campylobacter* spp. in domestic poultry in Lima and Iquitos ranges between 54.0% to 61.3% (165) (unpublished

results), while 16.7% of retail meat carcasses from Lima are positive to *C. jejuni* and *C. coli* (166). In the Peruvian Amazon, *Campylobacter lari* has also been isolated in 10.5% of domestic poultry fecal samples (167). Additionally, the isolation rate in pigs and dogs has been estimated to be 44% and 6% respectively. However, studies are limited by sample size and microbiological methods, given that most of them ignored molecular tools to confirm the species of *Campylobacter* isolated (165). Finally, the possibility of rats being a sources of *Campylobacter* infections is an interesting and plausible hypothesis. *C. jejuni*, *C. coli* and *C. hyointestinalis* have been isolated from rats in several studies, with isolation rates ranging from 3.4% - 12.5% (168-170) yet a comprehensive analysis of the role of this specie in the epidemiology of Campylobacteriosis in low and middle income nations is lacking.

Data available on the genetic composition of *Campylobacter* spp. strains colonizing human and animal carriers is limited. A study by Oberhelman and collaborators genetically characterized *Campylobacter* spp. strains using RFLP and determined the existence of a high variety of genetic types (164). No other molecular epidemiologic data in these highly endemic areas exists. Although household exposure to *Campylobacter* spp. positive chickens has been found to be a risk factor of human infection (171), a more recent study suggested that strains isolated from a human diarrhea case were unlikely to be the exact same as those being carried by domestic poultry, suggesting a state of acquired immunity and limiting potential household control measures, however this study is not definitive given sample size and technique applied (44, 164, 172).

Domestic poultry raising is most commonly observed in rural areas, in which space enables this practice. A study in the shanty-towns of Lima in the early 1990s among households raising poultry, reported an average 3.9 episodes/12-hours/household in which there was chicken feces were mouthed by toddlers (173). In the Peruvian Amazon, approximately a third of households hold domestic poultry within its premises and only 15% of these are contained in corrals (unpublished results). A behavioral-intervention study in the peri-urban shantytowns of Lima determined that corralling was mainly linked with preventing chicken from being stolen, yet these structures were made of scrap materials and highly limited by economic constrains, as well as food and water supply for the animals (44). Interestingly, with this same intervention *Campylobacter* rates in children remained the same, yet (according to RFLP patterns) the source of infections shifted, from outside the home to inside the home, suggesting that there was a shift in the ecology of the pathogen, as well as a protective effect of *Campylobacter* strains from within the household domain (172). That said, the poultry-human transmission route of *Campylobacter* spp. infections remains to be elucidated within a highly endemic setting such as the Peruvian Amazon, where a high percentage of atypical *Campylobacter* spp. strains are responsible for symptomatic and asymptomatic infections, (25) and were household raising practices include in-house slaughtering, evisceration and commercialization.

Increasing resistance rates to quinolones and macrolides has also been evidenced in Peru. Ciprofloxacin resistance among *C. jejuni* isolates from Lima increased from 73.1% in 2005 to 89.9% in 2010, while in Iquitos resistance rates of 24.1% were reported in 2005

and by 2010 resistance rated reached 48.9%. Additionally, in the Peruvian Amazon, rates of *C. coli* resistance to erythromycin are currently around 30% (unpublished results) (103). Recently, a *gyrA* mutation associated with quinolone resistance, as well as a 23S rRNA mutation associated with macrolide resistance was detected within a limited sample of phenotypic resistant *Campylobacter* spp. strains from a pediatric cohort in Lima.(174, 175) Preliminary data from our group currently indicates that *Campylobacter* spp. strains isolated from domestic poultry express 31.0% erythromycin resistance and 95.6% ciprofloxacin. Other studies assessing antimicrobial susceptibility of *Campylobacter* spp. strains from animal hosts in this region are non-existent.

Peruvian national legislation does not regulate antimicrobial use in animal feed, with the exception of chloramphenicol, nitrofurans, nitromidazole and olaquindox, under the resolution RD 0072-2013-MINAGRI-SENASA-DIAIA. However, almost any class of antibiotics can be acquired over the counter. Antimicrobials are either included in the commercial feed, or sold by the gram or kilogram in “agro-pharmacies”, commonly located in every city. Examples of antimicrobials regularly used for the prevention and treatment of respiratory and gastrointestinal disease in poultry include first and second generation fluoroquinolones including nalidixic acid and ciprofloxacin, enrofloxacin, colistin, tylosin, amoxicillin, streptomycin, gentamicin, fosfomycin, oxitetracycline, trimethoprim, sulfamethoxasol and lancomycin (*personal observations*). Using the most commonly formulated antibiotic mixes to be added to poultry water, 1 USD provided prophylaxis for 1 chick for 1 week.

2.5 Tables for Chapter 2

Table 1. Lists of Validated Host-Specific Microbial Source Tracking Makers in Low and Middle Income Settings

Host	Marker	Sensitivity	Specificity	Accuracy	Location	Reference
Human	*HF183* SYBR	0.87	0.93	NR	Bangladesh	
Human	*HF183* SYBR	0.89	0.03	0.35	India	Odagiri *et al*. 2015
Human	*HF183* SYBR	0.65	1.00	NR	Kenya	Pickering *et al.* 2012
Human	*HF183* Taqman	0.29	0.80	0.61	India	Odagiri *et al*. 2015
Human	*HF183* Taqman	1.00	0.32	0.44	Nepal	Malla *et al.* 2018
Human	*BacHum*	0.49	0.78	0.67	India	Odagiri *et al*. 2015
Human	*BacHum*	0.18	1.00	NR	Kenya	Pickering *et al.* 2012
Human	*BacHum*	1.00	0.77	0.81	Nepal	Malla *et al*. 2018
Human	*HumM2*	0.49	0.70	0.62	India	Odagiri *et al*. 2015
Human	*BacH*	0.17	0.83	0.59	India	Odagiri *et al*. 2015
Livestock	*BacCow*	0.95	1.00	0.96	India	Odagiri *et al*. 2015
Livestock	*BacCow*	0.94	1.00	NR	Kenya	Pickering *et al.* 2012
Livestock	*BacCow*	1.00	0.53	0.16	Nepal	Malla *et al*. 2018
Livestock	BacR	1.00	0.88	0.91	Nepal	Malla *et al*. 2018
Cattle	*CowM2*	0.50	1.00	0.94	India	Odagiri *et al*. 2015
Pig	*Pig2Bac*	1.00	0.75	0.80	Nepal	Malla *et al*. 2018
Dog	*BacCan*	0.90	0.99	0.98	India	Odagiri *et al*. 2015
Universal	*BacUni*	1.00	NR	NR	India	Odagiri *et al*. 2015
Universal	*GenBac3*	1.00	NR	NR	India	Odagiri *et al*. 2015

Table 2. List of Selected Avian Microbial Source Tracking Markers Developed

Host	Marker	Sensitivity	Specificity	Accuracy	Location	Reference
Avian	LA35	0.68-0.76	NR	NR	USA	Weidhaas *et al*. 2013
Avian	LA35	0.8	0.93	NR	USA	Weidhaas *et al*. 2010
Avian	ND5	1.00	0.85	NR	China	Zhuang *et al*. 2017
Avian	cytb	1.00	0.90	NR	China	Zhuang *et al*. 2017
Avian	Av4143	0.95	0.97	NR	Israel	Ohad *et al*. 2016

Chapter 3. Study context

3.1 Study setting

This research will take place in Santa Clara de Nanay, Santo Tomas and La Union, three peri-urban communities located in Iquitos, the capital of Loreto, Peru. Briefly, Peru which borders Brazil, Colombia, Ecuador, Chile and Bolivia, has a total extension of 1,285,000 km^2. Geographically, it is divided into three main regions: coastland, highlands and jungle. The Peruvian Amazon covers most of the national territory (60.3%) with 775 353,84 km^2, yet it holds only 14% of the total national population and has the lowest human population density (2.8 habitants/km^2) compared to Lima, the capital of Peru (282.4/km^2). Politically, Peru is divided by 24 departments (equivalent to US states), of which Loreto, located in the heart of the Peruvian Amazon, is the largest one (365,852km2). Iquitos, the capital of Loreto, is the home of 437,376 inhabitants (2015).(176) Surprisingly, this city is only accessible by boat or airplane, given that there are yet no highways that connect Iquitos with the rest of the country. The most recent national health survey (2016) revealed that in Loreto, 15.4% of children reported a diarrhea episode within the previous 2 weeks, while 2.4% reported a dysentery episode. Moreover, 31.4% of caregivers administered an antibiotic course during a diarrhea episode. Regarding the use of improved sanitation infrastructure, only 29.8% of the population of Loreto always use a latrine for fecal depositions, while only 48.9% have improved sanitation. Finally, the most recent health survey revealed that 91.6% of mothers know about oral rehydration salts (ORS), yet only 30.0% use them during a diarrheal episode (177).

This research will be conducted at the JHU-UVA-AB Prisma study site, an NIH-approved clinical trials site, of which Dr. Margaret Kosek and Pablo Yori are Principal Investigators. The site is located in Santa Clara de Nanay, a peri-urban, low-income community located 15km south east of the center of Iquitos, and bordered by two other communities: Santo Tomas and La Union (Figure 6). Most community members have access to basic needs for electricity and potable water sources from public or private wells (8). As of 2016, these communities have regular access to the main highway, given the construction of a new paved road and accessibility to the urban transport system. The effect of this newly implemented road on the communities has not been evaluated.
This site has been the subject of multiple investigations over the past two decades, including vector-borne disease studies (178) and two longitudinal cohort studies evaluating the burden and risk factors of diarrheal disease (5, 8, 179). At this point, the MAL-ED study (2009-2017) has been the largest study performed within these communities. It encompassed the longitudinal follow-up of 300 children from birth until 5 years of age, which included daily surveillance of diarrhea and bi-weekly sampling of surveillance stool samples.

3.2 Research ethical review and approvals

Human subject participation was required for specific aim 2. The ethical review board of Asociacion Benefica Prisma (Peru) and Johns Hopkins Bloomberg School of Public Health approved the protocol in June 2018 and September 2019, respectively. Participant consent was performed in Spanish, by a local field worker. Approval from the Animal Care and Use Committee was not obtained given that animals were not physically

handled. All animal fecal samples were collected from the ground, without touching any animal.

3.3 Contributions to Public Health

Overall, this dissertation seeks to validate a tool to measure the burden of animal fecal contamination in household environments, that has the potential to be used in future water, hygiene and sanitation interventions (WASH) and controlled trials. By proving, quantitatively that animal fecal waste is as or more important than human fecal waste, we propose a paradigm shift of how we think about an effective WASH intervention strategy. Additionally, this research seeks to provide new insights into the molecular epidemiology of drug-resistant Campylobacteriosis of poultry origin in an endemic, tropical environment. By comparing *Campylobacter* genomes originating from household and industrially-raised chickens we will be providing evidence to develop disease control interventions that are contextually and culturally appropriate. Finally, by comparing antibiotic resistance determinants from these two poultry ecosystems, we are in the position of informing national regulatory agencies about the need to control non-therapeutic use of antibiotics in the poultry production chain, as well as the unregulated commercialization of antibiotics.

Chapter 4. Validation of Microbial Source Tracking Markers for the attribution of fecal contamination in indoor household environments of the Peruvian Amazon

4.1 Abstract

The performance of eight microbial source tracking (MST) markers was evaluated in a low-resource, tropical community located in Iquitos, Peru. Fecal samples from humans, dogs, cats, rats, goats, buffalos, guinea-pigs, chickens, ducks, pigeons, and parrots were collected within the city (n=117). All samples were tested with human (*BacHum, HF183-Taqman*), dog (*BactCan*), pig (*Pig-2-Bac*), and avian (*LA35, Av4143, ND5, cytB*) MST markers using quantitative PCR (qPCR). Internal validity metrics were calculated using all animal fecal samples, as well as animal fecal samples contextually relevant for the Peruvian Amazon. Overall, *Pig-2-Bac* performed best, with 100% sensitivity and 88.5% specificity to detect the correct fecal source. Human-associated markers (*BacHum* and *HF183-Taqman*) showed a sensitivity of 80.0% and 76.7%, and specificity of 66.2% and 67.6%. Of the avian markers, *CytB* (mitochondrial) showed a sensitivity of 87% sensitivity and 82.4% specificity. When limiting the analysis to contextually relevant animal fecal samples for the Peruvian Amazon, *Av143* surpassed *cytB* with 95.7% sensitivity and 81.8% specificity. *BactCan* demonstrated 100% sensitivity and 47.4% specificity. All MST markers, except *LA35*, were able to detect higher concentrations of gene copy numbers (GCN) in target animal fecal samples in comparison to non-target animal species. The GCN detected by *BacHum* and *HF183-Taqman* were positively correlated (Pearson's correlation coefficient: 0.785), as well as avian markers *cytB* with *Av4143* (Pearson's correlation coefficient: 0.508) and *nd5* (Pearson's correlation

coefficient: 0.949). These findings suggest that markers such as Av4143, Pig2Bac, cytb and *BacHum* have acceptable performance to be impactful in source attribution studies for zoonotic enteric disease transmission in this and similar low-resource communities.

4.2 Introduction

Fecal contamination and associated exposure to enteric pathogens is commonly recognized within the domestic domain in settings were sanitation and household infrastructure is lacking and clean water availability is unstable (74). Traditional water, sanitation and hygiene interventions aim to reduce human fecal contamination of the environment, especially soils and other surfaces that are in routine close contact with children and other household members. However, identification and quantification of animal fecal burden in the household environment has garnered only limited attention and risk assessment to date (42, 180, 181).

Zoonotic enteric pathogens, including *Campylobacter* spp, *Cryptosporidium* sp. and Shiga (Vero) toxin producing *Escherichia coli* (STEC) are responsible for at least a large proportion of diarrhea-attributed deaths (31, 42). As a result, not taking into account the role of animal fecal waste greatly biases observational and experimental studies that aim to reduce diarrheal incidence in pediatric populations of the developing world. In fact, recent water sanitation and hygiene trials in low income settings that manage human fecal waste alone as a mechanism for reducing childhood diarrhea have proven inconsequential, and certain authors have suggested that sources of human pathogens likely extend beyond human fecal exposure to include animal sources (182-187).

Determination and quantification of fecal contamination using traditional fecal indicator bacteria such as *Escherichia coli* and *Enteroccoccus* has been applied despite significant assay shortcomings (9). These indicator bacteria multiply in tropical climates, so detected amounts do not in all cases directly reflect initial contamination levels. Furthermore, these traditional microbiologic methods do not identify the source of fecal contamination; thus, in settings where multiple exposures exist, it is impossible to assign risk to particular competing sources of fecal exposure in order to inform interventions. As a result, there is a need to develop and implement microbiologic tools that enable us to allocate and accurately quantify fecal contamination and attribute the contamination to specific animal species. We have focused within the indoor household environment, including household surface samples, for future evaluation of water, hygiene and sanitation trials that aim to reduce enteropathogen transmission (187).

Microbial source tracking (MST) has been developed as a tool to quantify and allocate the source of fecal contamination in water to animal sources at the species level. *Bacteroidales* are strictly anaerobic commensal bacteria of the human and animal gut that account for a large percentage of the human gastrointestinal flora (61, 62). These bacteria have been extensively used as a MST tool given the bacterium's ability to adapt to the intestinal environment of specific animal hosts (63). Other markers targeting host-specific enteric bacterial flora, as well as mitochondrial DNA segments, have also been developed (60, 71, 77, 79, 80, 188). Validation of MST markers has proven that their discriminatory power is site specific, and as a result, requires a performance evaluation to determine the sensitivity, specificity and accuracy of each marker (65). This study

validates eight MST markers for the determination and quantification of human and animal exposures in the Peruvian Amazon to inform their future implementation in risk assessment measures of animal fecal contamination in this and similar domestic settings in low-income, tropical communities.

4.3 Methods

Study setting

Between July and August of 2018, fecal samples used in the validation procedures were collected in Santa Clara de Nanay and Santo Tomas, two peri-urban communities with a population of 5000 individuals located near Iquitos, Loreto, the largest city in the Peruvian Amazon. The average annual precipitation is 3.4 meters and the average temperature is of 25.8ºC (min: 21.9ºC max: 32.4ºC).

Microbial Source Tracking Markers

Eight MST markers were selected for validation. Four markers targeted avian species (*LA35*, *Av4143*, *ND5* and *CytB*) and the other four targeted mammalian species including humans (*HF183-Taq* and *BacHum*), dogs (*BacCan*) and pigs (*Pig2Bac*)(60, 77, 79, 80, 189). Two markers (*cytB* and *ND5*) targeted avian mitochondrial DNA segments, and the remaining six targeted host-specific gastrointestinal bacteria, including *Brevibacterium avium (LA35)*, a species *of Lactobacillus* spp. (*Av4143*) and host-specific *Bacteroidales* (**Table 3**).

Fecal Sample Collection

Animal fecal samples (n=117) were collected in sterile 2mL tubes and stored at -20ºC until DNA extraction. Each specimen was from a single individual. Dog (*Canis lupus familiaris*) (n=10), chicken (*Gallus gallus*) (n=13), duck (*Anas platyrhynchos*) (n=10), parakeet (*Brotogeris versicolurus*) (n=2), and pig (*Sus domesticus*) (n=10) samples were collected from domestic animals of local households. Guinea-pig (*Cavia porcellus*) (n=5), buffalo (*Bubabuls bubalis*) (n=5) and goat (*Capra aegagrus hircus*) (n=10) fecal samples were collected from one single farm in Santo Tomas. Pigeon (*Columba livia*) (n=10) samples were collected from Iquitos city center. Animals were not touched during sample collection and feces were collected, as soon as the animal defacated, without touching the ground floor. Cat (*Felis catus*) (n=2) samples were donated by a local veterinarian. Rat (*Rattus* sp.) (n=10) and human (healthy children (n=15) and adults (n=15)) were obtained from the Kosek-Yori biorepository in Iquitos. Demographic characteristics of human fecal samples is shown in the **Table 4**. Based on a previous community census on animal ownership, only dogs, cats, chickens, ducks, pigs and parrots were found in more than 5% of households. Rats were not included in the census but are known to be commonly found within and around households. These animals will be referred to as "*relevant*" animals throughout the paper.

Sample Processing and Validation Assays

DNA was extracted from 0.10 grams of feces using PowerSoil® DNA extraction kit (Qiagen, Germantown, MD, USA) following beadbeating according to the manufacturer's instructions. For each extraction, a negative control consisting of RNA free water was used. TaqMan assays consisted of final reaction mixtures of 20uL, which

included TaqMan™ Advanced Fast Start Master Mix (2X) (Applied Biosystems, Foster City, CA), forward and reverse primers (200uM), probes (100uM), 5uL of DNA template and RNA free-water (Ambion™, Thermo Fisher Scientific, Waltham, MA, USA). Negative template controls (RNAse free water) were included in each amplification reaction. Reaction mixtures were placed in a 96-well plate, and amplified using a StepOnePlus real time PCR system (Applied Biosystems, Foster City, CA). Internal amplification controls (qHsaCtlP0001003, Bio-Rad Laboratories, Irvine, CA) to determine qPCR inhibition were run for every marker and fecal sample and runs were considered invalid if the internal amplification control was above the cycle threshold indicated by the manufacturers. Standard amplification conditions (95ºC for 5 minutes, 40 cycles of 95ºC for 15 seconds, 53ºC for 15 seconds, 60ºC for 45 seconds) were used for all reactions, except for *LA35* and *Av4143*, for which annealing temperatures were set at 56ºC and 55ºC respectively.

Standard Curve Analysis

MST markers were validated by assessing cross reactivity of each marker with target and non-target animal and/or human fecal samples through quantitative polymerase chain reaction (qPCR) amplification. Standard curves were prepared using 10-fold serial dilutions ($3.0 \times 10^5 – 3.0 \times 10^0$ gene copies/uL) of double-stranded synthetic DNA fragments (gBlocks®, Integrated DNA Technologies, Coralville, IA, USA) custom-manufactured for each specific marker. The specific sequences of each gBlock is found in **Table 5**. In order to prepare the working solution of the gBlock, the amount (fmoles) delivered in each control was diluted in 250uL of RNAse free water (Ambion™, Thermo

Fisher Scientific, Waltham, MA, USA). The molar concentration (mol/L) and gene copy concentration (copies/uL) of the stock solution were calculated. Ten-fold serial dilutions were prepared by diluting the stock solution with 10mM Tris-HCL (Quality Bological INC, Gaithersburg, MD, USA) + 0.05% Tween20 (Thermo Fisher Scientific, Waltham, MA, USA). DNA from fecal material was diluted 10-fold diluted and tested in duplicate.

For each specific MST assay, the lower limit of quantification (LLOQ) was set as the average cycle threshold value corresponding to the lowest concentration within the linear range of quantification where at least 95% of the dilution repetitions were detected. The limit of detection (LOD) was set as the LLOQ rounded to the nearest whole number. Additional standard curve parameters included percent efficiency, calculated as: -1 * 10^(-1/slope)), the slope of the curve and the y-intercept.

Statistical Analysis

Sensitivity [1], Specificity [2], Positive Predictive Value [3], Negative Predictive Value [4], and Accuracy of each MST marker was calculated based on the standard meaning in diagnostic testing and as explained. The Accuracy of each diagnostic marker was also estimated [5]. Internal validity metrics of each marker were calculated according to the species for which each was developed.

[1] $Sensitivity = \frac{True\ Positive}{True\ Positive + False\ Negative}$

[2] $Specificity = \frac{True\ Negative}{True\ Negative + False\ Positive}$

[3] $Positive\ Predictive\ Value = \frac{True\ Positive}{True\ Positive + False\ Positive}$

$$[4]\ Negative\ Predictive\ Value = \frac{True\ Negative}{True\ Negative + False\ Negative}$$

$$[5]\ Accuracy = \frac{True\ Positive + True\ Negative}{True\ Positive + True\ Negative + False\ Positive + False\ Negative}$$

Additionally, the predictive probability of detecting a target sample was calculated by fitting a logistic regression variable with the average Ct value of the sample as the independent variable, and the area under the curve (AUC) was estimated for each MST marker, as a post estimation exercise.

Gene quantities were normalized by log(10)-transforming all values . The abundance of gene copy numbers in target and non-target samples was compared using a one-way ANOVA. Pearson's correlation coefficients were estimated for gene copy abundances of all microbial source tracking markers tested, with Bonferroni-adjusted significance levels of 0.05. Data manipulation and statistical analysis was performed in STATA 14 (Stata Corp., College Station, TX) and R (Version 3.3.2).

4.4 Results

Standard curves for each microbial source marker tested are presented in **Figure 1**. Associated parameters for each curve, including the slope, y-intercept, efficiency (%), lower limit of quantification (LLOQ) and assay specific limit of detection (LOD) is presented in **Table 6**. Amplification efficiencies ranged between 91.3% and 101.2%. The lower limit of quantification was 3 gene copy numbers/uL for Av4143, *BactCan* and

HF183, 30 gene copy numbers/uL for ND5, and LA35, and 300 copy numbers/uL for *CytB* and Pig2Bac.

Internal validity metrics of all eight microbial source tracking markers are shown in **Table 7** and associated individual sample results used to calculate these parameters is shown in **Table 8**. Four avian markers were tested: two (*ND5* and *cytb*) targeting avian mitochondrial gene segments of chickens and ducks, one targeting a gene segment of *Lactobacillus* sp. associated with domestic and waterfowl birds (*Av4143*) and one targeting a gene segment of *Brevibacterium avium* associated with chickens only (*LA35*) (78-80). Of all four markers, *Av4143* and *cytB* presented the highest sensitivity (72.7% and 87.0%) and specificity (87.5% and 82.4%) combination. Cross reactivity of *Av4143, cytb* and *ND5* was mainly associated with dog and pig fecal samples. The *LA35* marker was only able to identify 23.1% (3/13) known avian target samples, yet it did not react with any non-target sample (100% specificity).

Of the mammalian markers, *Pig2Bac* showed the best performance parameters, (100.0% sensitivity and 88.5% specificity), detecting 100% of target samples and only 11.5% (11/87) of non-target samples. As specified in **Table 7**, cross-reactivity of *Pig2Bac* was mainly associated with goat fecal samples, a species that is not relevant in this study setting. Both human markers targeted a *Bacteroides* sp. gene segment, with similar sensitivity and specificity values. *BacHum* detected 80.0% (24/30) of target samples and *HF183-Taqman* 76.7% (23/30). Both markers detected 33.8% of non-target samples, with

highest cross-reactivity associated with goats and rats. *BactCan*, the dog-associated marker showed very low specificity, and cross-reacted with pig and goat samples. When excluding the results from goats, guinea-pigs, buffalos and pigeons, given their lack of representativeness in this community), the sensitivity of *Av4143* increased significantly (72.7% vs. 95/7%). Sensitivities for the rest of the markers did not change. Specificities increased for *Pig2Bac* (from 88.5% to 98.2%), *BactCan* (from 47.4% to 58.3%) and *HF183-Taqman* (from 67.6% to 68.8%), yet decreased for *Av4143* (from 87.5% to 81.8%), *cytb* (from 82.4% to 79.5%), *ND5* (from 75.7% to 70.5%) and *BacHum* (66.2% to 62.5%) (**Table 7**).

We did a quantitative assessment of the log(10) transformed gene copy numbers detected by each marker in both target and non-target species. Data is displayed in **Figure 2**. All markers (except of *LA35*) were able to detect higher gene copy numbers in target samples in comparison to non-target samples. Finally, avian markers *ND5* and *cytB* had a statistically-significant (Bonferroni adjusted 0.05 significance level) positive correlation (Pearson's correlation coefficient, 0.949), as well as *cytB* and *Av4143* (Pearson's correlation coefficient, 0.508). Human markers *HF183-Taqman* and *BacHum* also showed a statistically-significant positive correlation (Pearson's correlation coefficient, 0.786). A pairwise correlation coefficient matrix is presented in **Figure 3**. The association between the identification of a target sample and the sample's Ct value, as well as the post-estimation area under the curve (AUC) metric for each MST marker are shown in **Table 9**.

4.5 Discussion

This study validated eight microbial source tracking markers for the proposed principal sources of fecal contamination for communities in the Peruvian Amazon. This work informs use of these markers to understand the relative contribution of human and animal fecal contamination in household environments and further proves that the setting in which fecal samples were collected does influence the MST marker performance.

Various MST markers have been targeting human feces have developed for multiple purposes. Bacteroidales genetic markers are particularly common, yet prior work demonstrates that the performance of these markers may vary widely. In addition, few markers have been validated in low-resource developing areas of the world. In this study we validated the *BacHum* and *HF183-Taqman*, both of which have also been evaluated in Thailand (100 composite samples), Singapore (35 human sewage samples), Nepal (10 composite samples composed of 10 samples each), India (35 human samples) and Kenya (12 human samples) (69, 70, 190, 191). In this study, *BacHum* showed a sensitivity of 80% and specificity of 66%. Sensitivity parameters in these other countries have not been consistent, with values ranging from 95-100% in Thailand and Nepal, to 65% in Singapore, 50% in India and 18% in Kenya (69, 70, 72, 190, 191). Specificity parameters in these same countries ranges from 54% (Thailand), 77% (Nepal), 78% (India), 91% (Singapore) and 100% (Kenya) (69, 70, 72, 190, 191).

HF183 has been developed for both SYBR green and Taqman technologies. In this study, *HF183-Taqman* showed a sensitivity of 77% and specificity of 67%. As with *BacHum*,

internal validity metrics are not consistent across studies, with values ranging from 29% in India, 60% in Singapore and 84-100% in Thailand and Nepal. Specificity values are inconsistent, ranging from 70% in Nepal, 80% in India, 91% in Singapore and 77-100% in Thailand(69, 70, 190, 191). Validation studies done in Australia for both *HF183* and *BacHum* report 95-100% sensitivity and a specificity that ranges between 79% and 99% (66-68).

Reasons for the wide variation in parameters across studies could be multiple, such as differences in human microbiome, genetic variability of Bacteroides, and differences in the climate and local ecology which may indirectly affect microbial populations (71). Age differences could also account for such differences; yet in this study, results from adults and children were not significantly different (data not shown). The need for a consistently well-performing human microbial source tracking marker is evident.

Several swine microbial source tracking markers have been developed, yet *Pig-2-Bac* has shown consistently strong performance across studies. In this study, *Pig-2-Bac* showed a sensitivity of 100% and was the most specific marker (88.5%), and it only cross-reacted with goats (a non-relevant animal in this context) and one dog. Similar results were evidenced in Thailand and China (190, 192), with lower specificity in Nepal (75%) (70). The *BactCan* dog marker showed very low specificity in this study, cross reacting with goats, pigs, chickens and guinea pigs. Similarly, in Nepal this same marker shows a specificity of 45%, yet in India and Singapore it was quite found to be high (97%) (69, 70, 191). Multi-species cohabitation is common in this setting, with a high frequency of

coprophagia in both dogs and pigs. In this particular validation study, pigs were confined in a production facility and as a result, there is a low probability of ingestion of dog or any other animal fecal material. Dogs however, are seldom confined or fed pet kibble and scavenge extensively and are coprophagic.

Surprisingly, studies using microbial source tracking for source attribution generally have not employed avian MST markers, yet avian fecal samples are included among the animal fecal samples collected for validation assays. *LA35* was found to have extremely low sensitivity in this setting, contrasting with the parameters presented by the initial development and evaluation of this marker in the United States (77, 78, 193, 194). *Av4143*, was developed in Israel and validated in a single study in China, with a sensitivity of 100% and specificity of 95%, with cross-reactivity with human and cow samples (80, 195). In this study, *Av4143* cross-reacted with dogs, cats and pig fecal samples, and had a sensitivity of 72.7% when considering all fecal samples tested, and of 95.7% when considering only relevant animals for this community. The two mitochondrial avian markers, *cytB* and *ND5* have not been validated in other settings, besides China, where they were developed. There are other avian fecal markers that have been developed, such as those targeting a *Faecalibacterium* 16S rDNA gene (196, 197), as well as the other four markers developed concomitantly with Av4143(80).

The use of microbial source tracking markers to detect and quantify the source of fecal contamination in this setting has inherent limitations. Multi-species cohabitation alongside humans is common. As a result, ingestion of another species by a second

relevant species, or the feces of other species is not infrequent and likely is a factor that diminished the specificity of the markers in this field setting.

For instance, it is common to observe dogs, which are never confined, consuming matter on infant diapers or avian viscera. Although this may be seen as a limitation, it is a reflection of the real world performance in transmission studies in most regions where diarrhea is highly endemic. Future studies should track co-habitation and peri domestic livestock husbandry in attempt to elucidate the reason for highly variable false-positivity rates in order to improve source attribution. This issue could potentially have large implications for MST performance in low-income settings within One Health studies.

4.6 Conclusion

This study provides the first performance evaluation of six bacteria associated and two mitochondria associated microbial source tracking markers for the attribution of fecal contamination to a species-specific level in a low-resource tropical community of the Amazon. The best performing MST marker was Pig-2-Bac, a pig associated marker. Human and dog associated MST markers showed low specificity in this context, while avian markers show mid to high sensitivities. Importantly, a quantitative evaluation of all markers showed that higher gene copy concentrations were detected by MST markers in target fecal samples, showing these markers can appropriately distinguish host specific bacterial/mitochondrial genes. There is a need to develop and validate more avian markers in this context, as well as other low-resource tropical areas of the world. Most

importantly, there is a critical need to develop a human MST marker that shows higher reliability among studies for future use in quantitative risk assessment studies.

4.7 Tables for Chapter 4

Table 3. Characteristics, primers, probes and origin of Microbial source tracking markers (MST) validated in Iquitos, Peru

Host	Target	Marker	Primers and Probes		Reported Sensitivity	Reported Specificity	References
Chickens	*Brevibacterium avium*	LA35	LA35F LA35R Probe	5′-ACC GGA TAC GAC CAT CTG C-3′ 5′-TCC CCA GTG TCA GTC ACA GC-3′ 5′-FAM-CAG CAG GGA AGA AGC CTT CGG GTG ACG GTA-BHQ1-3′	60-76%	100%	(77, 78)
Chickens and Ducks	Mitochondrial DNA (NADH dehydrogenase subunit 5)	ND5	ND5-F ND5-R ND5-P	5'-ACCTCCCCCAACTAGC-3' 5'-TTGCCAATGGTTAGGCAGGAG-3' 5'-FAM-TCAACCCATGCCTTCTT-NFQ-MGB-3'	100%	84.60%	(79)
Chickens and Ducks	Mitochondrial DNA (cytochrome b)	CytB	cytb-F cytb-R cytb-P	5'-AAATCCCACCCCCTACTAAAAATAAT-3' 5'-CAGATGAAGAAGAATGAGGCG-3' 5'-FAM-ACAACTCCCTAATCGACCT-NFQ-MGB-3′	100%	89.80%	(79)
Domestic Birds and Waterfowl	*Lactobacillus* spp.	Av4143	Av4143F Av4143R Av4143P	5'-TGCAAGTCGAACGAGGATTTCT-3' 5'-TCACCTTGGTAGGCCGTTACC-3' 5'-FAM-AGGTGGTTTTGCTATCGCTTT-BHQplus-3′	95%	97%	(80)
Dogs	*Bacteroidales*	BacCan	BactCan545f1 Uni/Cow690r1 Uni/Cow 690r2 Uni/Cow 656p	5'-GGAGCGCAGACGGGTTTT-3' 5'-CAATCGGAGTTCTTCGTGATATCTA-3' 5'-AATCGGAGTTCCTCGTGATATCTA-3' 5'-FAM-TGGTGTAGCGGTGAAA-MGB-3'	57-63%	90-96%	(60, 198)
Pigs	*Bacteroidales*	Pig2Bac	Pig-2-Bac41F Pig-2-Bac163Rv Pig-2Bac113	5'-GCATGAATTTAGCTTGCTAAATTTGAT-3' 5'-ACCTCATACGGTATTAATCCGC-3' 5'-VIC-TCCACGGGATAGCC-NFQ-MGB-3'	100%	100%	(198)
Humans	*Bacteroidales*	*HF183*-Taq	*HF183*f BthetR1 BthetP1	5'-ATCATGAGTTCACATGTCCG-3' 5'-CGTAGGAGTTTGGACCGTGT-3' 5'-FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA-3'	29-100%	80-87%	(60, 69)
Humans	*Bacteroidales*	*BacHum*	*BacHum*160Fw *BacHum*241Rv *BacHum*193Probe	5'-TGAGTTCACATGTCCGCATGA-3' 5'-CGTTACCCCGCCTACTATCTAATG-3' 5'-FAM-TCCGGTAGACGATGGGGATGCGTT-TAMRA-3'	100%	87%	(60)

Table 4. Sex and age of human fecal samples used in the validation process

Adults	Sex	Age (years)	Children	Sex	Age (years)
Sample 1	Male	46	Sample 1	Male	3
Sample 2	Male	22	Sample 2	Female	4
Sample 3	Female	36	Sample 3	Male	3
Sample 4	Male	51	Sample 4	Male	3
Sample 5	Female	36	Sample 5	Female	5
Sample 6	Female	49	Sample 6	Female	2
Sample 7	Male	48	Sample 7	Male	4
Sample 8	Female	37	Sample 8	Male	3
Sample 9	Male	37	Sample 9	Female	5
Sample 10	Female	35	Sample 10	Female	3
Sample 11	Female	36	Sample 11	Female	3
Sample 12	Male	32	Sample 12	Female	1
Sample 13	Male	34	Sample 13	Female	2
Sample 14	Female	18	Sample 14	Male	5
Sample 15	Female	33	Sample 15	Male	3

Table 5. Gblock sequences used for the development of the eight standard curves.

MST	Final Control Sequence for gBlock
LA35_control	tgcatgatctacgtgcgtcacatgcagtacACCGGATACGACCATCTGCCGCATGGCGGGTGGTGGAAAGTTTTTCGATTGGGGATGGGCTCGCGGCCTATCAGTTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCGACGCAGCGTGCGGGATGACGGCCTTCGGGTTGTAAACCGCTTTCAGCAGGGAAGAAGCCTTCGGGTGACGGTACCTGCAGAAGAAGTACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTACGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGTGGTTGGTCACGTCTGCTGTGGAAACGCAACGCTTAACGTTGCGCGGGCAGTGGGTACGGGCTGACTAGAGTGCAGTAGGGGAGTCTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGACTCTGGGCTGTGACTGACACTGGGGAcactagctcagattcagtagaccgctgttg
ND-5_control	tgcatgatctacgtgcgtcacatgcagtacACCTCCCCCAACTAGCCTTCCTCCACATCTCAACCCATGCCTTCTTTAAAGCTATATTATTCCTATGCTCCGGCCTAATTATCCACAGCCTCAATGGAGAACAAGACATCCGCAAAATAGGATGTCTACAAAAAACCCTCCCAATAACCACCTCCTGCCTAACCATTGGCAAcactagctcagattcagtagaccgctgttg
cytbf_control	tgcatgatctacgtgcgtcacatgcagtacAAATCCCACCCCCTACTAAAAATAATTAACAACTCCCTAATCGACCTCCCAGCCCCATCCAACATCTCTGCTTGATGAAATTTCGGCTCCCTATTAGCAGTCTGCCTCATGACCCAAATCCTCACCGGCCTACTACTAGCCATGCACTACACAGCAGACACATCCCTAGCCTTCTCCTCCGTAGCCCACACTTGCCGGAACGTACAATACGGCTGACTCATCCGGAATCTCCACGCAAACGGCGCCTCATTCTTCTTCATCTGcactagctcagattcagtagaccgctgttg
Av4143_control	tgcatgatctacgtgcgtcacatgcagtacTGCAAGTCGAACGAGGATTTCTTACACTGAGTGCTTGCACTCACCGTAAGAAATTCGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCAAAAGAAGGGGATAACATTTGGAAACAAATGCTAATACCGTATAACCATGATGACCGCATGGTCATTATGTAAAAGGTGGTTTTGCTATCGCTTTTGGATGGACCCGCGGCGTATTAACTAGTTGGTAGGGTAACGGCCTACCAAGGTGAcactagctcagattcagtagaccgctgttg
BactCan545fl_control	tgcatgatctacgtgcgtcacatgcagtacGGAGCGCAGACGGGTTTTTAAGTCAGCTGTGAAAGTTTGGGGCTCAACCTTAAAATTGCAGTTGATACTGGAGACCTTGAGTGCAGTTGAGGCAGGCGGAGTTCGTGGTGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTGAcgctgtgtcgtaacTAGATATCACGAGGAACTCCGATTcactagctcagattcagtagaccgctgttg
Pig-2-Bac41F_control	tgcatgatctacgtgcgtcacatgcagtacGCATGAATTTAGCTTGCTAAATTTGATGGCGACCGGCGCACGGGTGAGTAACGCGTATCCAACCTTCCCTTATCCACGGGATAGCCCGTCGAAAGGCGGATTAATACCGTATGAGGTcactagctcagattcagtagaccgctgttg
*HF183f*_control	tgcatgatctacgtgcgtcacatgcagtacCGTAGGAGTTTGGACCGTGTCTCAGTTCCAATGTGGGGGACCTTCCTCTCAGAACCCCTATCCATCGTTGACTAGGTGGGCCGTTACCCCGCCTACTATCTAATGGAACGCATCCCCATCGTCTACCGGAAAATACCTTTAATCATGCGGACATGTGAACTCATGATcactagctcagattcagtagaccgctgttg
Bac Hum	ACGGGTGAGTAACACGTATCCAACCTGCCGTCTACTCTTGGACAGCCTTCTGAAAGGAAGATTAATCCAGGATGGCATCATGAGTTCACATGTCCGCATGATTAAGGTATTCCGGTAGACGATGGGGATGCGTTCCATTAGATAGTAGGCGGGGTAACGGCCCACCTAGTCTTCGATGGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAA

Table 6. Standard Curve Parameters of eight microbial source tracking markers standardized in this study

Target Specie	MST Marker	Slope	y-intercept	Efficiency (%)	LLOQ (Ct)	LLOQ (gene copy number/uL)	Assay LOD
Domestic Birds and Waterfowl	*Av4143*	-3.55	39.22	91.38	37.42	3	37
Chickens	*LA35*	-3.49	41.32	93.57	36.80	30	37
Chickens and Ducks	*ND5*	-3.29	43.44	101.23	37.55	30	37
Chickens and Ducks	*CytB*	-3.37	45.88	98.06	37.94	300	37
Humans	*HF183Taq*	-3.48	37.83	93.82	36.45	3	36
Humans	*BacHum*	-3.44	37.40	95.17	35.86	3	36
Pigs	*Pig2Bac*	-3.34	45.99	99.33	38.16	300	38
Dogs	*BacCan*	-3.39	38.39	96.89	36.67	3	37

Slope = y-intercept of the curve. LLOQ = Lower Limit of Quantification: Average cycle threshold value corresponding to the lowest concentration within the linear range of quantification where at least 95% of the dilution repetitions were detected. LOS = Limit of Detection: LLOQ rounded to the nearest whole number. Efficiency = (-1 * 10^(-1/slope)).

Table 7. Internal Validity Metrics of all Microbial Source Tracking markers tested in this study

Target Specie	Microbial Source Tracking marker	Including fecal samples from all species					Including fecal samples from contextually relevant species*				
		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Domestic Birds and Waterfowl	*Av4143*	72.7	87.5	75.0	86.2	82.5	95.7	81.8	73.3	97.3	86.6
Chickens	*LA35*	23.1	100.0	100.0	89.4	89.7	23.1	100.0	100.0	84.4	85.1
Chickens and Ducks	*cytB*	87.0	82.4	60.6	95.3	83.5	87.0	79.5	69.0	92.1	82.1
Chickens and Ducks	*ND5*	69.6	75.7	47.1	78.1	66.0	69.6	70.5	55.2	81.6	70.1
Pigs	*Pig2bac*	100.0	88.5	50.0	100.0	89.7	100.0	98.2	90.9	100.0	85.1
Dogs	*Bactcan*	100.0	47.4	19.6	100.0	53.4	100.0	58.3	33.3	100.0	69.2
Humans	*Bachum*	80.0	66.2	50.0	88.7	70.3	80.0	62.5	57.1	83.3	69.2
Humans	*HF183-Taqman*	76.7	67.6	50.0	87.3	70.3	76.7	68.8	60.5	82.5	71.8

Key: (*) Contextually Relevant Species = Humans, dogs, cats, rats, chickens, ducks, pigs, parrots. Sensitivity= (True Positive / True Positive + False Negative); Specificity = (True Negative / True Negative + False Positive); Positive Predictive Value (PPV) = (True Positive / True Positive + False Positive); Negative Predictive Value (NPV) = (True Negative / True Negative + False Negative); Accuracy = (True Positive + True Negative / True Positive + True Negative + False Positive + False Negative).

Legend: Performance characteristics for 8 microbial source tracking markers to quantify feces of chickens, pigs, dogs, and humans demonstrate excellent performance of pig Pig2Bac MST marker, and avian marker Av4143. Dog and human markers demonstrate moderate performance.

Table 8. Sample-specific results of all fecal samples tested in this study

Source	Microbial Source Tracking Markers															
	Av4143 (avian)		*LA35 (avian)*		*CytB (avian)*		*ND5 (avian)*		*pig2bac (swine)*		*bactcan (canine)*		*bachum (human)*		*HF183 (human)*	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Chicken	13/13	-	3/13	-	12/13	-	12/13	-	-	0/13	-	6/10	-	5/13	-	5/13
Duck	9/10	-	-	0/10	5/10	-	4/10	-	-	0/10	-	4/6	-	3/8	-	4/8
Pidgeon	2/10	-	-	0/10	-	4/10	-	4/10	-	0/10	-	2/10	-	0/3	-	0/3
Parrot	-	0/2	-	0/2	-	0/2	-	1/2	-	0/2	-	NA	-	0/1	-	0/1
Children	-	0/10	-	0/10	-	2/10	-	3/10	-	0/10	-	0/10	11/15	-	11/15	-
Adults	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	13/15	-	12/15	-
Guinea Pig	-	0/5	-	0/5	-	0/5	-	1/5	-	0/5	-	5/5	-	0/5	-	0/5
Rat	-	1/10	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10	-	5/10	-	4/10
Goat	-	0/10	-	0/10	-	0/10	-	0/10	-	9/10	-	9/10	-	5/10	-	6/10
Buffalo	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	5/5	-	1/5	-	2/5
Pig	-	3/10	-	0/10	-	4/10	-	4/10	10/10	-	-	10/10	-	2/5	-	2/5
Dog	-	3/10	-	0/10	-	3/10	-	5/10	-	1/10	10/10	-	-	2/9	-	0/9
Cat	-	1/2	-	0/2	-	0/2	-	0/2	-	0/2	-	0/2	-	1/2	-	0/2
Total Target Positive Samples	24/33	-	3/13	-	20/23	-	16/23	-	10/10	-	10/10	-	24/30	-	23/30	-
Total Non-Target Positive Samples	-	8/64	-	0/84	-	13/74	-	18/74	-	10/87	-	41/78	-	24/71	-	24/71

Legend: Sample specific results of each microbial source tracking marker, showing all eight markers, except LA35, are able to detect the majority of target samples. Dog marker (Bactcan), and human markers (*BacHum* and HF-Taqman) show a high proportion of cross reactivity with non-target samples.

Table 9. Association between the identification of a target sample and the sample's cycle threshold (Ct).

MST Marker	Odds Ratio	SE	*p-value*	AUC
Av4143	0.850	0.068	0.042	0.703
LA35	0.780	0.230	0.396	0.750
CytB	0.790	0.051	<0.001	0.805
ND5	0.760	0.045	<0.001	0.791
Pig2Bac	0.600	0.084	<0.001	0.975
BactCan	0.154	0.119	0.016	0.997
HF-183 Taqman	0.940	0.023	0.010	0.653
BacHum	0.910	0.026	<0.001	0.636

Odds a target sample based on the sample's cycle threshold obtained. For example: the odds of obtaining a positive Av4143 sample decreased by 15% for every additional cycle threshold. SE=standard errors; AUC= area under the curve.

4.1 Figures for Chapter 4

Figure 1. Standard Curves of eight MST markers validated in this study

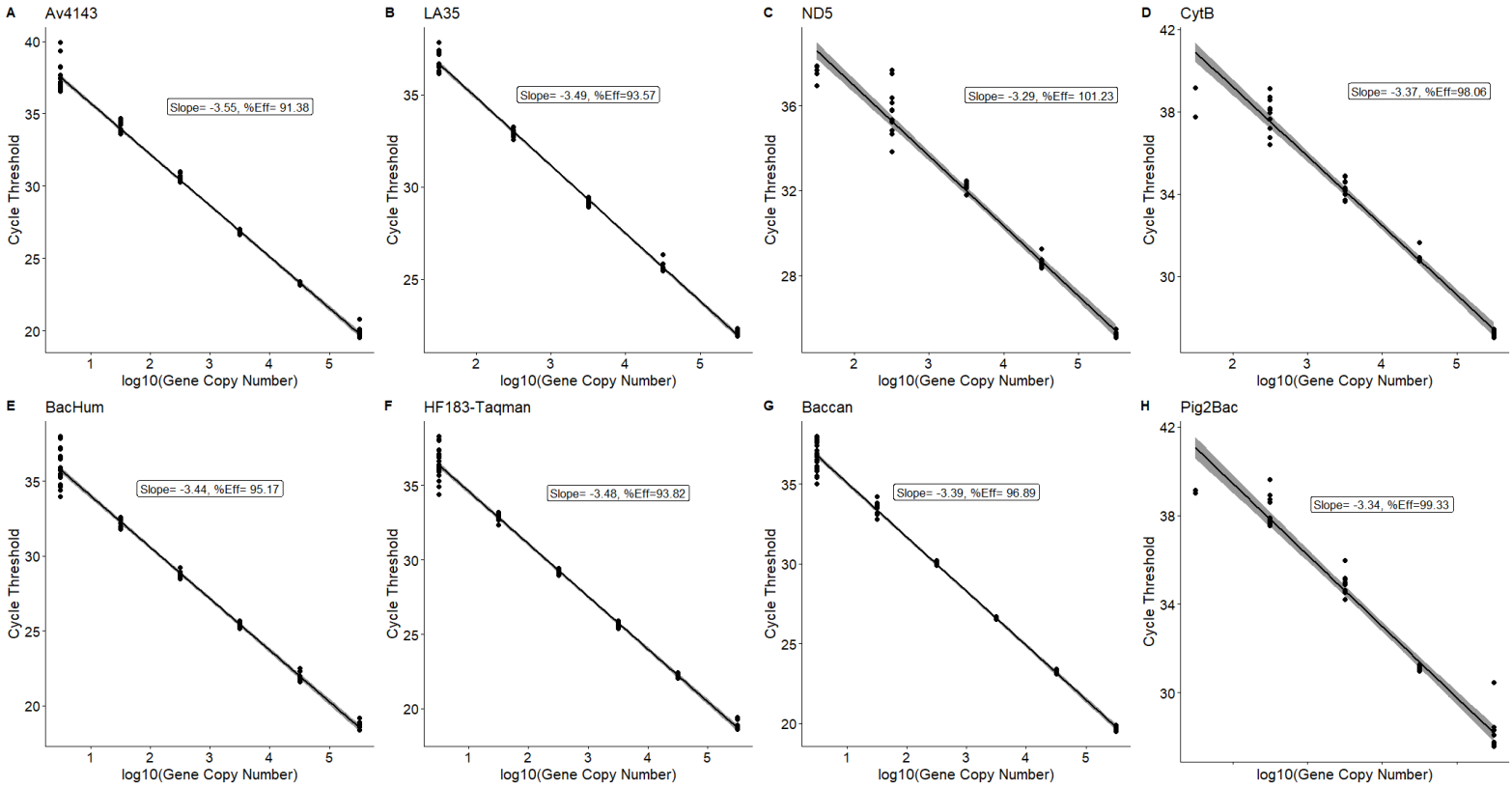
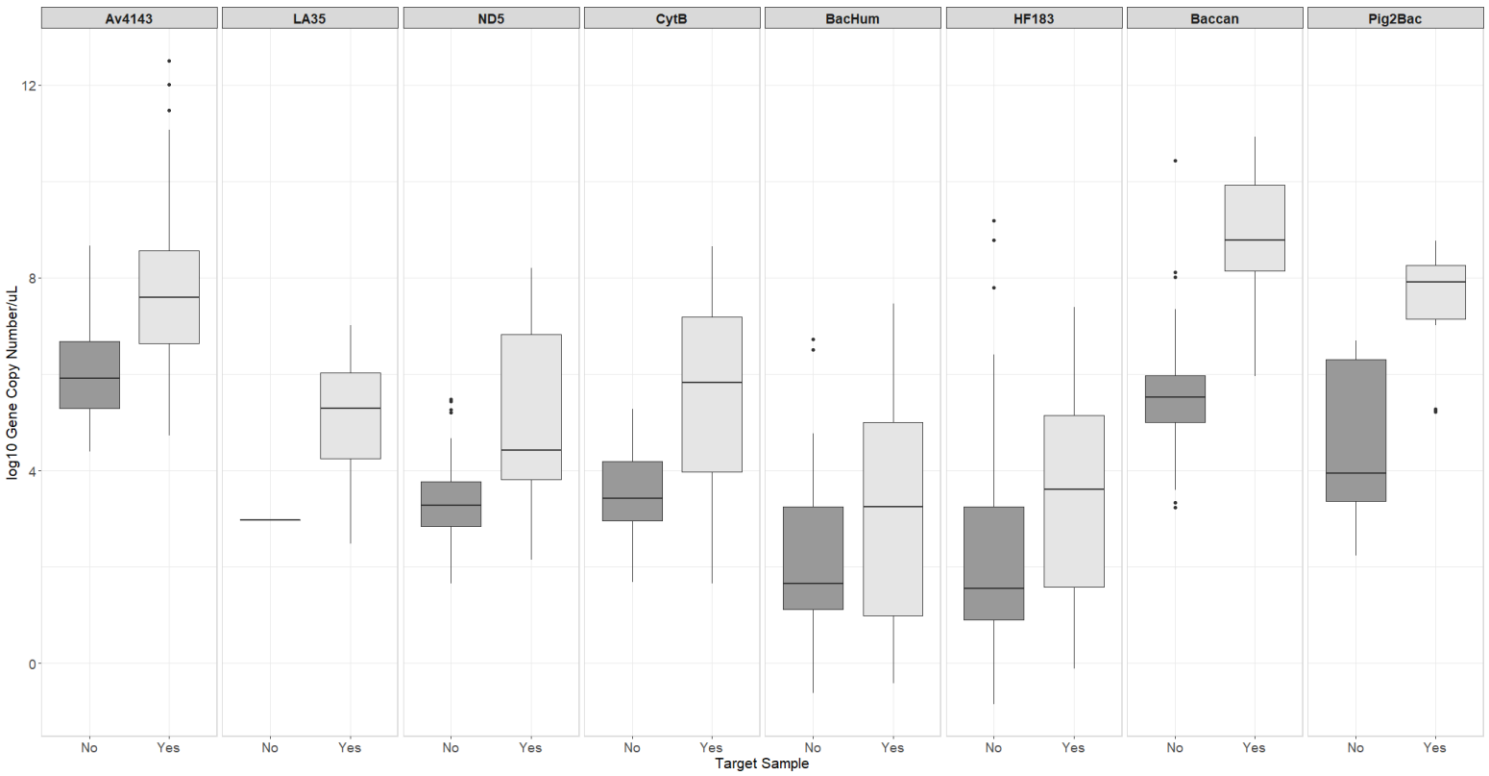


Figure 2. Quantitative analysis of (log10) gene copy number quantities of the eight MST markers validated in target and non-target fecal samples.



Key: Target samples refer to fecal samples of animal species for which the microbial source tracking was developed. Non-target samples refer to fecal samples of animal species for which the microbial source tracking marker was not develop

Legend: All microbial source tracking markers, except LA35, are able to detect statistically significant higher gene copy numbers among target samples in comparison to non-target samples.

Figure 3. Correlation Matrix with pairwise Pearson's correlation coefficients of the log(10) gene copy number of eight microbial source tracking markers among all fecal samples tested

	Av4143	*ND5*	*CytB*	*BacHum*	*HF183*	*Bactcan*	*Pig2Bac*
Av4143	1.0000						
ND5	0.5176	1.0000					
CytB	0.5083*	0.9492*	1.0000				
BacHum	0.1294	-0.2385	0.3554	1.0000			
HF183	-0.0187	0.0399	-0.0513	0.7855*	1.0000		
Bactcan	-0.1632	0.2950	0.0894	-0.2258	-0.3780	1.0000	
Pig2Bac	0.3473	-0.7071	0.1253	0.1175	-0.0751	-0.1594	1.0000

(*) Bonferroni Adjusted Significance level of 0.05.

Chapter 5. Associations between household animal ownership, infrastructure and hygiene characteristics with source attribution of household fecal contamination in communities in peri-urban Peru

5.1 Abstract

Using previously validated microbial source tracking markers (MST) we detected and quantified fecal contamination from avian species (*Av4143, LA35*), dogs (*Bactcan*), humans (*Bachum* and *HF183*-Taqman), as well as avian exposure (*CytB* and *ND5*) on household cooking tables (n=104) and floors (n=104) in Loreto, Peru. Quantitative data was log(10) transformed and categorized into "Low", "Medium" and "High" tertiles of contamination. The association between contamination and infrastructure and socio-economic covariates was assessed using simple and multiple ordinal logistic regressions. The presence of *Campylobacter* spp. in surface samples linked to avian markers to establish the importance of detected levels of fecal contamination. Animal feces were detected in 75% of households, and human feces were present in 20.2%. Floors were more contaminated than tables as detected by the avian marker *Av4143*, dog marker *Bactcan* and human marker *Bachum*, Wood tables were consistently more contaminated than non-wood surfaces, specifically for the mitochondrial avian marker *ND5* and CytB, fecal marker Av4143, and canine marker *Bactcan*. Final adjusted models for socioeconomic and infrastructure characteristics indicate that detection of avian feces and avian exposure was associated with the presence of chickens, maternal age and length of tenancy while detection of human markers was associated with unimproved water source.

Detection of *Campylobacter* in surface samples was associated with the avian fecal marker Av4143, after adjusting for the presence of chickens and other positive markers. We highlight the critical need to detect and measure the burden of animal fecal waste when evaluating household water, hygiene and sanitation interventions, and the possibility of decreasing risk of exposure through the modification of surfaces to permit more effective household disinfection practices, with the objective of reducing zoonotic enteric diseases in tropical low-resource communities.

5.2 Introduction

Intra-domiciliary fecal contamination is a well-known risk-factor for the transmission of enteric pathogens (199). Water, soil and household surfaces are regularly contaminated with bacterial pathogens of both human and other animal fecal material (9, 200-202). Detection and quantification of fecal contamination within the house is generally carried out using standard microbiologic methods targeting traditional fecal indicator bacteria (FIB), such as *Escherichia coli*. FIB are commonly detected and quantified in household water and surfaces, and epidemiologic studies have associated the degree of fecal contamination with hygiene, sanitation infrastructure and socio-demographic characteristics of household members (9, 74, 203). One of the principal assumptions underpinning these studies is that fecal contamination is mainly human-derived. However, animal fecal matter is often also highly prevalent within households, and the presence of animal, as well as animal fecal waste has been associated with increased risk of enteric illness (45, 52, 204). Although traditional fecal indicator bacteria are able to determine the degree of fecal contamination by taking into consideration all sources,

these methods are not capable of distinguishing between animal and human sources of detected feces (205, 206). Furthermore, samples analyzed using traditional microbiologic methods are easily contaminated by FIB from other environmental sources or reservoirs, such as soils (207, 208).

Microbial source tracking (MST) methods have been developed to determine the source of fecal contamination within waste-water and recreational water systems for remediation purposes (71, 209). By attributing and quantifying fecal contamination to a specific animal species, intervention strategies and remediation measures can be more easily targeted and applied. Several microbial source tracking markers have been developed and standardized in rural communities where there is a high degree of household fecal contamination (69, 72, 191, 210, 211). However, to date no studies have applied MST methods to quantify animal fecal burden in household surfaces. Furthermore, studies that have applied microbial source tracking markers in low income settings have not included avian specific markers of fecal contamination. This is a critical pitfall of previous studies given the role of poultry within domestic animal husbandry practices as an alternative source of income and nutritional source of protein, as well as a source of *Campylobacter* spp., one of the main causes of bacterial diarrhea, stunting and environmental enteropathy in pediatric populations of low-income settings (5, 12, 179, 212).

Using previously validated microbial source tracking markers in this same region, this study quantified the burden of animal fecal contamination of surfaces in households in a peri-urban, low-resource, tropical community of Loreto, Peru with a particular emphasis

on avian fecal contamination. We explored the associations between MST presence and burden with household infrastructure and socio-economic characteristics of the primary caregiver. Finally, we explore the association between the detection of *Campylobacter* spp. and different microbial source tracking markers in surface samples from the same households.

5.3 Methods

Study setting and population.

This study took place in Santa Clara de Nanay, Santo Tomas and La Union (3°47'S, 73°20'W), three peri-urban communities located 15km away from Iquitos city-center, Loreto, Peru. These communities combined are composed of approximately 1,300 households and 12,000 individuals. Common occupations for men in these communities include small-scale agricultural production, fishing and moto-taxi driving, while women most commonly report being homemakers, having a small corner shop ("bodega") or being unemployed (8).

Data and Sample Collection

Between October 2018 and September 2019 households from these three communities were randomly selected for sampling. Within each household two surface samples were obtained, and one socio economic questionnaire per household was completed by the head of household. Surface samples included a sample from the table where cooking took place, as well as a sample from the entrance floor. The cooking surface of preference (where food is manipulated for human consumption) was identified by the head of

household. As described previously and shown in **Figure 4**, a 30.0 cm by 30.0 cm square of scrap paper used to frame the sampling area was placed on top of the selected surface (203, 213). Using sterile nitrile gloves and applying moderate pressure, half of a dry autoclaved electrostatic cloth (Swiffer®, Procter and Gamble, Cincinnati, OH, USA) was spread over the framed surface area. The cloth was then placed in a sterile 24oz Whirl-Pak bag (Nasco, WI, USA) and 10mL of phosphate buffered solution (PBS) added. Samples were placed in a cooler with ice-packs and transported to the lab within 4 to 6 hours of collection for processing.

If there was a child under 2 years of age present in the household, a plastic toy was given and exchanged for an identical item within 24 hours as a sentinel object that reports more directly on the microbial exposure of the mobile child (214). The rubber toy was placed in a plastic bag and 10mL of PBS were added. All samples went through the same processing protocol.

Sample Processing

Samples were vigorously shaken for 5 minutes to ensure the sampled material was homogenized within the solution. Using a 10.0mL transfer pipette, the sample solution was transferred into sterile 2mL crioviales to DNA extraction. DNA was extracted from 200 microliters of solution using PowerSoil® DNA extraction kit (Qiagen, Germantown, MD, USA) following beadbeating according to the manufacturer's instructions. For each extraction, a negative control consisting of RNA free water was used.

qPCR using Microbial Source Tracking Markers

Eight microbial source tracking (MST) markers that were previously validated within this context were used to score surface samples (Schiaffino *et al*. *In Review*). Specifically, two avian fecal markers (*Av4143*, *LA35*), two avian mitochondrial fecal markers (*cytb* and *ND5*), two human fecal markers (*BachHum* and *HF83-Taqman*), one dog fecal marker (*BactCan*) and one pig fecal marker (*Pig2Bac*) were utilized (60, 77, 79, 80, 189). Details regarding the target species, gene, as well as internal validity parameters of all eight MST markers are presented in **Table 10**.

TaqMan assays consisted of final reaction mixtures of 20uL, which included TaqMan™ Advanced Fast Start Master Mix (2X) (Applied Biosystems, Foster City, CA), forward and reverse primers (200uM), probes (100uM), 5uL of DNA template and RNA free-water (Ambion™, Thermo Fisher Scientific, Waltham, MA, USA). Primers and probe sequences are presented in **Table 10**. Negative controls consisting of RNA and DNA free water were included in each amplification reaction. Reaction mixtures were placed in a 96-well plate, and amplified using a StepOnePlus real time PCR system (Applied Biosystems, Foster City, CA). Internal amplification controls (qHsaCtlP0001003, Bio-Rad Laboratories, Irvine, CA) were run for every marker and surface sample and runs were invalid if the internal standard did not amplify. Standard amplification conditions (95ºC for 5 minutes, 40 cycles of 95ºC for 15 seconds, 53ºC for 15 seconds, 60ºC for 45 seconds) were used for all reactions, except for *LA35* and *Av4143*, for which annealing temperatures were set at 56ºC and 55ºC respectively.

Standard Curve Analysis

Standard curves of each microbial source tracking marker were prepared using 10-fold serial dilutions ($3.0 \times 10^5 - 3.0 \times 10^0$ gene copies/uL) of double-stranded synthetic DNA fragments (gBlocks®, Integrated DNA Technologies, Coralville, IA, USA) manufactured for each specific marker. The specific sequences of each gBlock is found in **Table 5**. In order to prepare the working solution of the gBlock, the amount (fmoles) delivered in each control was diluted in 250uL of RNA free water (Ambion™, Thermo Fisher Scientific, Waltham, MA, USA). The molar concentration (mol/L) and gene copy concentration (copies/uL) of the stock solution were calculated. Ten-fold serial dilutions were prepared by diluting the stock solution with 10mM Tris-HCL (Quality Biological Inc, Gaithersburg, MD) + 0.05% Tween20 (Thermo Fisher Scientific, Waltham, MA, USA).

Detection of Campylobacter spp.

Surface samples were tested for the presence or absence of *Campylobacter* spp. using a semi-quantitative PCR that targeted a 16S sRNA segment that identifies all members of the *Campylobacter* genus(215) (16S_Fw: 5'- CAC GTG CTA CAA TGG CAT AT -3'; 16S_Rv: 5'- GGC TTC ATG CTC TCG AGT T -3';16S_Probe: 5'- /56-FAM/CAG AGA ACA /ZEN/ ATC CGA ACT GGG ACA /3IABkFQ/ -3'), as well as the cadF gene (adhesion to fibronectin) (*cadF*_Fw: 5'- CTG CTA AAC CAT AGA AAT AAA ATT TCT CAC -3'; *cadF_Rv*: 5'- CTT TGA AGG TAA TTT AGA TAT GGA TAA TCG -3'; *cadF_Probe*: 5' -/56-JOEN/CAT TTT GAC /ZEN/ GAT TTT TGG CTT GA/3IABkFQ/ -3') to detect thermotolerant species (most likely *Campylobacter jejuni*/ *Campylobacter*

coli only(216)). Final reaction mixtures of 25uL consisted of 12.5uL of Taq Environmental Master Mix 2.0 (Thermo-Fisher Scientific, Waltham, MA, USA), primers at a concentration of 0.2uM each and the probes at a concentration of 0.1uM each, 1uL of DNA and nuclease free water. The assay was performed under the following cycling conditions: 95ºC for 10 minutes, 45 cycles of 95ºC for 15 seconds, and 55ºC for 1 minute (Step-One Instrument, Applied Biosystems, Foster City, Ca). A target was determined to be positive if a cycle threshold (Ct) of less than 38 was obtained for the 16S gene.

Data Management Analysis

A binary variable indicating the presence and absence of a given MST marker in a surface sample was created using the assay-specific limit of detection (Table 1), indicating a positive sample if the cycle threshold obtained was below the limit of detection (lower Ct) and a negative sample if the cycle threshold was above that limit or was undetermined. Floor and table surface samples were treated as distinct independent samples. Floor were categorized as either finished - made of a material that separated the dirt floor from household members or animals – or unfinished – uncovered earth (217). The surface material of tables was classified as being either of wood or non-wood, a category that was mainly comprised of wood tables covered with plastic sheeting. Pearson's chi-square and Fisher's exact test were used to test the differences in positivity for any specific MST marker between floors and tables, as well as between unfinished and finished floors, and wood and non-wood tables. Gene quantities were log(10) transformed (log(10)GCN/uL). The distribution of this continuous variable was assessed using Shapiro-Wilk, Skewness and Kurtosis normality tests. Data were found to be right-

skewed for all six markers. The difference in the median log(10)GCN/uL in tables and floors, as well as unfinished vs. finished floors and non-wood vs. wood floors was assessed using the Wilcoxon rank-sum test. Gene quantities were further categorized into tertiles and modelled as an ordinal outcome variable, where the first tertile was interpreted as “low”, the second as “medium” and the third one as “high” gene copy number quantities.

Covariates analyzed included age of the primary caregiver (years), maternal education (years), age of the primary caregiver at first pregnancy (years), and average monthly income (US Dollars). Household infrastructure characteristics included a binary variable for household crowding (less than six people living in the household/ more than six people living in the household), length of household tenancy (less than 1 year, between 1 and 5 years, between 5 and 10 years, between 10 and 20 years, more than 20 years), floor material (unfinished/finished), table material (wood/ non-wood) and wall type (cement/ other). Hygiene covariates included treatment of drinking water, water source (improved/unimproved) and sanitation facility (improved/unimproved). Finally, the presence and absence of chickens within the household was also included as a covariate. Baseline associations between the main exposures, floor material and table material, were performed using Pearson’s chi-square or Fisher’s exact test for binary covariates and Wilcoxon rank-sum test for continuous covariates. Regression models were fitted for all microbial source tracking markers separately using simple and multiple ordered logistic regressions to test the association between the degree of contamination (“Low”, “Medium” or “High”) in floor and table samples and the specified household and socio-

economic covariates. Multivariate regression models were fitted by adjusting for household and socio-economic covariates. The proportional odds assumption was tested for all adjusted and unadjusted regression models.

Campylobacter positive surface samples were treated as a bivariate outcome and modeled using generalized lineal models with binomial family and link logit (Odds Ratios). Separate models were run with the presence or absence of each MST as covariates, adjusting for the presence of chickens. Type I error was set at 0.05 for all statistical analysis. Data management and statistical analysis were performed in STATA 14 and (Stata Corp., CollegeStation, TX) and R (version 3.3.2).

Ethical Considerations

Ethical approval was obtained from the International Review Boards of Asociacion Benefica Prisma and Johns Hopkins Bloomberg School of Public Health. A local field worker from Asociacion Benefica Prisma explained the study procedures to the household head and a signed informed consent was requested before any study procedure took place. All participants were voluntary and free to stop any study procedure at any moment. All field workers spoke Spanish and all informed consent documents and socio-economic questionnaires were written in Spanish.

5.4 Results

A total of 104 cooking tables surface samples and 104 floor samples were obtained from 104 unique households. Of the 104 floors samples, 54 (51.9%) were made of dirt, 39 (37.5%) of cement, 6 (5.8%) of wood, four (3.8%) of tile and one (1.0%) floor was covered in plastic material. A total of 54 floors were classified as unfinished (i.e. bare earth) and 50 were classified as finished. Of the 104 table samples, 75 (72.1%) were made of wood, 22 (21.2%) of plastic, 4 (3.8%) of fabric, 2 (1.9%) of tile and 1 (1.0%) was covered in paper (**Table 11**). Out of all household samples, 76.9% (80/104) were positive for any fecal marker (*Av4143, Bactcan, BacHum* and *HF183-Taqman*). Animal feces were detected in 75% (78/104) of households and human feces were detected in 20.2% (21/104) of households. Bivariate results of each MST marker by table and floor surface categories are shown in **Table 12**, quantitative and categorical (*High*, *Medium*, *Low* data) results for each MST marker are shown in **Figure 5** and **Figure 6** respectively**.** All samples were negative for the avian fecal marker *LA35* and pig fecal marker *Pig2Bac*. Covariate characteristics of household and primary caregiver are presented in **Table 13**. The univariate associations between MST gene quantity tertials of floors and tables surface samples, and the household and primary caregiver covariates are shown in the supplementary material (**Table 14** and **Table 15**), while the adjusted associations are presented in **Table 16**. All results that follow are presented by microbial source tracking marker.

Microbial Source Tracking Markers in Surface Samples

i. *Avian fecal marker Av4143*

Out of the 104 floor samples, 49.0% (n=51) of floors and 15.4% (n=16) of tables (p-value <0.001) were positive for the avian marker *Av4143*. The median log(10)GCN/uL of Av4143 was 1.80 among floor samples and 1.33 among table samples (p-value <0.001). The number of finished and unfinished floors, as well as wooden and non-wooden tables positive for *Av4143* were not significantly different. Similarly, the median log(10)GCN/uL of Av4143 among finished and unfinished floors, and wood and non-wood tables were not statistically different. As shown in **Figure 6**, 50% of floor samples and 16.3% of table samples were classified as having a "High" quantity of Av4143 marker, while 17.3% of floors and 12.5% of table samples were "Medium" and 31.5% of floors and 71.2% of tables were "Low" (p-value <0.001). The unadjusted Av4143 models are shown in **Table 14** and **Table 15.** The odds of a floor sample being classified in the "High" tertile in comparison with the "Middle" and Low" tertile were 3.70 (95% confidence interval [CI]: 1.71-7.99; p-value = 0.001) among households who owned chickens in comparison to those who did not. This same statistically significant association was found among table samples scored for Av4143 (OR: 4.42; 95% confidence interval [CI]: 1.70-11.49; p-value=0.003). The odds of a table sample being classified in the "High" group in comparison to the "Medium" and "Low" group were 3.32 (95% confidence interval [CI]: 1.05-10.32; p-value=0.041) among surfaces made of wood in comparison to other material than wood.

The adjusted models showed that the presence of chickens in the household (OR: 4.03; 95% CI: 1.51-10.79; p-value = 0.005), maternal age (OR: 0.93; 95% CI: 0.87-0.99; p-value = 0.044), and length of property tenancy (more than 20 years in comparison to less

than 1 year, OR: 0.14; 95% CI: 0.03-0.75; p-value = 0.022) retained statistical significance among floor samples, whereas the presence of chickens (OR: 4.73; 95% CI: 1.40-15.91; p-value = 0.012) and table material (OR: 0.15; 95% CI: 0.03-0.82; p-value = 0.028) retained statistical significance among table samples, adjusted for all other covariates.

ii. Avian mithocondrial markers CytB and ND5

Fifty-nine percent (62/104) of floor samples and 67.3% (70/104) of table samples were positive for *CytB*, while 83.7% (87/104) floor samples and 77.9% (81/104) table samples were positive for *ND5*. A greater number of wood tables (84.0% (63/75)) were positive for the avian *ND5* marker, in comparison to non-wood tables (62.1% (18/29)) (p-value 0.016). The median quantity of the avian markers *CytB* (wood: 4.31 log(10)GCN/uL; non-wood 3.37 log(10)GCN/uL; p-value=0.038) and *ND5* (wood: 4.24 log(10)GCN/uL; non-wood 3.43 log(10)GCN/uL p-value=0.010) were statistically different between wood and non-wood tables.

The unadjusted *CytB* and *ND5* models are shown in **Table 14** and **Table 15**. Of significance, wood tables in comparison to table surfaces made of other material than wood had a higher odds of being classified "High" tertile, for both the ND5(OR: 3.11; 95% CI: 1.38-7.02; p-value=0.006), and *CytB* markers (OR: 2.53; 95% CI: 1.12-5.68; p-value=0.025). Within the final adjusted model for *ND5*, the presence of chickens in the household (OR: 3.33; 95% CI: 1.33-8.30; p-value = 0.10), treatment of drinking water (OR: 3.62; 95% CI: 1.44-9.12; p-value =0.006) retained statistical significance among

floor samples, whereas table material (OR: 0.27; 95% CI: 0.10-0.76; p-value = 0.013) and treatment of drinking water (OR: 2.46; 95% CI: 1.04-5.79; p-value = 0.040) retained statistical significance among table samples. Within the final adjusted model for *CytB*, the odds of a floor sample being classified in the "High" tertile in comparison with the "Middle" and Low" tertile were of 0.94 (95% confidence interval [CI]: 0.89-0.99; p-value = 0.015), for every additional age of the primary caregiver. The effect of treatment of drinking water (OR: 2.67; 95% CI: 1.12-6.39; p-value = 0.027) also retained statistical significance among table samples.

iii. Dog fecal marker Bactcan

A total of 27.9% (29/104) of floors and 11.5% (12/104) of tables (p-value 0.003) were positive for the dog fecal marker *Bactcan*. From all unfinished and finished floors samples, 35.2% (19/54) and 20.0% (10/50) were positive. Wood tables were 12.0% (9/75) positive and non-wood tables 10.3% (3/29). Once the quantities of *Bactcan* were categorized, 38.5% of floor samples and 11.5% of table were classified as having a "High", 8.7% of floors and 5.8% of tables were classified as "Medium" and 52.9% of floors and 82.7% of tables were classified as having "Low" quantities of *Bactcan* marker (p-value <0.001). From the unadjusted models, the odds of having a "High" *Bactcan* floor sample in comparison to a "Medium" or "Low" *Bactcan* floor sample were reduced by 70% (95% CI: 0.11-0.82; p-value=0.020) among households with wall materials other than cement in comparison to those households with cement walls. No statistically significant associations were found between the categories of *Bactcan* and household infrastructure and socioeconomic characteristics.

iv. Human fecal markers Bachum and HF183-Taqman

A total of 17.3% (18/104) of floors and 3.9% (4/104) of tables were positive for the human fecal marker *Bachum*. Eight of the floors were unfinished and 10 were finished. All four tables positive for the human fecal marker were made of wood. When categorized, 31.7% of floors and 8.7% of tables had a high *Bachum* burden, while 68.3% of floors and 91.3% of tables had a low *Bachum* burden (p-value<0.001). Only one table and one floor were positive for the *HF183*-Taqman marker, which consisted of one finished floor and one wood table. In the final adjusted mode, the odds of a table sample being classified in the "High" tertile in comparison with the "Middle" and Low" tertile were of 0.81 (95% confidence interval [CI]: 0.68-0.97; p-value = 0.020), for every additional age of the primary caregiver. Finally, floor variables scored with the human *Bachum* fecal marker the odds being classified in the "High" tertile in comparison with the "Medium" and "Low" tertial were 13.81 (95% CI: 1.72-110.92; p-value = 0.014) among samples from households with unimproved water sources in comparison to households with improved water sources, holding all other variables constant.

Microbial Source Tracking Markers in Toy Samples

A total of 55 toys were given to a child and collected within 24 hours. Avian fecal contamination exceeded microbial contamination from other sources: *ND5* (avian exposure) 49.1% (27/55), *CytB* (avian exposure) 29.1%(16/55), *Av4143* (Avian feces) 9.1% (5/55), and *BacHum* (Human feces) 5.5% (3/55). All toy samples were negative for *HF183*-Taqman (human feces), *Bactcan* (canine feces), *LA35* (avian feces), and *Pig2Bac* (swine feces).

Association between Campylobacter spp. detection and MST markers

A total of 60.6% (63/104) floor surface samples (43 unfinished and 20 finished; p-value <0.001) and 18.3% (19/104) table surface samples (16 wood and 3 non-wood; p-value = 0.193) were positive for *Campylobacter* spp (*16S* gene) (p-value <0.001). Among floor samples, the odds of detecting *Campylobacter* spp. were 29.34 (95%CI: 7.61-113.17; p-value<0.001) if the sample was positive for Av4143, adjusting for the presence of chickens and floor material. Among table samples, the odds of detecting *Campylobacter* spp. were 29.49 (95%CI: 6.71-129.55; p-value<0.001) if the sample was positive for Av4143, adjusting for the presence of chickens and table material. Similarly, there was a higher odds of detecting *Campylobacter* spp. in floor and table samples if samples were positive for *CytB* (Floors: OR 12.61; 95%CI: 4.11-38.68; p-value<0.001; Tables: OR: 10.72; 95% CI: 1.36-84.75; p-value=0.025) and among floor samples if they were positive for *ND5* (OR 10.25; 95%CI: 2.43-43.32; p-value=0.002). Having a positive floor sample for the human *Bachum* also increased the odds of having a *Campylobacter* positive floor sample (OR: 25.25; 95%CI: 2.94-216.81; p-value=0.003). A final model adjusting for the presence of all markers, as well as the presence of chickens in the household and the floor material, showed that the odds of having a *Campylobacter* spp. positive sample increased by 11.71 (95%CI: 2.59-53.09; p-value=0.001) among floor samples positive for the *Av4143* marker, and decreased by 0.11 (95%CI: 0.03-0.39; p-value=0.001) if floors were finished in comparison to unfinished floors. All other markers showed no association with the odds of detecting *Campylobacter* spp. in a floor sample in models adjusted for all markers, suggesting that identifiable exposures rather than

universal community wide contamination drove household levels of risk for campylobacteriosis. Similarly, among table samples, the odds of having a *Campylobacter* spp. positive sample was 21.74 times greater (95%CI: 4.62-101.38; p-value<0.001) among samples positive for *Av4143* in comparison to negative samples, adjusting for all other markers, presence of chickens and table material. No other covariate was associated with the presence of *Campylobacter* spp. from a table surface sample.

5.5 Discussion

In three peri-urban communities of Iquitos, Peru 76.9% household surfaces were positive for any fecal microbial source tracking marker. Specifically 75.0% of households were positive for animal feces while 20.2% were posisitve for human feces. The degree of animal fecal material, with particular emphasis on feces from an avian species, is greater in comparison to human fecal material in household surfaces. Detection of avian markers was associated with the material of table samples, presence of chickens in the household, maternal age and length of property tenance, while detection of human markers was associated with unimproved water source. This study further demonstrated that detection of viable *Campylobacter* spp. was associated with detection of avian marker *Av4143*, even after adjustment for presence of chickens and the presence of other markers. Overall, this study strongly suggests that animal sources are important to fecal contamination in households in a tropical low-resource community.

Chickens are highly ubiquitous animals in communities in the Peruvian Amazon, given that poultry is the main source of animal protein besides fish, and are commonly raised as

an alternative source of income or as pets. Chickens are seldom corralled and there is no physical barrier that prohibits their entrance to the living or cooking spaces. These same husbandry practices have been identified previously in peri-urban Lima, Peru, and associated with *Campylobacter* spp. exposure (44, 164).

The odds of detecting high quantities of *Av4143*, *CytB* and *ND5* was strongly associated with the presence of chickens in the household, corroborating the utility of these MST markers in this particular setting. Additionally, having a positive *Av4143* floor or table sample had a strong association with *Campylobacter* spp. positive surface samples. Although chickens are considered a risk factor for *Campylobacter* spp. infections, we do not advocate discouraging the practice of chicken-rearing. On the contrary, chickens and eggs are an important nutritional source and their consumption has been associated with increased statural growth in children under 2 years of age both in a randomized community trial in Ecuador and an observational study in Kenya (47, 48, 218). However, these data suggest the potential importance of mitigation strategies to reduce exposure to avian fecal material, and future studies will be needed to identify strategies that are effective but that do not have unintended consequence for economic stability of households, animal health or human health. In fact, studies in Peru and Ethiopia present evidence that suggests corralling chickens might increase the risk of *Campylobacter* spp. transmission and infection, potentially by affecting the ecology of *Campylobacter* spp. in the host, or by increasing the degree of animal crowding and therefore overall concentration of fecal burden in a single location within the household (55, 172). Results from this study indicate that a higher burden of avian exposure, as measured by *CytB* and

ND5, is associated with wood tables in comparison to non-wood tables. This is one potential risk factor amenable to intervention that could be tested directly in future trials given cooking tables made or covered of materials such as plastic or tiles are more frequently cleaned than non-wood tables (*personal observation*). Although a great percentage of houses report having bleach and using it in water, few houses actually use bleach to clean tables. Other cleaning products are seldom found within these households. Recent randomized clinical trials that aim to reduce the burden of enteric disease using water, hygiene and sanitation practices have not been unsuccessful (183-185). Although reasons for these are multifaceted, some studies have already suggested the need to incorporate household infrastructure improvements in order to reduce the burden of fecal contamination. Among many, these include building cement floors, improved water distribution systems and improved waste management systems. A recent call to for an "integrative management of animals, wash, sanitation and hygiene" highlights the need transformative WASH interventions (187). This study provides data to support the importance of addressing animal fecal waste within household environments and to introduce a One Health approach to water sanitation and hygiene research.

We found few samples positive for the human fecal markers *Bachum* and *HF183*-Taqman. However, among samples that were positive for *Bachum*, the odds of having high human fecal contamination were highly increased if the household had an unimproved water. Although human MST markers have been the most frequently used in resource poor environments, to our knowledge they have not been applied to household surface samples (69, 70, 191, 219). *Pig2Bac* and *LA35* markers were not detected in any

surface sample. The lack of positive samples to *Pig2Bac* is not surprising given that few households own and raise pigs. The absence of positive LA35 markers is attributed to the particularly low sensitivity of this marker (Chapter 4).

Limitations of these study include the fact that the performance of MST markers is setting specific and requires a previous validation step. Additionally, new human MST markers that have higher degrees of sensitivity and specificities are required. Given the emerging microbiome research, the development of markers taking into consideration age specific features of fecal microbiota, age specific Bifidobacterium and other fastidious members of the flora, could significantly enhance our understanding of the source of human fecal contamination. Finally, future studies should include water samples along with surface samples as a further method of comparison, as well as samples from toilet areas and human hands, as hand-to-mouth ingestion of avian fecal material has been shown previously (173). Comparing the burden of MST markers in these additional sampling environments will help elucidate the degree of animal and human fecal waste among potential transmission pathways that exist in these environments.

Human and avian fecal markers as well as avian exposure markers were detected among the 55 toys sampled. The high degree of positivity to avian and human markers suggest that toys serve as a relevant sentinel of animal fecal exposure, and that both avian and human feces are frequently in contact with household members. This sampling strategy was inspired by the work of Vujcic *et al*. 2014 in Bangladesh (214).

5.6 Conclusion

Avian fecal material is highly prevalent in floor and cooking spaces of households located in peri-urban, low-resource tropical communities. There is a need to include animal fecal waste in interventions that target water, hygiene and sanitation aiming to reduce the burden of enteric disease and environmental enteropathy. However, care should be taken to do this in a way that recognizes the key food security role chickens and eggs play in pediatric and adult populations living in resource poor settings and should not discourage animal husbandry practices. This study adds to mounting evidence for the need to treat the domestic environment as a single entity using a One Health strategy, and to support simple interventions to decrease exposure to avian fecal material and potentially pathogenic bacteria such as the use of easy-to-clean plastic surfaces on cooking tables.

5.7 Tables for Chapter 5

Table 10. Target Gene, Host, Primers and Probes, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Accuracy of Eight Microbial Source Tracking (MST) Markers used for Source Attribution

Marker	Target	Host	Primers and Probes		Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
LA35	*Brevibacterium avium*	Chickens	LA35F LA35R Probe	5′-ACC GGA TAC GAC CAT CTG C-3′ 5′-TCC CCA GTG TCA GTC ACA GC-3′ 5′-FAM-CAG CAG GGA AGA AGC CTT CGG GTG ACG GTA-BHQ1-3′	23.10%	100.00%	100.00%	89.40%	89.70%
ND5	Mitochondrial DNA (NADH dehydrogenase subunit 5)	Chickens and Ducks	ND5-F ND5-R ND5-P	5'-ACCTCCCCCAACTAGC-3' 5'-TTGCCAATGGTTAGGCAGGAG-3' 5'-FAM-TCAACCCATGCCTTCTT-NFQ-MGB-3'	69.60%	75.70%	47.10%	78.10%	66.00%
CytB	Mitochondrial DNA (cytochrome b)	Chickens and Ducks	cytb-F cytb-R cytb-P	5'-AAATCCCACCCCCTACTAAAAATAAT-3' 5'-CAGATGAAGAAGAATGAGGCG-3' 5'-FAM-ACAACTCCCTAATCGACCT-NFQ-MGB-3′	87.00%	82.40%	60.60%	95.30%	83.50%
Av4143	*Lactobacillus* spp.	Domestic Birds and Waterfoul	Av4143F Av4143R Av4143P	5'-TGCAAGTCGAACGAGGATTTCT-3' 5'-TCACCTTGGTAGGCCGTTACC-3' 5'-FAM-AGGTGGTTTTGCTATCGCTTT-BHQplus-3′	72.70%	87.50%	75.00%	86.20%	82.50%
BacCan	*Bacteroidales*	Dogs	BactCan545f1 Uni/Cow690r1 Uni/Cow 690r2 Uni/Cow 656p	5'-GGAGCGCAGACGGGTTTT-3' 5'-CAATCGGAGTTCTTCGTGATATCTA-3' 5'-AATCGGAGTTCCTCGTGATATCTA-3' 5'-FAM-TGGTGTAGCGGTGAAA-MGB-3'	100.00%	47.40%	19.60%	100.00%	53.40%
Pig2Bac	*Bacteroidales*	Pigs	Pig-2-Bac41F Pig-2-Bac163Rv Pig-2Bac113	5'-GCATGAATTTAGCTTGCTAAATTTGAT-3' 5'-ACCTCATACGGTATTAATCCGC-3' 5'-VIC-TCCACGGGATAGCC-NFQ-MGB-3'	100.00%	88.50%	50.00%	100.00%	89.70%
HF183-Taq	*Bacteroidales*	Humans	*HF183*f BthetR1 BthetP1	5'-ATCATGAGTTCACATGTCCG-3' 5'-CGTAGGAGTTTGGACCGTGT-3' 5'-FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA-3'	76.70%	67.60%	50.00%	87.30%	70.30%
BacHum	*Bacteroidales*	Humans	*BacHum*160Fw *BacHum*241Rv *BacHum*193Probe	5'-TGAGTTCACATGTCCGCATGA-3' 5'-CGTTACCCCGCCTACTATCTAATG-3' 5'-FAM-TCCGGTAGACGATGGGGATGCGTT-TAMRA-3'	80.00%	66.20%	50.00%	88.70%	70.30%

Table 11. Materials of Paired Floor and Table Surface Samples from Households (N=104) in in three communities of Iquitos, Loreto, Peru

Material	Surfaces (n=208)	
	Floors (n=104)	Tables (n=104)
Cement	39 (37.5%)	0 (0.0%)
Tile	4 (3.8%)	2 (1.9%)
Plastic	1 (9.6%)	22 (21.2%)
Dirt	54 (51.9%)	0 (0.0%)
Wood	6 (5.8%)	75 (72.1%)
Paper	0 (0.0%)	1 (9.6%)
Cloth	0 (0.0%)	4 (3.8%)

Table 12. (A) Percent Positive (A) Floor and Table Samples, (B) Unfinished and Finished Floors, (C) Wood and Non-Wood samples, from Households in three communities of Iquitos, Loreto, Peru, Scored with Eight Microbial Source Tracking Markers.

MST	Target Species	Floors Percent Positive (n/N)	Tables Percent Positive (n/N)	p-value*
Av4143	Domestic birds and waterfowl	**49.03 (51/104)**	**15.38 (16/104)**	**<0.001**
CytB	Chickens and ducks	59.62 (62/104)	67.31 (70/104)	0.249
ND5	Chickens and ducks	83.65 (87/104)	77.88 (81/104)	0.291
Bactcan	Dogs	**27.88 (29/104)**	**11.54 (12/104)**	**0.003**
Bachum	Humans	**17.31 (18/104)**	**3.85 (4/104)**	**0.003**
HF-183 Taqman	Humans	0.96 (1/104)	0.96 (1/104)	NA

MST Marker	Target Species	Unfinished Floors Percent Positive (n/N)	Finished Floors Percent Positive (n/N)	p-value*
Av4143	Domestic birds and waterfowl	57.4% (31/54)	40.0% (20/50)	0.082
CytB	Chickens and ducks	64.8% (35/54)	54.0% (27/50)	0.319
ND5	Chickens and ducks	88.9% (48/54)	78.0% (39/50)	0.185
Bactcan	Dogs	35.2% (19/54)	20.0% (10/50)	0.125
Bachum	Humans	15.0% (8/54)	20.0% (10/50)	0.606
HF-183 Taqman	Humans	0.00% (0/54)	2.0% (1/50)	0.481

MST Marker	Target Species	Wood Tables Percent Positive (n/N)	Non-Wood Tables Percent Positive (n/N)	p-value*
Av4143	Domestic birds and waterfowl	18.7% (14/75)	7.0% (2/29)	0.224
CytB	Chickens and ducks	72.0% (54/75)	55.2% (16/29)	0.101
ND5	Chickens and ducks	84% (63/75)	62.1% (18/29)	**0.016**
Bactcan	Dogs	12.0% (9/75)	10.3% (3/29)	1.000
Bachum	Humans	5.3% (4/75)	0.0% (0/29)	0.574
HF-183 Taqman	Humans	1.3% (1/75)	0.0% (0/29)	1.000

Table 13. Female head of household, infrastructure and hygiene characteristics by unfinished and finished floors, and wood and non-wood tables, from households located in three communities of Iquitos, Loreto, Peru

Covariates	Floors			Tables		
	Finished (n=50)	Unfinished (n=54)	p-value	Wood (n=75)	Non-Wood (n=29)	p-value
Female Head of Household Characteristics						
Maternal Age (Mean, 95% CI)	28.2 (26.1-30.4)	30.5 (27.6-33.3)	0.217	29.0 (26.9-31.1)	30.4 (26.8-34.0)	0.515
Maternal Education (Mean, 95% CI)	8.7 (7.9-9.5)	7.75 (7.04-8.46)	0.085	8.0 (7.3-8.6)	8.9 (8.0-9.8)	0.103
Age of First Pregnancy (Mean, 95% CI)	18.6 (17.2-20.1)	17.6 (16.8-18.4)	0.212	17.7 (16.8-18.7)	19.0 (17.4-20.5)	0.175
Monthly Income (Peruvian Sol) (Mean, 95% CI)	374.0 (333.5-414.5)	314.1 (275.9-352.2)	**0.033**	323.5 (296.3-350.6)	398.3 (327.8-468.8)	**0.016**
Household Infrastructure Characteristics						
Number of people sleeping in household (Mean (SD))	6.4 (5.7-7.0)	5.8 (5.1-6.5)	0.209	6.2 (5.6-6.8)	5.6 (4.8-6.3)	0.216
Length of Household Tenancy	-	-	0.228			0.739
Less than 1 year (n=12)	6.0% (3/50)	16.7% (9/54)		10.7% (8/75)	6.9% (2/29)	
Between 1 and 5 years (n=28)	32.0% (16/50)	22.2% (12/54)		29.3% (22/75)	20.7% (6/29)	
Between 5 and 10 years (n=22)	16.0% (8/50)	25.9% (14/54)		22.7% (17/75)	20.7% (6/29)	
Between 10 and 20 years (n=14)	14.0% (7/50)	13.0% (7/54)		12% (9/75)	17.2% (5/29)	
More than 20 years (n=28)	32.0% (16/50)	22.2% (12/54)		25.3% (19/75)	34.5% (10/29)	
Wall Type	-	-				
Cement	36% (18/50)	11.1% (6/54)	**0.003**	16% (12/75)	48.3% (14/29)	**<0.001**
Other	64% (32/50)	88.9% (48/54)		84% (63/75)	51.7% (15/29)	
Hygiene Characteristics						
Household treats drinking water	62.0% (31/50)	61.1% (33/54)	0.926	62.7% (47/75)	58.6% (17/29)	0.704
Water Source	-	-				
Improved	90.0% (45/50)	88.9% (48/54)	0.854	86.7% (65/75)	96.6% (28/29)	0.142
Unimproved	10.0% (5/50)	11.1% (6/54)		13.3% (10/75)	3.4% (1/29)	
Sanitation Facility	-	-				
Improved	60.0% (30/50)	38.9% (21/54)	**0.031**	42.7% (32/75)	65.5% (19/29)	**0.037**
Unimproved	40.0% (20/50)	61.1% (33/54)		57.3% (43/75)	34.5% (10/29)	
Chickens in Household (n=50)	54% (27/50)	42.6% (23/54)	0.245	49.3% (37/75)	51.7% (15/29)	0.827

Table 14. Unadjusted associations between female head of household, infrastructure and hygiene characteristics and the change in log(10) gene copy number (GCP) of each microbial source tracking marker among floor samples

	AV4143			ND5			CYTB			BactCan			BacHum			HF183-Taqman		
	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	Odds Ratio (SD)	β (SD)	95% CI	p-value
Female Head of Household Characteristics																		
Age (years)	**0.95 (0.02)**	**[0.91-0.99]**	**0.039**	0.97 (0.02)	[0.93-1.01]	0.094	0.96 (0.02)	[0.93-1.00]	0.08	1.04 (0.02)	[0.99-1.08]	0.101	1.00 (0.02)	[0.96-1.05]	0.914	0.98 (0.03)	[0.92-1.04]	0.42
Maternal Education (years)	1.02 (0.07)	[0.90-1.18]	0.681	0.98 (0.07)	[0.86-1.13]	0.802	0.95 (0.06)	[0.82-1.09]	0.427	0.99 (0.07)	[0.89-1.14]	0.848	0.99 (0.08)	[0.84-1.16]	0.866	0.96 (0.09)	[0.80-1.15]	0.656
Age of First Pregnancy (years)	1.02 (0.05)	[0.93-1.12]	0.691	1.10 (0.05)	[0.93-1.11]	0.785	0.98 (0.04)	[0.89-1.07]	0.662	1.01 (0.05)	[0.92-1.10]	0.904	0.99 (0.05)	[0.90-1.10]	0.922	1.03 (0.06)	[0.91-1.16]	0.648
Average Monthly Income (US Dollars)	0.99 (0.00)	[0.99-1.00]	0.893	0.99 (0.00)	[0.99-1.00]	0.413	0.99 (0.00)	[0.99-1.00]	0.789	0.99 (0.00)	[0.99-1.00]	0.068	0.99 (0.00)	[0.99-1.00]	0.718	0.99 (0.00)	[0.99-1.00]	0.444
Household Infraestructure Characteristics																		
Number of people sleeping in household																		
≤ 5 (n=44)	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
> 5 (n=59)	1.68 (0.64)	[0.80-3.53]	0.172	1.60 (0.58)	[0.77-3.25]	0.214	1.24 (0.45)	[0.61-2.54]	0.556	0.90 (0.34)	[0.42-1.90]	0.774	0.78 (0.34)	[0.34-1.81]	0.567	1.03 (0.53)	[0.38-2.83]	0.952
Length of Household Tenancy																		
Less than 1 year	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Between 1 and 5 years	0.43 (0.30)	[0.11-1.66]	0.222	1.02 (0.64)	[0.30-3.51]	0.975	1.28 (0.79)	[0.39-4.28]	0.684	1.10 (0.80)	[0.27-4.55]	0.886	3.75 (3.24)	[0.69-20.38]	0.126	1.67 (1.48)	[0.29-9.52]	0.566
Between 5 and 10 years	1.62 (0.30)	[0.35-7.53]	0.538	1.62 (1.05)	[0.46-5.75]	0.456	2.82 (1.81)	[0.81-9.90]	0.104	1.60 (1.19)	[0.37-6.91]	0.527	1.88 (1.71)	[0.31-11.17]	0.490	1.88 (1.71)	[0.31-11.17]	0.490
Between 10 and 20 years	0.38 (0.29)	[0.08-1.73]	0.210	0.73 (0.53)	[0.18-3.00]	0.661	1.21 (0.84)	[0.31-4.73]	0.787	3.74 (0.3.02)	[0.77-18.14]	0.101	3.75 (3.54)	[0.59-23.87]	0.162	1.36 (1.38)	[0.19-9.91]	0.759
More than 20 years	**0.18 (0.13)**	**[0.05-0.74]**	**0.017**	0.64 (0.41)	[0.18-2.23]	0.481	1.16 (0.72)	[0.35-3.90]	0.806	1.73 (1.23)	[0.42-6.99]	0.444	1.67 (1.48)	[0.29-9.52]	0.566	0.38 (0.41)	[0.05-3.11]	0.371
Floor Material																		
Unfinished	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Finished	0.63 (0.24)	[0.30-1.31]	0.214	0.73 (0.27)	[0.36-1.49]	0.387	0.77 (0.28)	[0.37-1.57]	0.474	0.54 (0.21)	[0.25-1.15]	0.112	0.72 (0.30)	[0.31-1.65]	0.432	3.11 (1.67)	[1.09-8.89]	0.034
Wall Type																		

Cement	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Other	0.89 (0.40)	[0.37-2.13]	0.796	0.99 (0.45)	[0.41-2.43]	0.993	1.60 (0.71)	[0.67-3.84]	0.29	**0.30**	**[0.11-0.82]**	**0.020**	0.65 (0.35)	[0.23-1.84]	0.421	0.53 (0.36)	[0.14-1.99]	0.346
Hygiene Characteristics																		
Household treating drinking water																		
No	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Yes	1.27 (0.48)	[0.60-2.69]	0.531	2.07 (0.79)	[0.98-4.39]	0.058	1.36 (0.51)	[0.66-2.82]	0.408	0.67 (0.26)	[0.31-1.46]	0.316	1.68 (0.75)	[0.70-4.05]	0.246	1.20 (0.62)	[0.43-3.33]	0.724
Water Source																		
Improved	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Unimproved	4.59 (3.72)	[0.94-22.44]	0.06	**3.56 (2.23)**	**[1.04-12.13]**	**0.042**	1.98 (1.21)	[0.60-6.59]	0.263	1.11 (0.71)	[0.32-3.89]	0.872	**7.25 (5.20)**	**[1.78-29.53]**	**0.006**	**6.77 (4.55)**	**[1.82-25.24]**	**0.004**
Sanitation Facility																		
Unimproved	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Improved	0.56 (0.21)	[0.27-1.16]	0.120	1.40 (0.51)	[0.68-2.84]	0.358	1.02 (0.37)	[0.50-2.07]	0.964	0.87 (0.33)	[0.41-1.84]	0.725	0.68 (0.29)	[0.29-1.56]	0.359	0.64 (0.32)	[0.24-1.71]	0.37
Chickens in Household																		
No	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Yes	**3.70 (1.45)**	**[1.71-7.99]**	**0.001**	**3.29 (1.25)**	**[1.56-6.92]**	**0.002**	1.94 (0.71)	[0.94-3.98]	0.072	1.62 (0.62)	[0.76-3.43]	0.211	2.10 (0.90)	[0.91-4.88]	0.084	2.36 (1.22)	[0.85-6.51]	0.097

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Table 15. Unadjusted Associations between female head of household, infrastructure and hygiene characteristics and the change in log(10) gene copy number (GCP) of each microbial source tracking marker among table samples

	AV4143			ND5			CYTB			BactCan			BacHum			HF183-Taqman		
	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value	Odds Ratio (SD)	95% CI	p-value
Female Head of Household Characteristics																		
Age (years)	0.98 (0.02)	[0.93-1.03]	0.334	0.98 (0.02)	[0.94-1.01]	0.196	0.97 (0.02)	[0.93-1.00]	0.075	1.03 (0.03)	[0.98-1.08]	0.287	0.92 (0.05)	[0.83-1.02]	0.105	1.03 (0.03)	[0.97-1.10]	0.319
Maternal Education (years)	1.00 (0.08)	[0.85-1.18]	0.967	0.99 (0.07)	[0.86-1.13]	0.832	0.99 (0.07)	[0.86-1.13]	0.830	1.06 (0.11)	[0.86-1.30]	0.607	1.04 (0.14)	[0.79-1.36]	0.788	0.87 (0.10)	[0.69-1.10]	0.248
Age of First Pregnancy (years)	0.97 (0.05)	[0.88-1.08]	0.572	0.97 (0.04)	[0.89-1.05]	0.414	0.94 (0.04)	[0.87-1.03]	0.178	0.99 (0.06)	[0.88-1.13]	0.910	1.00 (0.09)	[0.95-1.19]	0.974	0.95 (0.08)	[0.80-1.12]	0.527
Average Monthly Income (US Dollars)	1.00 (0.00)	[0.99-1.00]	0.202	0.99 (0.00)	[0.99-1.00]	0.878	0.99 (0.00)	[0.99-1.00]	0.771	0.99 (0.00)	[0.99-1.00]	0.127	0.99 (0.00)	[0.99-1.00]	0.142	0.99 (0.00)	[0.99-1.00]	0.571
Household Infraestructure Characteristics																		
Number of people sleeping in household																		
<=5 (n=44)	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
>5 (n=59)	**3.16 (1.49)**	**[1.26-7.96]**	**0.014**	1.45 (0.54)	[0.70-2.99]	0.317	0.91 (0.34)	[0.44-1.88]	0.803	0.55 (0.29)	[0.20-1.54]	0.774	0.93 (0.65)	[0.23-3.67]	0.913	0.46 (0.31)	[0.121-1.74]	0.254
Length of Household Tenancy																		
Less than 1 year	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Between 1 and 5 years	0.64 (0.48)	[0.14-2.78]	0.551	1.33 (0.68)	[0.35-5.13]	0.676	1.00 (0.07)	[0.25-3.98]	1.000	1.53 (1.81)	[0.15-15.61]	0.720	0.87 (0.81)	[0.14-5.40]	0.881	1.47E+07 (4.38E+10)	NA	0.996
Between 5 and 10 years	0.74 (0.57)	[0.16-3.34]	0.698	0.96 (0.66)	[0.25-3.72]	0.952	0.62 (0.44)	[0.15-2.52]	0.507	0.81 (2.05)	[0.07-10.14]	0.872	4.14E-08	NA	0.993	1.00 (3561.53)	NA	1.000
Between 10 and 20 years	0.25 (0.25)	[0.03-1.73]	0.16	0.68 (0.52)	[0.15-3.09]	0.615	0.44 (0.35)	[0.90-2.12]	0.305	1.53 (1.99)	[0.12-19.65]	0.745	4.14E-08	NA	0.995	6.80E+06 (2.02E+10)	NA	0.996
More than 20 years	0.40 (0.31)	[0.09-1.80)	0.232	0.86 (0.58)	[0.23-3.23]	0.827	0.62 (0.44)	[0.16-2.44]	0.498	3.5 (3.94)	[0.39-31.77]	0.265	0.29 (0.32)	[0.04-2.45]	0.259	1.84E+07 (5.48E+10)	NA	0.996
Table Material																		
Wood	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Non-Wood	**3.32 (1.95)**	**[1.05-10.52]**	**0.041**	**3.11 (1.29)**	**[1.38-7.02]**	**0.006**	**2.53 (1.05)**	**[1.12-5.68]**	**0.025**	2.16 (1.45)	[0.58-8.08]	0.253	3.34 (3.63)	[0.40-27.99]	0.266	1.61 (1.33)	[0.32-8.08]	0.562
Wall Material																		

Cement	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Other	1.41 (0.67)	[0.56-3.58]	0.471	1.38 (0.58)	[0.60-3.16]	0.446	1.34 (0.57)	[0.58-3.10]	0.484	0.55 (0.37)	[0.15-2.07]	0.376	1.55 (1.16)	[0.36-6.76]	0.548	0.73 (0.60)	[0.14-3.67]	0.702
Hygiene Characteristics																		
Household treating drinking water																		
No	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Yes	1.50 (0.69)	[0.61-3.68]	0.379	1.97 (0.74)	[0.95-4.10]	0.069	**2.32 (0.88)**	**[1.10-4.88]**	**0.027**	1.31 (0.71)	[0.45-3.79]	0.625	0.47 (0.33)	[0.12-1.85]	0.279	0.59 (0.40)	[0.16-2.19]	0.434
Water Source																		
Improved	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Unimproved	**3.48 (2.14)**	**[1.04-11.61]**	**0.043**	1.00 (0.58)	[0.33-3.09]	1.000	0.70 (0.40)	[0.23-2.16]	0.538	1.16 (0.97)	[0.23-5.91]	0.855	1.06 (1.18)	[0.12-9.39]	0.957	1.68E-07 (0.00)	NA	0.994
Sanitation Facility																		
Unimproved	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Improved	1.55 (0.67)	[0.67-3.60]	0.308	0.75 (0.27)	[0.37-1.53]	0.434	0.61 (0.22)	[0.30-1.23]	0.169	0.83 (0.43)	[0.30-2.29]	0.713	1.33 (0.93)	[0.34-5.27]	0.683	1.04 (0.69)	[0.28-3.84]	0.949
Chickens in Household																		
No	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Yes	**4.42 (2.15)**	**[1.70-11.49]**	**0.002**	1.49 (0.54)	[0.73-3.04]	0.274	1.00 (0.36)	[0.50-2.05]	0.983	1.46 (0.76)	[0.53-4.03]	0.470	1.28 (0.90)	[0.32-5.05]	0.728	1.00 (0.67)	[0.27-3.68]	1.000

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Table 16. Adjusted associations between female head of household, infrastructure and hygiene characteristics and the change in log(10) gene copy number (GCP) of each microbial source tracking marker among floor and table samples

	AV4143				ND5				CYTB				BactCan				BacHum				HF183-Taqman			
	Floors		Tables		Floors		Tables		Floors		Tables		Floors		Tables		Floors		Tables		Floors		Tables	
	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95% CI	Odds Ratio (SD)	95% CI	Odds Ratio (SD)	95 % CI	Odds Ratio (SD)	95 % CI
Female Head of Household Characteristics																								
Age (years)	**0.93 (0.03)**	**[0.87-0.99]**	1.02 (0.04)	[0.95-1.10]	0.95 (0.03)	[0.90-1.00]	0.97 (0.03)	[0.92-1.02]	**0.94 (0.03)**	**[0.89-0.99]**	0.99 (0.03)	[0.94-1.04]	1.04 (0.03)	[0.98-1.10]	1.07 (0.04)	[0.99-1.16]	1.02 (0.03)	[0.96-1.09]	**0.81 (0.07)**	**[0.68-0.97]**	1.02 (0.05)	[0.93-1.12]	1.03 (0.04)	[0.96-1.12]
Maternal Education (years)	0.98 (0.11)	[0.79-1.22]	1.06 (0.13)	[0.84-1.35]	0.86 (0.08)	[0.72-1.04]	0.98 (0,09)	[0.82-1.19]	0.85 (0.08)	[0.71-1.02]	1.02 (0.09)	[0.85-1.22]	1.06 (0.11)	[0.86-1.30]	1.19 (0.20)	[0.86-1.65]	1.06 (0.12)	[0.85-1.31]	0.76 (0.15)	[0.52-1.12]	0.96 (0.14)	[0.72-1.28]	0.79 (0.14)	[0.56-1.10]
Age of First Pregnancy (years)	1.11 (0.07)	[0.97-1.26]	0.94 (0.07)	[0.82-1.07]	1.07 (0.06)	[0.96-1.20]	0.99 (0.05)	[0.90-1.11]	1.06 (0.06)	[0.95-1.19]	0.94 (0.05)	[0.84-1.05]	0.96 (0.06)	[0.84-1.08]	0.88 (0.09)	[0.72-1.06]	0.99 (0.07)	[0.86-1.13]	1.36 (0.21)	[0.99-1.84]	0.99 (0.09)	[0.83-1.18]	0.93 (0.11)	[0.56-1.10]
Average Monthly Income (Peruvian Sol)	0.99 (0.00)	[0.99-1.00]	1.00 (0.00)	[0.99-1.01]	0.99 (0.00)	[0.99-1.00]	1.00 (0.00)	[0.99-1.00]	0.99 (0.00)	[0.99-1.00]	0.99 (0.00)	[0.99-1.00]	0.99 (0.00)	[0.99-1.00]	0.99 (0.00)	[0.99-1.00]	0.99 (0.00)	[0.99-1.00]	**0.99 (0.01)**	**[0.98-0.99]**	1.00 (0.00)	[0.99-1.01]	0.99 (0.00)	[0.99-1.00]
Household Infraestructure Characteristics																								
Number of people sleeping in household																								
<=5 (n=44)	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
>5 (n=59)	1.60 (0.78)	[0.63-	**3.93 (2.55)**	**[1.10-**	1.20 (0.55)	[0.49-	1.54 (0.68)	[0.65-			0.91 (0.40)	[0.39-	0.85 (0.41)	[0.33-	0.72 (0.48)	[0.19-	0.94 (0.52)	[0.32-	0.44 (0.42)	[0.07-	0.77 (0.56)	[0.18-	0.31 (0.24)	[[0.07-

		4.04]		**14.02]**		2.94]		3.66]				2.16]		2.16]		2.67]		2.76]		2.89]		3.24]		1.39]
Length of Household Tenancy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Less than 1 year	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Between 1 and 5 years	0.40 (0.33)	[0.08-2.04]	0.27 (0.26)	[0.04-1.76]	0.72 (0.54)	[0.17-3.13]	1.10 (0.85)	[0.23-5.03]	1.04 (0.74)	[0.26-4.18]	0.80 (0.64)	[0.17-3.80]	0.93 (0.77)	[0.18-4.71]	1.14 (1.49)	[0.09-14.8]	2.86 (2.73)	[0.22-18.59]	NA	NA	NA	NA	NA	NA	NA	NA
Between 5 and 10 years	1.38 (1.22)	[0.24-7.82]	0.16 (0.17)	[0.02-1.20]	1.00 (0.77)	[0.22-4.48]	0.89 (0.73)	[0.18-4.42]	2.54 (1.90)	[0.59-10.97]	0.50 (0.41)	[0.10-2.51]	1.02 (0.87)	[0.19-5.47]	0.30 (0.45)	[0.02-5.71]	0.57 (0.61)	[0.06-4.69]	NA	NA	NA	NA	NA	NA	NA	NA
Between 10 and 20 years	0.33 (0.29)	[0.57-1.89]	0.14 (0.16)	[0.01-1.38]	0.47 (0.39)	[0.09-2.42]	0.76 (0.66)	[1.14-4.15]	1.22 (0.96)	[0.26-5.66]	0.41 (0.36)	[0.07-2.31]	2.93 (2.58)	[0.52-16.43]	1.00 (1.45)	[0.06-17.07]	2.74 (2.81)	[0.37-20.43]	NA	NA	NA	NA	NA	NA	NA	NA
More than 20 years	**0.14 (0.12)**	**[0.03-0.75)**	0.12 (0.14)	[0.01-1.14]	0.52 (0.42)	[0.11-2.51]	0.78 (0.65)	[0.56-3.14]	0.98 (0.75)	[0.22-4.41]	0.47 (0.39)	[0.09-2.44]	2.11 (1.86)	[0.37-11.88]	3.14 (4.08)	[0.25-40.00]	1.14 (1.19)	[0.15-8.88]	NA	NA	NA	NA	NA	NA	NA	NA
Table Material	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wood	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	[REF]
Non-Wood	-	-	**6.48 (5.51)**	**[1.22-34.37]**	-	-	**3.73 (1.97)**	**[1.32-10.52]**	-	-	2.55 (1.31)	[0.93-6.99]	-	-	4.10 (4.22)	[0.55-30.83]	-	-	6.30 (9.16)	[0.37-108.75]	-	-	1.61 (1.59)	[0.23-11.14]	-	[0.23-11.14]
Floor Material	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unimproved	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	[REF]	[REF]	-	-	-	-
Improved	0.45 (0.23)	[0.16-1.25]	-	-	0.60 (0.27)	[0.25-1.46]	-	-	0.57 (0.25)	[0.24-1.34]	-	-	0.71 (0.33)	[0.28-1.78]	-	-	0.53 (0.30)	[0.18-1.59]	-	-	5.62 (4.21)	[0.05-3.53]	-	-	-	-
Wall Material	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cement	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Other	2.67 (1.84)	[0.69-10.34]	3.18 (2.56)	[0.66-15.40]	2.05 (1.27)	[0.61-6.90]	2.65 (1.61)	[0.81-8.74]	3.41 (2.12)	[1.01-11.60]	2.51 (1.51)	[0.78-8.12]	0.22 (0.16)	[0.05-0.94]	0.39 (0.41)	[0.05-3.12]	0.53 (0.30)	[0.18-1.59]	7.18 (9.44)	[0.54-94.54]	0.88 (0.87)	[0.13-6.11]	0.52 (0.56)	[0.06-4.24]		
Hygiene Characteristics																										
Household treating drinking water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]

Yes	1.39 (0.65)	[0.55-3.50]	2.39 (1.49)	[0.71-8.09]	**3.62 (1.71)**	**[1.44-9.12]**	**2.46 (1.08)**	**[1.04-5.79]**	1.56 (0.68)	[0.66-3.68]	**2.67 (1.19)**	**[1.12-6.39]**	0.65 (3.02)	[1.82-4.72]	2.21 (1.52)	[0.57-8.55]	1.95 (1.11)	[0.64-5.95]	0.31 (0.28)	[0.05-1.78]	1.34 (0.94)	[0.34-5.29]	0.34 (0.26)	[0.08-1.51]
Water Source	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Improved	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Unimproved	5.90 (80.03)	[0.41-84.88]	5.73)5.15)	[0.98-33.41]	3.30 (2.79)	[0.63-17.28]	1.36 (1.12)	[0.27-6.84]	1.29 (1.07)	[0.25-6.61]	1.48 (1.21)	[0.30-7.31]	0.86 (0.75)	[0.16-4.75]	3.57 (4.18)	[0.36-35.54]	**13.81 (14.68)**	**[1.72-110.92]**	0.33 (0.56)	[0.01-8.94]	6.44 (6.66)	[0.85-48.95]	NA	NA
Sanitation Facility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unimproved	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Improved	0.43 (0.20)	[0.17-1.08]	1.31 (0.74)	[0.44-3.94]	1.98 (0.85)	[0.85-4.60]	0.81 (0.35)	[0.35-1.87]	1.20 (0.51)	[0.52-2.74]	0.64 (0.27)	[0.28-1.46]	1.11 (0.51)	[0.45-2.73]	0.84 (0.56)	[0.22-3.13]	0.49 (0.25)	[0.17-1.35]	1.31 (1.25)	[0.20-8.47]	0.31 (0.22)	[0.07-1.25]	2.53 (2.08)	[0.50-12.68)
Chickens in Household	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]	[REF]
Yes	**4.03 (2.02)**	**[1.51-10.79]**	**4.73 (2.93)**	**[1.40-15.91]**	**3.33 (1.55)**	**[1.33-8.30]**	1.32 (0.58)	[0.56-3.13]	1.53 (0.69)	[0.64-3.68]	0.89 (0.38)	[0.38-2.07]	2.42 (1.21)	[0.91-6.45)	2.35 (1.64)	[0.59-9.26)	1.63 (0.87)	[0.58-4.63]	1.31 (1.23)	[0.21-8.29]	2.68 (1.89)	[0.67-10.71]	3.09 (2.65)	[0.57-16.60]

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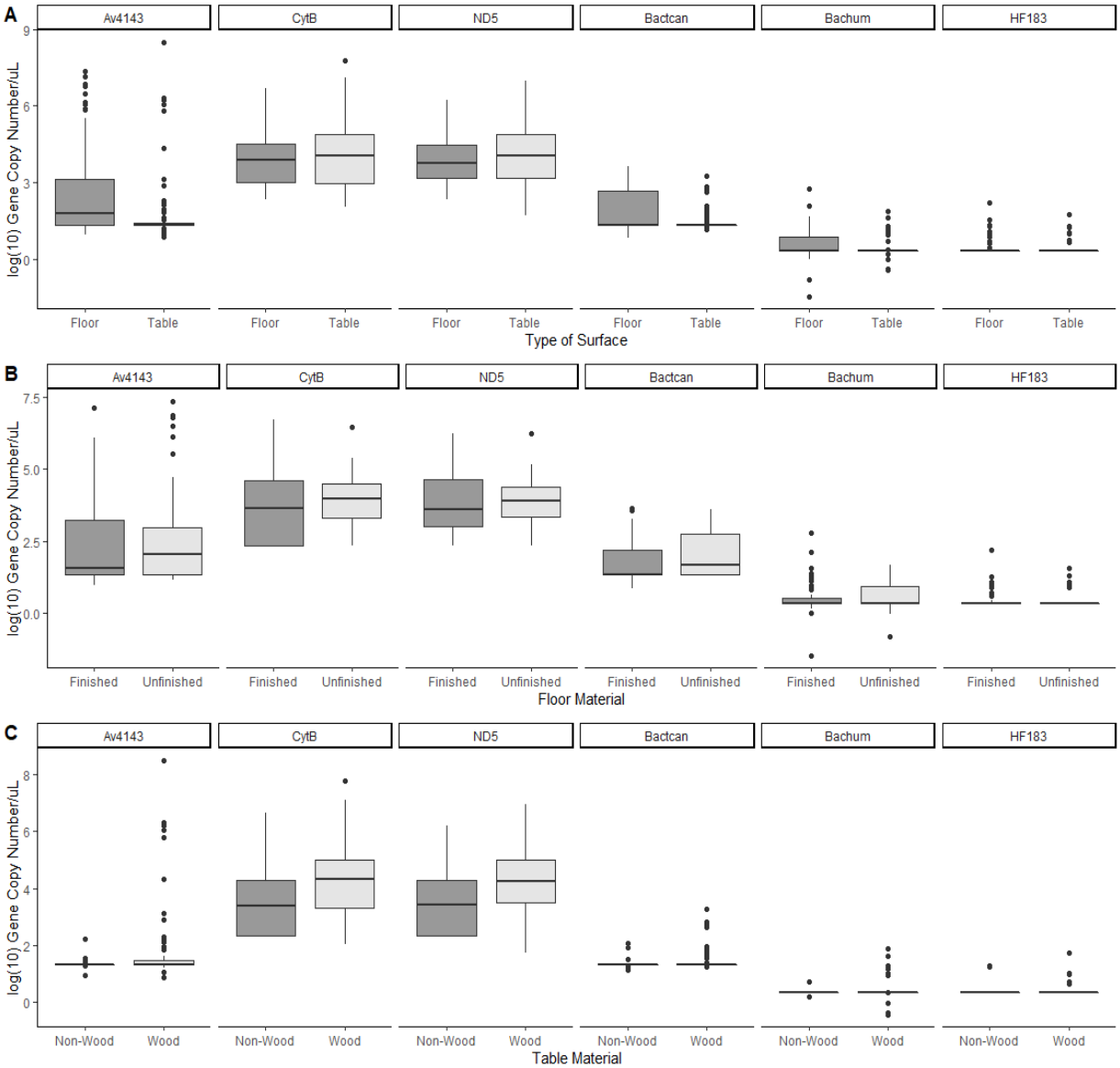
5.8 Figures for Chapter 5

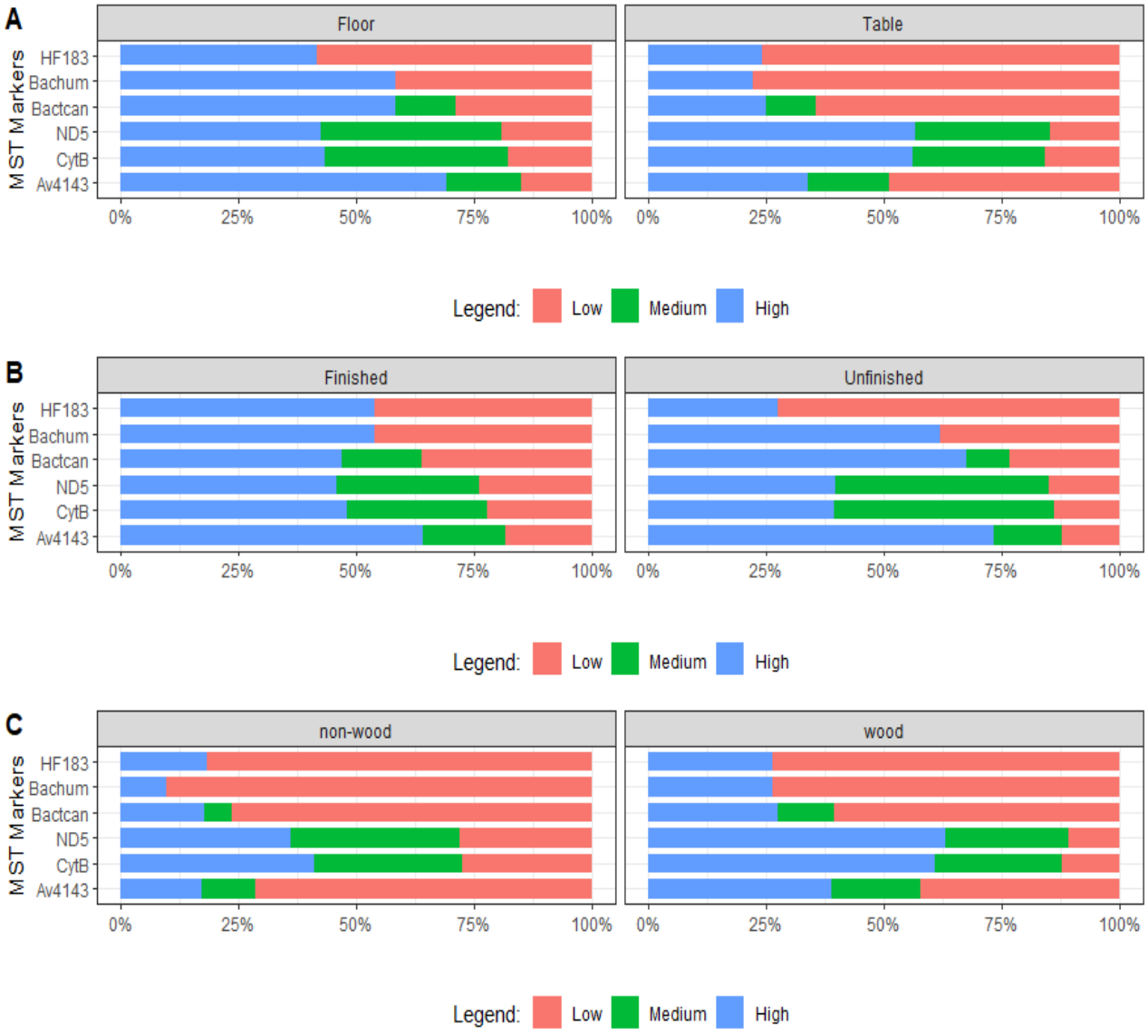
Figure 4. Photograph showing sampling of a two table surface in two households located in Santa Clara de Nanay, Iquitos, Peru

Figure 5. Quantitative Burden of Eight Microbial Source Tracking Markers in (A) Floor and Table Surfaces, (B) Unfinished and Finished Floors, and (C) Wood and Non-Wood Tables from Households Three Communities off Iquitos, Loreto, Peru



*Unfinished= dirt floor samples; Finished = cement, tile, wood or plastic floor surface samples;
*Non-Wood= tile, plastic, paper or clot table surface samples

Figure 6. Categorized (Low, Medium, High) Burden of Eight Microbial Source Tracking Markers in (A) Floor and Table Surfaces (B) Unfinished and Finished Floor Surfaces, and (C) Wood and Non-Wood Table Surfaces from Households of Three Communities off Iquitos, Loreto, Peru.



*Unfinished= dirt floor samples; Finished = cement, tile, wood or plastic floor surface samples;
*Non-Wood= tile, plastic, paper or clot table surface samples

Chapter 6. Genomic Epidemiology and Antimicrobial Resistance of *Campylobacter* spp. isolated from household and industrially raised poultry from Iquitos, Peru

6.1 Abstract

Campylobacter spp. was cultured from industrially-raised and household-raised poultry fecal samples from Iquitos, Peru. Phenotypic patterns of antimicrobial resistance (AMR) were obtained. Whole genome sequencing was completed for 63 isolates, identifying AMR genes and *C. jejuni* (n=43) and *C. coli* (n=20) species. A sequence type (ST) and clonal complex (CC) was assigned to isolates and genetic relatedness of isolated from different sources estimated. Results indicate that *Campylobacter* spp. from household-raised and industrially raised poultry have distinct population structures. *C. jejuni* isolates belonged to widely disseminated poultry specialist lineages, while *C. coli* isolates belonged to generalist ST-828. Thirteen *C. coli* and 13 *C. jejuni* isolates did not have a ST assigned. *C. coli* with undetermined ST of poultry clustered with previously isolated highly multi-drug-resistant *C. coli* (also of undetermined ST) from children. Siaylated lipooligosacharides (LOS) associated with post-infection neuropathies were more commonly found among *C. jejuni* from household-raised poultry, in comparison to non-siaylated LOS in *C. jejuni* from industrially-raised chicken. A greater number of antimicrobial resistance genes were identified in both *C. jejuni* and *C. coli* genomes from industrially-raised poultry. Of importance, a 23S ribosomal subunit conferring macrolide resistance was only found in all *C. coli* isolates from industrially-raised poultry and a statistically significant proportion of *C. jejuni* isolates from this same production ecosystem. These results add to the mounting evidence that non-therapeutic use of antibiotics within the poultry production chain could be promoting the emergence of

antimicrobial resistance that is, potentially, translatable to both the phenotype and genotype of *Campylobacter* spp. We propose and highlight the need to control *Campylobacter* spp. within the home environment, including intra-domiciliary food hygiene practices, given the risk of infection with *C. jejuni* associated with the development of Guillan-Barre syndrome. Finally, higher level regulation of antimicrobial use among poultry producers is needed to curve to spread of multi-drug resistant Campylobacteriosis from poultry to humans.

6.2 Introduction

Campylobacter spp. is as an important cause of diarrhea, environmental enteropathy and stunting among children living in poverty (5, 7, 12). This Gram-negative, microaerophilic bacterium has been described as being highly host specific. However, studies suggest that ecological factors such as intensive agriculture can promote bacterial recombination and evolution of generalist lineages of *Campylobacter jejuni*, such as sequence type (ST), ST-45 and ST-828 clonal complexes, hindering source attribution efforts (220-223). *Campylobacter jejuni* and less frequently *Campylobacter coli* are commensal flora of avian species. Consequently, undercooked poultry meat and poultry by-products derived from industrial poultry production are most frequently identified as sources of human infection in high-income, industrialized settings (158, 224, 225).

In low-resource settings, where the incidence of pediatric *Campylobacter* spp. infections is highest, the genomic epidemiology and source attribution of pediatric Campylobacteriosis remains poorly characterized. Genomic data of human *Campylobacter* spp. isolates from low-resource settings that are based on population based studies is limited to a few number of geographical settings (*Hannah et al; Arora-Williams et al; manuscript in preparation*) (226). Most importantly, genomic data from poultry within these highly characterized human cohorts are lacking. Genomic characterization of poultry associated *Campylobacter* spp. isolates from tropical environments is fundamental to distinguish between the multiple poultry exposures and risk of human infection. For instance, household poultry production is a common cultural practice, and it is highly frequent as an alternate source of poultry meat and income

diversification strategy. It is unclear if household chicken exposure or industrially produced chicken purchased from market pose a greater risk of Campylobacter associated diarrhea in children.

Rates of antimicrobial resistance from clinically derived human *Campylobacter* spp. isolates are high. In Iquitos, Peru, results from The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) cohort show that 77.4% and 79.8% of 917 *C. jejuni* and *C. coli* isolates, respectively were resistant to ciprofloxacin, and 24.8% of *C. coli* isolates were resistant azithromycin(29). Given the lack of new drugs to treat clinically-relevant *Campylobacter* spp. infections, these rates of resistance are concerning.

The role of atypical *Campylobacter* species of unknown animal and environmental sources has been suggested to play an important role in the epidemiology of pediatric *Campylobacter* infections in Iquitos, Peru (25). However, we hypothesize that poultry remains the main source of infection in this setting. As a result, this study aimed to compare the phylogenetic relatedness of *Campylobacter* spp. isolates from household-raised poultry and industrially raised broilers (herein denominated as farm-raised) in a low-resource tropical community of the Peruvian Amazon in order to associate the origins of the highly resistant *Campylobacter* infections within the local pediatric population.

6.1 Methods

Data and Sample Collection

Households that had chickens in their backyards ("*chacras*") or inside the living domain of the household were identified by local field workers and randomly included in the study. Between 3 to 5 fecal samples from backyard poultry (crossbreed, *Gallus gallus*) were collected from households located in Santa Clara de Nanay, Santo Tomas and La Union, three peri-urban communities located in Iquitos, Loreto, Peru. Characteristics poultry breeding and management within the household collected by the field worker included number of chickens, chicken breed, location of the birds within the household, as well as use of pharmaceuticals (by visual inspection and question to the owner of the household). Fecal samples from industrially raised broilers (White Leghorn or Cornish; *Gallus gallus*) were collected from two live poultry market located at Iquitos city center. Specifically, 10 poultry vendors were selected for sampling, and within each vendor, 10 fecal samples were collected using a sterile cotton swab as soon as the bird voided, and placed directly in Cary Blair transport medium. All samples where processed within 12h (29, 215).

Additionally, 25 *Campylobacter coli* isolates resistant to three key clinically-important antibiotics—ciprofloxacin, azithromycin and gentamycin—obtained from a pediatric cohort of children in Iquitos between 2009 and 2012 were selected to explore whether populations of *Campylobacter spp.* among children and chickens in Iquitos were similar. Phenotypic and genetic antimicrobial resistance information from these isolates has been published previously (Gray *et al*. *In Preparation*)(29).

Laboratory Procedures

Stools were inoculated on *Campylobacter* Blood Free Selective agar base (Oxoid, Lenexa, KS, USA) without any supplementation. Plates were incubated for 48 to 72 hours at 42ºC at 5% O_2, 10% CO_2, 85% N_2. Colonies demonstrating typical *Campylobacter* morphology were assessed using oxidase and catalase tests, as well as Gram staining. Using the hippurate hydrolysis tests, colonies were initially distinguished as *Campylobacter jejuni* (*C. jejuni*) (hippurate positive) and *C. coli* (hippurate negative) (5). Colonies were cryopreserved in Tripticase Soy Broth (Oxoid, Lenexa, KS, USA) supplemented with 20% glycerol (Oxoid, Lenexa, KS, USA) at -70ºC.

Phenotypic antimicrobial susceptibility patters were assessed using standard disc-diffusion (Kirby-Bauer) methods, as described previously(29). Resistance to the following antibiotics was tested: ciprofloxacin (CIP), nalidixic acid (NAL), erythromycin (ERY), azithromycin (AZM), tetracycline (TE), gentamicin (GM), amoxicillin and clavulanic acid (AMC), cefotaxime (CTX), chloramphenicol (C) and trimethoprim/sulfamethoxazole (TMS), colistin (CL) and Imipenem (IMP). Zone diameter breakpoints (mm) for *Campylobacter* spp. validated the Clinical & Laboratory Standards Institute (CLSI M45) were applied to assess ciprofloxacin, erythromycin, azithromycin and tetracycline resistance. CLSI zone diameter breakpoints (mm) for *Enterobacteriaceae* were used for the remaining antibiotics for which there are no established breakpoints for *Campylobacter* spp. Colistin zone diameter breakpoints (mm)

for were adapted from Galani *et al*. 2008(227). Zone diameter breakpoints are displayed in Table 20.

DNA was extracted from all bacterial cultures was done using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) as specified by manufacturer's instructions. As described previously, a duplex qPCR targeting a 16S rRNA and the *Campylobacter* adhesion to fibronectin (*cadF)* genes was performed to confirm all bacterial cultures as *Campylobacter* spp. or *C. jejuni*/*C. coli* (25).

Sequencing and Bioinformatic Analysis

Libraries were prepared with the Nextera XT kit, and batches of 24 isolate gDNA were barcoded and sequenced in multiplex to achieve 80-120x coverage. Genomes were sequenced using an Illumina MiSeq generating 250nt paired-end reads. Nullarbor v2.0 pipeline was used for reads quality, read-processing, de novo assembly, reference-alignment, SNP calling and phylogenetic analyses (Seemann T, Goncalves da Silva A, Bulach DM, Schultz MB, Kwong JC, Howden
BP. *Nullarbor* **Github** https://github.com/tseemann/nullarbor). Raw reads were quality-filtered and trimmed prior to de novo assembly and mapping to *C. jejuni* NTCC 11168 reference genome. Core genome Multi Locus Sequence Types and associated Clonal Complexes were determined through the PubMLST allelic database (228). Conserved sequences shared by all *Campylobacter* spp. genomes were concatenated to construct a SNP based phylogenetic tree using CSI Phylogeny v1.4 (229). Tree visualization and aesthetic edition was done in Microreact (230). *C. jejuni* lipooligosacharide biosynthesis

loci (LOS class), a highly variable region, was identified from raw sequence reads as described previously (231, 232).

Genomes were mined for antimicrobial resistance chromosomal gene mutations and antibiotic resistance genes using Comprehensive Antibiotic Resistance Database (CARD) and ResFinder(233, 234). Matches were determined when genes had >90% nucleotide identity and >60% coverage. Frequency of isolation of each mutation or gene were tabulated for *Campylobacter jejuni* and *Campylobacter coli* isolates separately.

6.2 Results

Between August and October 2019, 202 poultry fecal samples were collected. Specifically, 102 fecal samples from household-raised poultry were collected from 32 distinct households (average of 3.2 fecal samples per household) and 100 fecal samples from industrially-raised broilers were collected from 10 distinct market vendors.

Campylobacter spp. culture results are shown in **Table 17**. *Campylobacter* was isolated in 91 (45.0%) of the samples by culture. Of the 32 households from which poultry fecal samples were obtained, 65.5% (21/32) housed at least one *Campylobacter* culture-positive chicken, while from the 10 distinct market vendors, 9 vendors had at least one sample culture positive for *Campylobacter* spp. From the 10 samples collected per chicken vendor, on average 50.3% were culture positive for *Campylobacter* spp. Seventy-five (82.4%) isolates underwent whole genome sequencing. From the 31 sequenced *Campylobacter* spp isolates obtained from household-raised chickens, 16.1%

(5/31) were identified as *Campylobacter coli*, and 61.3% (19/31) as *Campylobacter jejuni*. From the 44 sequenced *Campylobacter* spp isolates obtained from industrially-raised chickens, 34.1% (15/44) were identified as *Campylobacter coli*, and 54.4% (24/44) as *Campylobacter jejuni*. The seven remaining isolates sequenced were excluded from the analysis for either contamination with *Pseudomonas* spp. or mixed isolation of *Campylobacter jejuni* and *Campylobacter coli* within the same sample.

(i) *Isolated Campylobacter spp. between industrially raised and household raised chickens*

As shown in **Table 18**, core genome MLST assignment was achieved for 30 out of the 43 *C. jejuni isolates* sequenced. *C. jejunis* from household-raised poultry were identified as belonging to ST-607 (n=7), ST-1036 (n=2), ST-1212 (n=2), ST-1365 (n=2), ST-4722 (n=1) and ST-535 (n=2). Twelve *C. jejuni* isolates from farm-raised poultry were also found to be ST-607, and closely related to those isolated from household-raised chickens (**Figure 8**). The two other *C. jejuni* from farm-derived poultry belonged to ST-6177 and one was classified as both ST-1232 and ST-7865. Thirteen (10 farm-raised and 3 household-raised) *C. jejuni* genomes did not have a sequence type assigned. Of these, a cluster of 6 farm derived isolates, and a cluster of two household derived isolates were most closely related to ST-535.

Core genome MLST assignment was achieved for only 7 out of the 20 *C. coli* sequenced. Those with an identifiable sequence type from household-raised poultry were allocated to ST-1055 (n=1) and ST-1173 (n=3), while those from industrial production settings were

allocated to ST-825 (n=1) and ST-8408 (n=2). Of the 7 genomes assigned to a clonal complex, all were identified as CC-828. The 13 genomes that did not have an MLST assigned to were submitted to PubMLST for further curation, and are awaiting assignment. As shown in the **Figure 9** and **Figure 10**, there is a group of 8 isolates from industrially-raised chickens that is closely related to ST-8408, while a cluster of 2 isolates from household-raised chickens are most closely related to ST-1173. Additionally, as shown in Figure 10, highly resistant isolates obtained between 2009-2015 derived from a pediatric population belonged mainly to ST-1497, ST-8738 and ST-825, while a single ST-825 farm-raised derived isolate was found within this cluster. Interestingly, human and farm-raised poultry derived *C. coli* isolates with undetermined ST clustered together and seem different from other isolates.

Simpson's diversity index was 0.208 among *C. jejuni* isolates from household-raised poultry, and 0.725 among those from industrially-raised poultry, indicating a higher genetic diversity among *C. jejuni* isolates from industrially-raised chickens. Due to the high number of undetermined sequence types among *C. coli* isolates, Simpsons diversity index was not calculated. A list of the sequence type and clonal complexes assigned to each specific isolate is available in the **Table 19**.

The neighbor joining phylogenetic tree in **Figure 9** shows distinct population structures between industrial and household-raised *C. coli* genomes. However, no clearly-distinct population structure differentiated *C. jejuni* isolates from these two production settings.

The six chicken *C. jejuni* and eight *C. coli* isolates demonstrating phenotypic resistance to CIP, AZM, TE and GM were not assigned to a known sequence type.

Among households for which more than one *C. jejuni* isolate was sequenced, two or more distinct sequence types (including undetermined ones) were identified in 2 out of the 12 houses. While among market stalls from which broiler fecal samples were obtained 3 out of the 7 had more than one distinct sequence type assigned. However, most of these were classified as undetermined. Too many *C. coli* isolates had unassigned sequence type in order to infer any relationship about polyclonal carriage within households and market vendors.

(ii) *Distinct Lipooligosacharide biosynthesis loci (LOS class) among C. jejuni isolates from farm and household raised chickens*

LOS class was determined for 42/43 *C. jejuni* isolates. Class H was the predominantly present among farm-derived isolates (83.3% (20/24). Two other isolates had a class C LOS (2/24), and one was a mixed H and A culture and the last one a mixed H and C culture. Among isolates from household derived *C. jejuni*, 66.5% had a class B LOS (10/18), 11.1% had a class K LOS (2/18), as well as a class E (2/18) LOS. Class H and class C LOS were found in 11.1% (2/18) and 5.6% (1/18) of isolates, respectively.

(iii) *Higher Antimicrobial Resistance determinants among Campylobacter spp. from farm raised chickens*

Phenotypic patters of resistance are shown in **Table 21**. Among *C. coli* isolates, 100% were resistant to ciprofloxacin, while among *C. jejuni* isolates, 100% of those from industrially-raised broilers were resistant, while only 53.8% of those obtained from household chickens were resistant (Fisher's exact test: p-value = 0.003). Regarding phenotypic resistance to macrolides, 90% (9/10) of *C. coli* isolates from industrially-raised broilers were resistant, while none of the *C. coli* isolates obtained from household chickens demonstrated resistance (Fisher's exact test: p-value = 0.014). These same 9 macrolide resistant isolates were also resistant to ciprofloxacin. Only 29.4% (5/17) of *Campylobacter jejuni* isolates from industrially-raised broilers were resistant to both ciprofloxacin and azithromycin, while only 15.4% (2/13) from household-raised poultry. Tetracycline resistance was higher among *C. coli* and *C. jejuni* isolates obtained from industrially-raised chickens (*C. coli*: 100% (10/10); *C. jejuni*: 88.2% (15/17) in comparison to household-raised chickens (*C. coli*: 33.3% (1/3); *C. jejuni*: 46.2% (6/13)) (Fisher's exact test: p-value = 0.038). Finally, the difference in phenotypic gentamicin resistance among *C. coli* isolates between industrially-raised (80% (8/10) and household-raised (0% (0/3)) chickens was also statistically significant (p-value 0.035). Multi-drug resistance (MDR) to ciprofloxacin, azithromycin, tetracycline and gentamicin was observed in 80% *C. coli* isolates from industrially-raised chickens in comparison to 0% from household-raised chickens (Fisher's exact test: p-value = 0.035).

The presence or absence of antimicrobial resistance genes identified in the available genomes is shown in **Table 22**. Among both *C. jejuni* and *C. coli* genomes, an average number of 9 genes or mutations per isolate were identified. However, among *C. coli*

isolates from industrially-raised chickens, an average of 10.3 genes were found, while only 7.6 genes among *C. coli* isolates from household chickens (Two sample t-test: p-value=0.038). This difference was also identified among *C. jejuni* isolates, given that genomes from industrially-raised chickens had an average of 11.0 genes, while those from household chickens had 6.42 (Two sample t-test: p-value<0.001). The chromosomal point mutation of the *gyrA* gene that confers ciprofloxacin resistance (Th186L) was identified in all *C. coli* isolates. However, among *C. jejuni* isolates, 100% of those from industrially-raised chickens had the mutation, yet only 47.4% of *C. jejuni* isolates from household-raised chickens carried the gene (Fisher's exact test: *p-value* = 0.027). The *23S* ribosomal subunit mutation (A207G) that confers resistance to macrolides was identified in 73.3% (15/15) of *C. coli* genomes from industrially-raised broilers while in none of those from household-raised chickens (Fisher's exact test: p-value=0.008). This difference was also statistically significant among *C. jejuni* isolates, where 25% (6/24) genomes from industrially-raised chickens were identified as having the point mutation and none of those from household-raised chickens (Fisher's exact test: p-value=0.027). Similarly, genes conferring resistance to aminoglycosides were more prevalent among *C. coli* and *C. jejuni* genomes from industrially-raised chickens. Specifically, among all *Campylobacter* genomes, *aad(9)*, *aph(2'')-If* and *aph(3")-IIIa* were more prevalent among industrially-raised broilers in comparison to household-raised chickens. A greater number of *C. coli* (55% (11/20)) isolates had the *aph(2'')-If* in comparison to *C. jejuni* genomes (30.3% (10/33)) (Fisher's exact test: p-value=0.021), while only *C. jejuni* genomes contained an *aadE* gene (23.3% vs. 0%) Fisher's exact test: p-value=0.023) Interestingly, an *rpsL* chromosomal mutation conferring resistance to Streptomycin was

prevalent only among *C. coli* genomes from industrially-raised chickens (66.7% (10/15), Fisher's exact test: p-value=0.033).

Among *C. jejuni* isolates, phenotypic resistance to either azithromycin, erythromycin and gentamicin was positively correlated with the presence of the *23S* ribosomal mutation A207G (Spearman rank correlation coefficient, 0.01 significance level: AZM=0.792, ERY: 0.709). As expected, phenotypic resistance to fluoroquinolones ciprofloxacin and nalidixic Acid was also highly associated with the *gyrA* mutation (Th186L) (Spearman rank correlation coefficient, 0.01 significance level: CIP and NAL: 0.829). Statistically significant correlations were also identified between gentamicin resistance and the presence of *aph(2'')-If* (Spearman rank correlation coefficient, 0.01 significance level= 0.814), while the presence of *aph(3'')-IIIa* was highly correlated with ciprofloxacin, nalidixic acid and tetracycline phenotypic resistance (Spearman rank correlation coefficient, 0.01 significance level CIP and NAL=0.612, TE=0.505).

6.3 Discussion

Among *C. jejuni* isolates from household-raised chickens, CC-607, CC-460, CC-443, and CC-353 were identified, while among isolates derived from industrially-raised poultry CC-607 and CC-353 predominated. All of these clonal complexes are poultry specialist lineages, and have been identified throughout the world (235-240). Clinical cases associated with CC-607 predominated in this setting but have been characterized in less than 3% of patients in the United Kingdom (241). All *C. coli* isolates belonged to the generalist lineage ST-828. Strains belonging to this sequence type have been isolated from pigs, cattle, and poultry, globally, and studies on the evolution of this pathogen suggest a high degree of recombination with agriculturally related *C. jejuni* isolates (222, 242-245)

The most prevalent LOS class among *C. jejuni* isolates from household chickens was LOS class B, a locus class capable of producing a siaylated LOS, and less frequently class K and class E, both of which do not enable LOS siaylation. Sialylation of LOS is a highly related and characterized virulence factor related to post-infection neuropathies such as GBS and MFS (246, 247). Contrasting with our observations, class B was predominantly found among farm-derived *Campylobacter jejuni* isolates from studies in Sweden, France, US and Australia (225, 248-250). Farm-raised poultry predominantly had class had a class H LOS, which does not produce a siaylated LOS. This LOS locus has also been identified among Swedish *C. jejuni* isolates from farm-raised poultry(250). For instance, patients in Bangladesh and China with GBS due to *C. jejuni* infections have been identified as having class B LOS (251, 252). Therefore, among our population of

Campylobacter jejuni isolates, it would appear as if those derived from household-raised chickens would pose the biggest threat to risk for a post-infectious neuropathy.

C. coli isolates had a greater number of resistance genes in comparison to *C. jejuni* isolates from any poultry environment, which is consistent with prior observations (253). We did, however, identify a higher number of resistance genes among industrially-raised poultry in comparison to household-raised poultry, for both *C. jejuni* and *C. coli* isolates. A study by Kassem and collaborators only identified phenotypic differences in resistance between *Campylobacter* spp. from conventional-and organic-raised chickens(254). Of particular interest is a cluster *C. coli* and *C. jejuni* isolates with unidentified sequence types derived *only* from farm-raised chickens harboring 23S rRNA A2075G mutation conferring macrolide resistance. This is compelling evidence that suggest administration of macrolides to chickens within a confined feeding operation promotes the emerge of macrolide resistance among *Campylobacter* isolates. No other mutation or gene conferring resistance only to macrolides was identified among our isolates. The number of AMR genes conferring aminoglycoside resistance was also higher among *C. coli* and *C. jejuni* isolates derived from industrially-raised chickens, also indicating external antibiotic pressure from the production environments. The *tetO* gene was highly ubiquitous among our isolates, consistent with other studies (253). Resistance to aminoglycosides is expanding worldwide, and a higher prevalence of genes conferring resistance to these type of antibiotics has also been identified in *Campylobacter* from retail poultry from around the world, as well as in human clinical isolates (*aph(2'')-If*) (134, 136)

The main limitation associated with this analysis is the temporal difference between human and poultry derived *Campylobacter* isolates. Further studies evaluating concomitantly isolated *Campylobacter* strains from humans, farm-raised and household-raised poultry, as well as other animal sources, are needed to estimate analyze disease transmission pathways and transference of antimicrobial resistance genes. Additionally, the location of AMR genes within the *Campylobacter* genome needs to be determined. Finding the majority of these genes within mobile elements would address the potential transmissibility of resistance among *Campylobacter* spp. isolates (253).

6.4 Conclusion

Campylobacter jejuni and *Campylobacter coli* from industrially-raised and household-raised poultry generally to distinct population structures. A greater number of antimicrobial resistance genes are found among both *C. jejuni* and *C. coli* isolates from industrially-raised chickens in comparison to household-raised chickens. However, a greater proportion of household derived *C. jejuni* isolates hold sialylated LOS locus, suggesting a potentially greater risk of post-infection neuropathies such as Guillain-Barre-Syndrome. Policy regulations limiting the usage of antibiotics among farm-raised poultry could limit the risk of infection with multi-drug-resistant Campylobacteriosis to workers and consumers. However, a household base prevention and control strategy is also required to limit human infection with strains linked to post-infection neuropathies.

6.5 Tables for Chapter 6

Table 17. *Campylobacter* spp. isolates from fecal samples of household-raised and industrially-raised poultry from Iquitos, Peru

	Household	**Industrial**
Fecal samples (N)	102	100
Culture positive (% (n/N))	37.3% (38/102)	53.0% (53/100)
Campylobacter spp. positive community households (% (n/N))	65.5% (21/32)	-
Campylobacter spp. positive poultry vendors (% (n/N))	-	90% (9/10)
Hippurate hydrolysis positive isolates (% (n/N))	39.5% (15/38)	47.2% (25/53)
Whole Genome Sequencing (n)	81.6% (31/38)	83.0% (44/53)
Campylobacter jejuni (% (n/N))	61.3% (19/31)	54.5% (24/44)
Campylobacter coli (% (n/N))	16.1% (5/31)	43.2% (19/44)
Polyclonal	9.7% (3/31)	9.1% (4/44)
Contaminated	12.9% (4/31)	2.3% (1/44)

Table 18. Summary of Multi-Locus Sequence Types and Clonal Complexes of *Campylobacter jejuni* and *Campylobacter coli* isolates from household-raised and industrially-raised poultry from Iquitos, Peru

	Campylobacter coli (n=20)		*Campylobacter jejuni* (n=43)	
	Industrial	Household	Industrial	Household
Clonal Complex				
Not-Determined	12	1	10	3
ST-828 complex	3	4	-	-
ST-353 complex	-	-	2	3
ST-443 complex	-	-	0	2
ST-460 complex	-	-	0	2
ST-607 complex	-	-	12	9
Sequence-Type				
Not-Determined	12	1	10	3
ST-1055	0	3	-	-
ST-1173	0	1	-	-
ST-825	1	0	-	-
ST-8408	2	0	-	-
ST-1036	-	-	0	2
ST-1212	-	-	1	2
ST-1365	-	-	0	2
ST-4722	-	-	0	1
ST-535	-	-	0	2
ST-607	-	-	12	7
ST-6177	-	-	1	0
ST1232/ ST-7865	-	-	1	0

Table 19. Multi-Locus Sequence Types and Clonal Complexes of *Campylobacter jejuni* and *Campylobacter coli* of individual isolates from household-raised and industrially-raised poultry from Iquitos, Peru

Sample	Production Type	MLST Allelles							Sequence Type	Clonal complex
		aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*		
Campylobacter coli										
PA010	Industrial	247		30	82	113	47	17	ND	
PA026	Industrial	33	110	30	82	113	631	17	ND	
PA028	Industrial	33	110	30	82	113	631	17	ND	
PA029	Industrial	33	110	30	82	113	631	17	ND	
PA031	Industrial	103	110	103				74	ND	
PA032	Industrial	33	39	30	82	113	631	17	8408	ST-828 complex
PA033	Industrial	103	110	103				74	ND	
PA034	Industrial	33	39	30	82	113	631	17	8408	ST-828 complex
PA040	Industrial	103	110	103		113	3	79	ND	
PA048	Industrial	33	110	30	82	113	631	17	ND	
PA061	Industrial	33	110	30	82	113	631	17	ND	
PA062	Industrial	33	110	30	82	113	631	17	ND	
PA065	Industrial	33	110	30	82	113	631	17	ND	
PA070	Industrial	33	110	30	82	113	631	17	ND	
PA093	Industrial	33	39	30	82	113	47	17	825	ST-828 complex
PV004	Household	247		30	82	113	47	17	ND	
PV086	Household	33	39	30	82	113	56	17	1173	ST-828 complex
PV091	Household	33	39	30	82	104	47	17	1055	ST-828 complex
PV093	Household	33	39	30	82	104	47	17	1055	ST-828 complex
PV094	Household	33,103	39	30	82	104	47	17	1055	ST-828 complex
Campylobacter jejuni										
PA001	Industrial	24	2	5	10	10	120	6	ND	
PA002	Industrial	24	2	5	10	10	120	6	ND	
PA004	Industrial	24	2	5	10	10	120	6	ND	
PA006	Industrial	24	2	5	10	10	120	6	ND	
PA009	Industrial	24	2	5	10	10	120	6	ND	
PA030	Industrial	24	2	5	10	10	120	6	ND	
PA046	Industrial	7	17	5	10	10	412	6	6177	ST-353 complex
PA049	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA050	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA064	Industrial	2	1	5	25	10	203	5	ND	
PA067	Industrial	2	1	5	25	10	203	5	ND	
PA074	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA075	Industrial	7,8	17	5	10	11	3	6	1232, 7865	ST-353 complex
PA079	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA081	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA082	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA083	Industrial	2	4	5	25		203	5	ND	
PA084	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA085	Industrial	8	2	5	53	11	3	1	607	ST-607 complex

PA088	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA089	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA090	Industrial		4	5	25	640	203		ND	
PA092	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PA096	Industrial	8	2	5	53	11	3	1	607	ST-607 complex
PV007	Household	7	84	5	10	11	3	6	1036	ST-353 complex
PV010	Household	8	2	5	53	11	3	1	607	ST-607 complex
PV012	Household	8	2	5	53	11	3	1	607	ST-607 complex
PV020	Household	8	2	5	53	11	3	1	607	ST-607 complex
PV021	Household	8	2	5	53	11	3	1	607	ST-607 complex
PV048	Household	7	17	5	10	11	412	6	4722	ST-353 complex
PV064	Household	8	2	5	53	11	3	1	607	ST-607 complex
PV071	Household	8	17	2	2	11	3	6	ND	
PV072	Household	8	17	2	2	11	3	6	ND	
PV075	Household	8	2	5	53	11	3	105	1212	ST-607 complex
PV076	Household	8	2	5	53	11	3	105	1212	ST-607 complex
PV077	Household	9	30	2	2	89	59	6	535	ST-460 complex
PV083	Household	8	2	5	53	11	3	1	607	ST-607 complex
PV084	Household	9	30	2	2	89	59	6	535	ST-460 complex
PV087	Household	7	84	5	10	11	3	6	1036	ST-353 complex
PV088	Household	24	2	2	15	23	3	12	1365	ST-443 complex
PV090	Household	24	2	2	15	23	3	12	1365	ST-443 complex
PV092	Household	7	84	5	2	11	3	6	ND	
PV099	Household	8	2	5	53	11	3	1	607	ST-607 complex

Table 20. Disc Diffusion Breakpoints Used to Assign a Resistance Phenotype to *Campylobacter* spp. isolates from Iquitos, Peru.

Antibiotic (Abbreviation)	Concentration (μg)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)	Source
Fluoroquinolones					
Ciprofloxacin (CIP)	5	≤20	20-24	≥24	1
Nalidixic Acid (NA)	30	≤19	-	≥20	2
Macrolides					
Erythromycin (ERY)	15	≤12	13-15	≥16	1
Azythromycin (AZM)	15	≤12	13-15	≥16	1
Tetracyclines					
Tetracycline (TE)	30	≤22	23-25	≥26	1
Aminoglycosides					
Gentamicine (GM)	10	≤12	13-14	≥15	3
Beta-Lactams					
Amoxicilin Clavulanic Acid (AMC)	20/10	≤13	14-17	≥18	3
Cefotaxime (CTX)	30	≤22	23-25	≥26	1
Carbapenems					
Imipenem (IMP)	10	≤19	20-22	≥23	3
Others					
Cloranphenicol (C)	30	≤12	13-17	≥18	3
Trimetoprim-sulphamethoxasol (STX)	1.25 – 23.75	≤10	11-15	≥16	3
Colistin (CL)		≤11	-	≥14	4

Source:

1. CLSI. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016
2. BSAC. British Society for Antimicrobial Chemotherapy. Methods for Antimicrobial Susceptibility Testing. 2013
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2016
4. Galani I, Kontopidou F, Souli M, Rekatsina PD, Koratzanis E, Deliolanis J, et al. Colistin susceptibility testing by Etest and disk diffusion methods. Int J Antimicrob Agents. 2008;31(5):434-9

Table 21. Phenotypic antibiotic susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolates from household-raised and industrially-raised poultry from Iquitos, Peru

Antibiotics	*Campylobacter coli* (n=13)					*Campylobacter jejuni* (n=30)				
	Household (%(n/N))		Industrial (%(n/N))		p-value	Household (%(n/N))		Industrial (%(n/N))		p-value
Fluoroquinolones										
Ciprofloxacin (CIP)	100%	(3/3)	100%	(10/10)	-	53.8%	(7/13)	100%	(17/17)	**0.003**
Nalidixic Acid (NA)	66.7%	(2/3)	100%	(10/10)	0.231	53.8%	(7/13)	100%	(17/17)	**0.003**
Macrolides										
Erythromycin (ERY)	0%	(0/3)	90.0%	(9/10)	**0.014**	15.4%	(2/13)	29.4%	(5/17)	0.427
Azythromycin (AZM)	0%	(0/3)	90.0%	(9/10)	**0.014**	7.7%	(1/13)	29.4%	(5/17)	0.196
Tetracyclines										
Tetracycline (TE)	33.3%	(1/3)	100%	(10/10)	**0.038**	46.2%	(6/13)	88.2%	(15/17)	**0.020**
Aminoglycosides										
Gentamycin (GM)	0%	(0/3)	80.0%	(8/10)	**0.035**	15.4%	(2/13)	29.4%	(5/17)	0.427
Beta-Lactams										
Amoxicilin Clavulanic Acid (AMC)	33.3%	(1/3)	90.0%	(9/10)	0.108	30.8%	(4/13)	29.4%	(5/17)	1.000
Cefotaxime (CTX)	66.7%	(2/3)	100%	(10/10)	0.231	46.2%	(6/13)	52.9%	(9/17)	1.000
Carbapenems										
Imipenem (IMP)	0%	(0/3)	0%	(0/10)	-	0%	(0/13)	0%	(0/13)	-
Others										
Cloramphenicol (C)	0%	(0/3)	0%	(0/10)	-	0%	(0/13)	0%	(0/17)	-
Trimethoprim-sulphamethoxasol (STX)		(2/3)	100%	(10/10)	0.231	76.9%	(10/13)	82.4%	(14/17)	1.000
Colistin (CL)	0%	(0/3)	0%	(0/10)	-	30.8%	(4/13)	29.4%	(5/17)	1.000

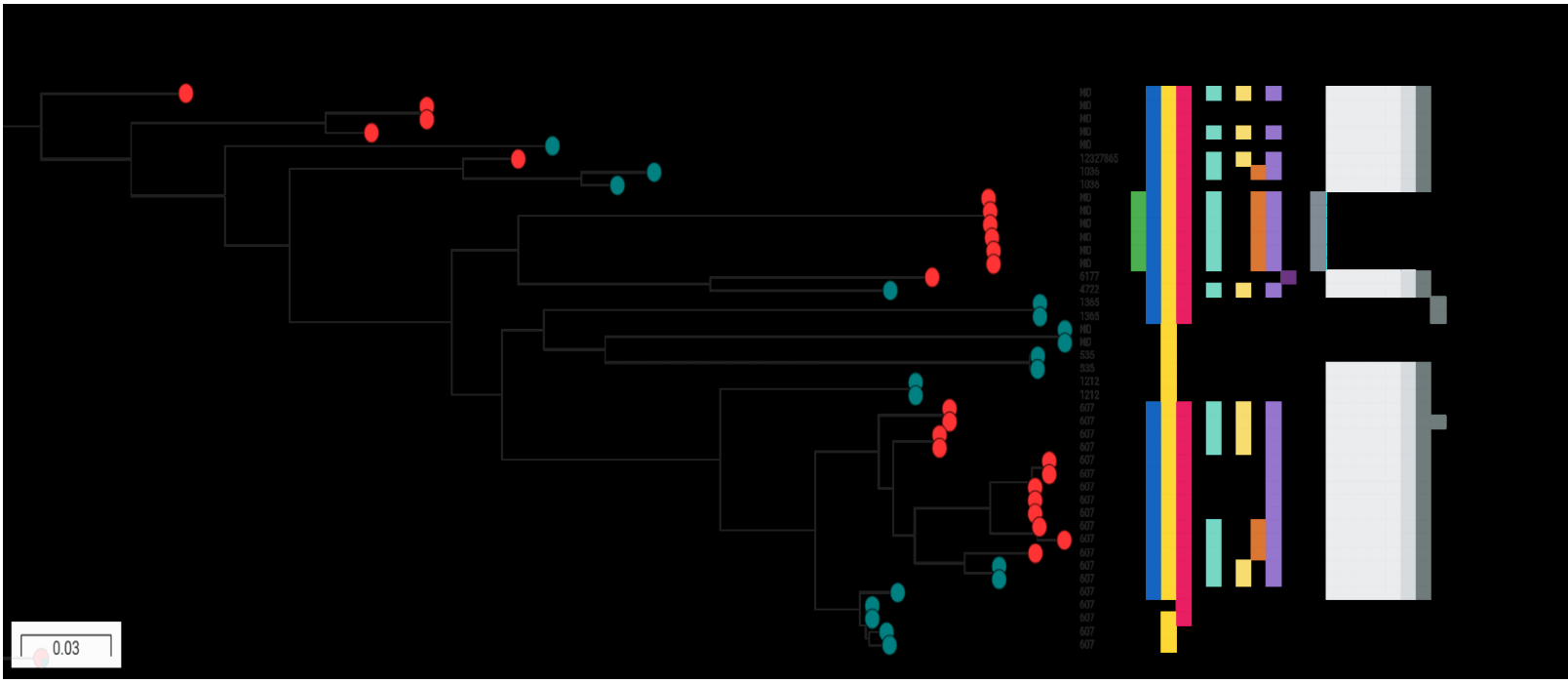
Table 22. Antimicrobial Resistance genes identified in *Campylobacter jejuni* and *Campylobacter coli* isolates from household-raised and industrially-raised poultry from Iquitos, Peru

	***Campylobacter coli* (n=20)**			***Campylobacter jejuni* (n=43)**		
	Household (% (n/N))	Farm (% (n/N))	p-value	Household (% (n/N))	Farm (% (n/N))	p-value
Fluoroquinolones						
gyrA Th186L	100% (5/5)	100% (15/15)	-	47.4% (9/19)	100% (24/24)	**<0.001**
Macrolides						
23S rRNA A2075G	0% (0/5)	73.3% (11/15)	**0.008**	0% (0/19)	25% (6/24)	**0.027**
MDR						
cmeR	0% (0/5)	13.3% (2/15)	0.553	100% (24/24)	94.7% (18/19)	0.442
Tetracyclines						
tet(O)	60% (3/5)	86.7% (13/15)	0.249	57.9% (11/19)	100% (24/24)	**0.001**
Aminoglycoside						
aad(6)	0% (0/5)	13.3% (2/15)	0.553	0% (0/19)	0% (0/24)	-
aad(9)	0% (0/5)	80.0% (12/15)	**0.004**	21.1% (4/19)	66.7% (16/24)	**0.005**
aad(E)	0% (0/5)	0% (0/15)	-	15.8% (3/19)	29.2% (7/24)	0.470
aph(2'')-If	0% (0/5)	73.3% (11/15)	**0.008**	47.4% (9/19)	37.5% (9/24)	**0.026**
aph(3')-IIIa	0% (0/5)	93.3% (11/15)	**<0.001**	21.1% (4/19)	87.5% (21/24)	**<0.001**
spw	0% (0/5)	13.3% (2/15)	0.553	0% (0/19)	0% (0/24)	-
Beta-lactams						
blaOXA-184	0% (0/5)	6.7% (1/15)	1.000	0% (0/19)	25% (6/24)	**0.027**
blaOXA-193	80% (4/5)	40% (6/15)	0.303	52.6% (10/19)	75% (18/24)	0.198
blaOXA-450	80% (4/5)	40% (6/15)	0.303	52.6% (10/19)	75% (18/24)	0.198
blaOXA-451	80% (4/5)	40% (6/15)	0.303	52.6% (10/19)	75% (18/24)	0.198
blaOXA-452	80% (4/5)	40% (6/15)	0.303	52.6% (10/19)	75% (18/24)	0.198
blaOXA-453	80% (4/5)	40% (6/15)	0.303	52.6% (10/19)	75% (18/24)	0.198
blaOXA-489	100% (5/5)	93.35% (14/15)	1.000	52.6% (10/19)	75% (18/24)	0.198
blaOXA-605	100% (5/5)	100% (15/15)	-	52.6% (10/19)	75% (18/24)	0.198
blaOXA-61	0% (0/5)	0% (0/15)	-	10.5% (2/19)	4.2% (1/24)	0.575
Streptomycine						
rpsL	0% (0/5)	66.7% (10/15)	**0.033**	0% (0/19)	4.2% (1/24)	1.000
Streptothricin						
sat4	0% (0/5)	20% (3/15)	0.539	0% (0/19)	0% (0/24)	-

6.1 Figures for Chapter 6

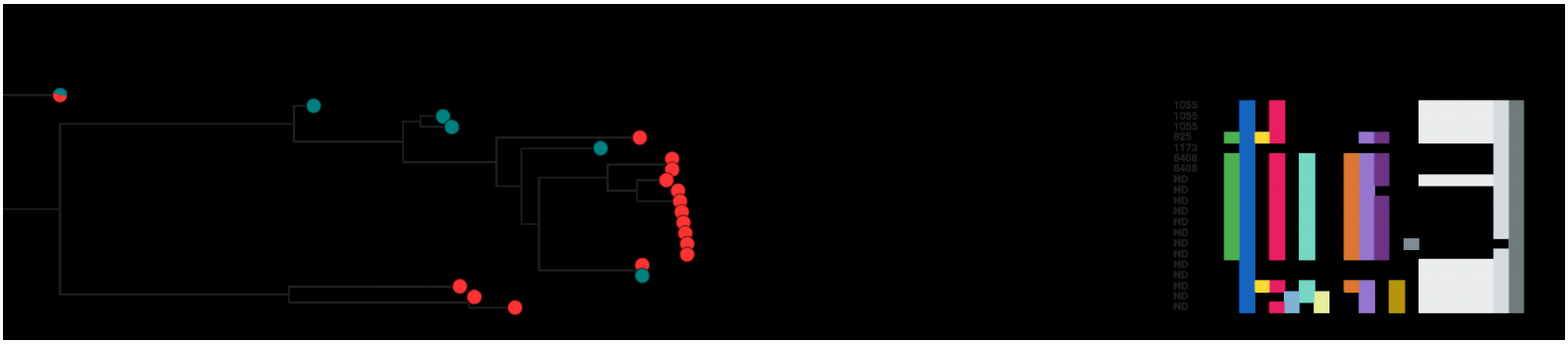
Figure 7. Photograph showing household raised chickens in the backyard of a house from Santa Clara de Nanay

Figure 8. SNP based Phylogeny tree of *Campylobacter jejuni* isolates from household-raised and industrially-raised poultry from Iquitos, Peru (https://microreact.org/project/FIDKh2q0k)



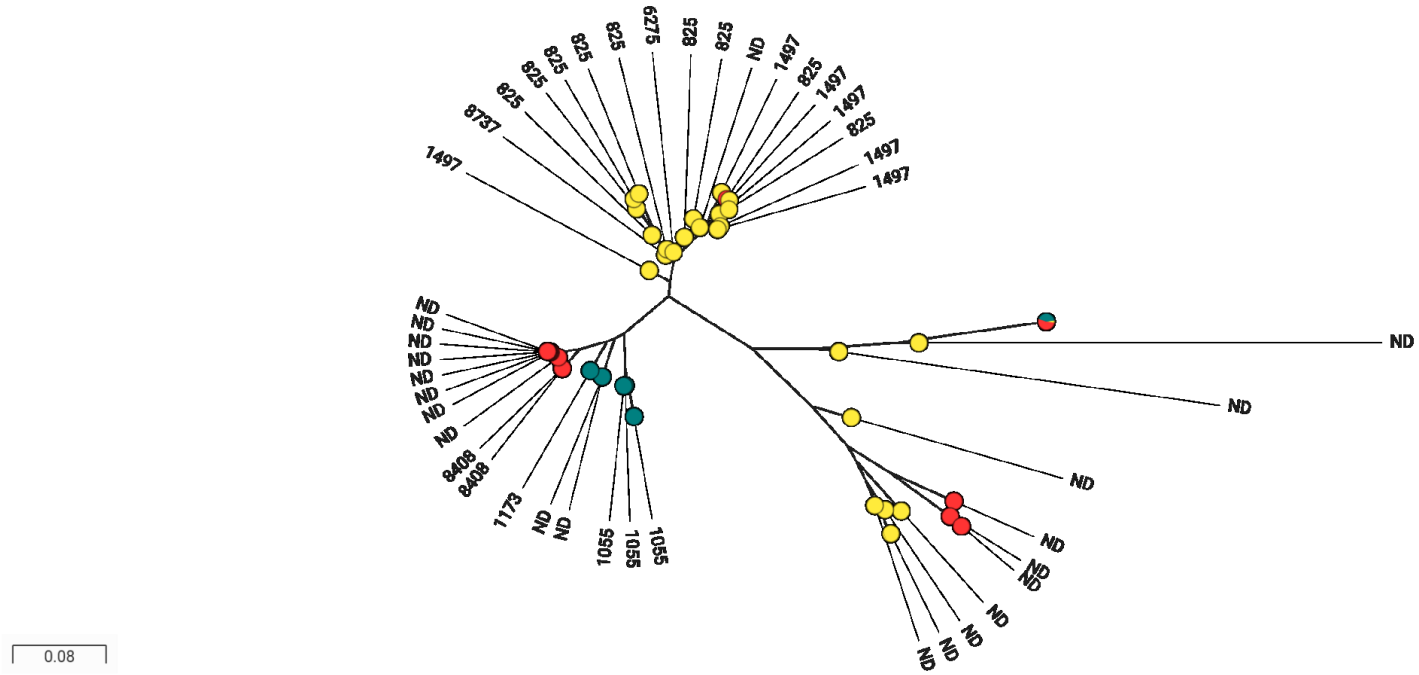
SNP based Phylogenetic Tree. Red nodes = *Campylobacter jejuni* from industrially-raised poultry; Green nodes = *Campylobacter jejuni* from household-raised poultry. Labels indicate the multilocus sequence type, where ND = undetermined sequence type. Metadata blocks indicate presence or absence of the antibiotic resistance gene listed. Reference genome NTCC11168 used.

Figure 9. SNP based Phylogeny tree of *Campylobacter coli* isolates from household-raised and industrially-raised poultry from Iquitos, Peru (https://microreact.org/project/FIDKh2q0k



SNP based Phylogenetic Tree. Red nodes = *Campylobacter coli* from industrially-raised poultry; Green nodes = *Campylobacter coli* from household-raised poultry. Labels indicate the multilocus sequence type, where ND = undetermined sequence type. Metadata blocks indicate presence or absence of the antibiotic resistance gene listed. Reference genome NTCC11168 used.

Figure 10. SNP based Phylogeny tree of *Campylobacter coli* isolates from household-raised and industrially-raised poultry, and children 0-2 years of age from Iquitos, Peru (https://microreact.org/project/FIDKh2q0k)



SNP Phylogenetic Tree. Red nodes = *Campylobacter coli* from industrially-raised poultry; Green nodes = *Campylobacter coli* from household-raised poultry; Yellow nodes = *Campylobacter coli* from children 0-2 years of age collected between 2010-2015. Labels indicate the multilocus sequence type, where ND = undetermined sequence type. Reference genome NTCC11168 used.

Chapter 7. Conclusions

7.1 Summary of results

This dissertation validated a tool to attribute fecal contamination to a specific animal source in household surfaces samples, measured source specific fecal contamination and avian exposure in household surfaces and compared the genomic epidemiology and antimicrobial resistance determinants of *Campylobacter* spp. isolated from household-raised and industrially-raised poultry in Iquitos Peru.

Aim 1:

In this study we validated eight microbial source tracking markers for the attribution of fecal contamination to a specific animal host: *Av4143* (avian), *LA35* (avian), *Pig2Bac* (swine), *Bactcan* (canine), *HF183*-Taqman (human) and *Bachum* (human). Additionally, two markers of avian exposure, *ND5* and *CytB*, were also validated. Results from aim 1 further prove that the performance of all eight microbial source tracking markers is specific to the geographic setting in which they are being deployed. Microbial source tracking markers were able to achieve adequate sensitivities and specificities, and were able to detect higher gene quantities among target samples in comparison to non-target samples. Human fecal markers where correlated with each other, as well as avian fecal markers and avian exposure markers, as expected. Additionally, the validation of avian markers of fecal contamination and markers of avian exposure, was the first of its kind to be developed in a low resource tropical environment. Given the sensitivities and specificities obtained for human markers, this study provides further evidence to suggest

that the development of additional human fecal source markers is needed, specifically markers that are able to distinguish adult fecal samples from pediatric fecal samples.

Aim 2:

For aim 2, we used microbial source tracking markers previously validated to score detect and attribute fecal contamination in household surface samples to a specific source. This is a novel method for measuring animal fecal contamination in household environments. Results indicate that animal fecal contamination is more prevalent among household surfaces than human fecal contamination. Specifically, animal feces were detected in 75% of households, while human feces were detected in 20.2%. A higher burden of avian, canine and human fecal contamination was found in floors in comparison to tables. Additionally, we determined that the material of floors and tables was an important determining factor for identifying the presence of fecal material as well as the quantities of fecal material. Specifically, wood table and unfinished floors (i.e. dirt floors), had higher quantities of avian and canine fecal material. The utility of these markers was further validated by determining that both the presence and quantities of *Av4143* (fecal marker), *ND5* and *Cytb* (avian exposure), where strongly associated with the presence of chickens within the household. The quantities of avian fecal contamination were algo correlated with the age of the primary caregiver of the household and the length of tenancy of the household. Moreover, the quantities of human fecal contamination were associated with having an unimproved source of water. The odds of detecting *Campylobacter* spp. (a zoonotic bacterium of which chickens are the main source) DNA in these same surface samples was highly associated with the presence of *Av4143* fecal

marker, even after adjusting for the presence of other markers, as well as socioeconomic and infrastructure covariates. Finally, toys given to a child for 24 hours were positive for avian exposure markers (*ND5* and *CytB*), avian fecal marker *Av4143* and human fecal marker *BacHum*.

Aim 3:

In this chapter we decided to explore the population structure and diversity of *Campylobacter* spp. isolates from household-raised poultry from Santa Clara de Nanay, Santo Tomas and La Union, as well as from industrially raised poultry from Iquitos. We also compared the phenotypic and genetic determinants of antimicrobial resistance among *Campylobacter* spp. isolates from these two poultry populations. *Campylobacter* spp. was obtained from 37.3% of household chickens and 53.0% of farm chickens. Seventy-five isolates had whole genome sequencing and assembly performed. Phylogenetic analysis of *C. jejuni* and *C. coli* revealed that isolates obtained from household and farm chickens are genetically isolated by three independent genomic assessments. Among *C. jejuni* isolates from both industrial and household samples, we isolated poultry specialist lineages, such as clonal complexes ST-607, ST-460, ST-443 and ST-353. *C. coli* isolates from farm and household samples were mainly associated with clonal complex 828, a generalist lineage that is known to cause disease in humans. Multi-drug resistant *Campylobacter coli* isolates resistant to Ciprofloxacin, azithromycin and gentamicin obtained from a pediatric population between 2011-2016 revealed that poultry isolates of undetermined sequence type cluster together with human samples also of an undetermined sequence type that awaits assignment from PubMLST at this time.

Moreover, a higher proportion of siaylated LOS class (predominantly B) associated with post infection neuropathies such as Guillain Barre Syndrome, was identified among *C. jejuni* isolates from households, whereas *C. jejuni* from farm chickens had mainly LOS class H, a class that does not enable siaylation. Finally, *C. coli* harbored more antibiotic resistance genes than *C. jejuni*, and both *C. jejuni* and *C. coli* obtained from industrially produced chickens had a higher proportion of genes and mutations that confer resistance to macrolides, aminoglycosides and fluoroquinolones.

7.2 Overall conclusions

Microbial source tracking has been validated as a tool for determining the presence, quantifying and attributing fecal contamination to a specific source, within the indoor environments of households from rural tropical communities where multi-specie fecal contamination is ubiquitous. Measuring the degree of fecal contamination from household surfaces is an efficient and useful strategy for determining indoor fecal contamination. Adding the utility of source attribution of microbial source tracking markers, makes this technique a promising tool for evaluation the overall effect on hygiene and sanitation interventions within low-resource communities. Animal fecal contamination needs to be incorporated in future disease control prevention programs that take place within the household.

Our results show that household infrastructure is associated with the burden of fecal contamination, indicating that precarious building materials and infrastructure are related to an increased risk of human infection. Although this is in itself a higher level

hypothesis, we would like to use our findings to advocate for a return back to tackling the social determinants of health as a mechanism of reducing the prevalence of enteric disease and achieving health equity, and not just deploying intermittent magic bullets as a problem solving approach. Future randomized controlled trials that aim to reduce enteric disease, enteropathy, and stunting among underserved pediatric populations should take into consideration both the source of fecal contamination and the infrastructure characteristics of households.

Poultry are the principal source of animal protein for families within Santa Clara de Nanay, Santo Tomas and La Union. Poultry rearing within backyards also serves as an alternate source of income for many of these families. This research identified a high burden of avian fecal material within households, and a strong association with *Campylobacter* spp. exposure in household surfaces. Additionally, we identified that in these specific communities, backyard chickens are a of drug-resistant *Campylobacter* spp. and of greater importance, a source of *C. jejuni* with the potential to cause post-infection neuropathies. However, we do not encourage the elimination of poultry from households. We propose that poultry should be kept within backyards and away from children with the objective of reducing household fecal contamination, and diminishing the risk of *Campylobacter* spp. transmission. Moreover, the highest risk of acquiring a multi-drug resistant *Campylobacter* spp. comes from commercially produced poultry, sold within the main markets of the city and accessible to the entire population of Iquitos. Reducing the non-therapeutic use of antibiotics, as well as technically improving poultry production facilities is needed to curve down the emergence and spread of multi-drug

resistant Campylobacteriosis. Given the disproportionate burden of *Campylobacter* spp. among children from low-resource communities, achieving a healthy poultry production chain is an issue of health equity in this setting.

7.3 Strengths and limitations

Aim 1 & Aim 2:

The use of multiple microbial source tracking markers for the detection and attribution of fecal contamination has the potential to be transferred to an easy-to use diagnostic platform, such as a Taqman Array card to efficiently test and assign source of microbial contamination at the household level. Developing an array card with various MST markers that target not only avian and human feces, but also, canine, swine and other animals would be an ideal scenario for large trials including hundreds of samples, either from surfaces, water or sewage.

Additionally, measuring fecal contamination in household surfaces has been previously shown to be an efficient method (9, 203). Using MST markers in surfaces samples is a novel way of looking at household fecal contamination. Children, as well as animals are most frequently in contact with floors, and food, as well as animals are most frequently in contact with tables. As a result, using these surfaces as a proxy for risk of infection from zoonotic pathogens is ideal.

One of the main limitations associated with using microbial source tracking markers for source attribution is the imperfect sensitivities and specificities of these markers, as well

as the dependency of a prior validation step before this tool can be used in various geographic regions. Additionally, multispecies cohabitation and coprophaga within households is a common occurrence, inherently reducing the specificity of these markers. Moreover, there is a need to developed human markers with increased sensitivities, and if possible, markers that are able to distinguish bacteria specific to infants or specific to adults. The development of a marker of rat feces would also be ideal given the high prevalence of these animals within households.

Finally, the location of where the floor sample was obtained was based on previous similar studies evaluating traditional fecal indicator bacteria in floor surfaces. However, we did not pursue an analysis of which area of the house (front entrance, back entrance, cooking area) would be the most ideal scenario to measure the degree of fecal contamination, based on frequency of use by members of the household.

Aim 3:

Whole genome sequence data from *Campylobacter* spp. isolates from poultry or any other animal source did not exist in this region of South America in public data repositories. Preliminary studies suggest that the main source of human infection with this pathogen is poultry. As a result, characterizing *Campylobacter* spp. from poultry origin was a much needed research gap to be able to improve the ability to appropriately attribute source allocation. Comparing isolates from both household and industrially-raised poultry provided the opportunity to evaluate the similarity of strains and genetic admixture in among the two populations of *Campylobacter* in chickens in the same

community. Surprisingly, based on 3 independent analysis the concurrently collected strains were genetically isolated. This allowed us to compare the strains derived from a subset of highly antimicrobial resistant *Campylobacter* to evaluate the origin of antimicrobial resistance determinants.

Limitations associated with Aim 3 are mainly associated with the lack of time-matched human and other animal isolates from this community, and from within households. True transmission studies require human and animal samples to be measured concurrently. Additionally, another limitation of this study lies with the inability to assign a sequence type to 26 *Campylobacter* spp isolates. Although genomes have been submitted for further curation, determining these sequence types would have ideal to better understand the population structure of these isolates. Finally, having a greater number of isolates from both poultry ecosystems an expanded number of communities and contexts would strengthen our findings.

7.4 Recommendations for future research

Aim 1 and Aim 2:

Future research that uses microbial source tracking markers as a mechanism of attributing fecal contamination to a source and measuring the burden of fecal contamination within each source should aim to include an additional set of samples. First, getting repeated hand "surface" samples throughout the day, from the primary caregiver of the child, as well as the child itself would be a simple yet powerful way of observing the degree and source of fecal contamination. Additionally, sampling surfaces from toilet areas would be an ideal comparison with household surfaces and toy samples. We did not identify a high prevalence or burden of fecal contamination in comparison to animal fecal contamination, and having human toilet areas as a predicted area of high fecal contamination would be ideal.

Randomized controlled trials implementing water, hygiene and sanitation interventions tend to measure the reduction of fecal contamination within drinking water and other household environments. That said, we recommend that future trials of this sort implement microbial source tracking as a measurement and attribution tool. On what proportion is human vs. animal fecal contamination reduced based on these WASH interventions? Do they tackle animal fecal contamination at all? It is time that we take a One Health approach the prevention and control of infectious disease in environments where animal husbandry is ubiquitous, and both socially, nutritionally and economically important.

Aim 3:

Future research on the ecology and molecular epidemiology of *Campylobacter* spp. in Iquitos, as well as similar settings should consist of a longitudinal cohort of families, where follow-up and surveillance of fecal samples for the isolation of this pathogen is not only focused on the child, but on the entire household environment. Specifically, household members, animals, food and environmental samples should be monitored continuously to detect *Campylobacter* spp. infections and characterize transmission dynamics. The phylogenetic relationship and structure of the population should be established, as well as the genetic patterns of antibiotic resistance. This would offer researchers a high resolution image of the most probable source of *Campylobacter* spp. and help elucidate the potential role of human-to-human transmission of this pathogen.

Future research should also be focused on sensitive diagnostic techniques that are able to detect and quantify *Campylobacter* spp. within a human or animal stool sample. Although some qPCR techniques exist, they are not sensitive enough, and are not able to distinguish different *Campylobacter* species, especially the atypical ones such as C. upsaliensis and C. infans. Once the transmission dynamics of this pathogen are established, producing a sensitive and specific multiplex assay would reduce the hassle of bacterial culture and aid in determining if prevention and control strategies are being effective.

Finally, future research should also focus on the development of a simple, easily distributable and safe disinfection mechanism that reduces the amount of *Campylobacter*

on poultry meat. If primary caregivers could apply this method of disinfection it would reduce the risk of transmission of drug-resistant Campylobacteriosis, until more structural and policy related changes are implemented.

Chapter 8. Policy recommendations

Through the results of Aim 3 we identified that the highest proportion of multi-drug resistant *Campylobacter* spp., both in terms of phenotypic resistance and number of resistance genes or mutations conferring resistance, was associated with industrially-raised chickens. As a result, we highlight the need of the Peruvian FDA counterpart ("Servicio Nacional de Sanidad Agraria (SENASA)) to incorporate a surveillance system that monitors poultry farms and slaughterhouses for the contamination poultry by-products with *Campylobacter* spp. Setting a specific limit of *Campylobacter* spp. contamination within a flock or setting a quality standard within slaughterhouses are potential tools that could help reduce the risk of *Campylobacter* infection. Improving slaughtering facilities has been shown to reduce contamination levels in chicken meat in the United Kingdom. Although the technologies and resources available for slaughtering facilities in Europe are far more advanced than those available in Peru, regulating agencies need to start thinking towards improving the poultry production chain to achieve a high quality, safe food product.

Additionally, this same agencies, with support of the Ministry of Agriculture, should promote legislation that controls non-therapeutic use of antimicrobials within the poultry sector, as well as limiting the commercialization of over-the-counter veterinary antibiotics. Although some steps have been taking towards this goal, such as the ban of chloramphenicol, nitrofurans and nitroimidazol in animal production, antibiotics are still easily attainable over-the-counter, even without the prescription of a veterinarian.

Chapter 9. Appendices

9.1 Data collection instruments

DEMOGRAPHIC AND SOCIO-ECONOMIC STATUS FORM (PSE)	
If no response for any question, write NA as response.	
Fieldworker ID	☐☐☐
Date (DD/MMM/YY)	☐☐/☐☐☐/☐☐

DEMOGRAPHIC QUESTIONS			
#	**Question**	**Code**	**Response**
Questions for head of household (If mother/primary caregiver of child is the head of household skip to question 9)			
1	What is your age?	10-99 *(years)*	☐☐
2	*(Record sex)*	Male = 01; Female = 02	☐☐
3	What is your relationship to [CHILD'S NAME]? *Refers to child aged 24-60 months.*	Father = 01; Mother = 02; Grandmother = 03;Grandfather = 04; Sibling = 05; Other = 06	☐☐
4	Are you currently married, divorced, widowed, or never married? *If never married, skip to question 6.*	Never married = 01; Married = 02 Divorced = 03; Widowed = 04	☐☐
5	How old were you when you got married for the first time?	08-40 *(years)*	☐☐
6	Have you ever attended school? *If no, skip to question 9.*	Yes = 01; No = 00	☐☐
7	How many years of schooling have you completed?	00-20	☐☐
8	*If younger than 25 years old:* Are you currently attending school or college?	Yes = 01; No = 00	☐☐
9	What number is [CHILD'S NAME] in the birth order of her or his siblings?	First or only child = 01; Second = 02; Third = 03; Fourth = 04; Fifth = 05; Sixth = 06; Seventh = 07; Eighth = 08; Ninth = 09; Tenth or more = 10	☐☐
10	How old is [NAME]?*Age range for children included is 24-60 months.*	24-60 months	☐☐
11	Is [NAME] male or female?	Male = 01; Female = 02	☐☐
12	*Record weight of selected child aged 24-60 months. Weight recorded in kg. to one decimal place.*		☐☐.☐
13	*Record height of selected child aged 24-60 months. Height recorded in cm. to one decimal place.*		☐☐☐.☐

SOCIO-ECONOMIC STATUS QUESTIONS			
14	How long has your family lived in this house?	Less than one year = 01 Between one year and five years = 02 Between five years and ten years = 03 Between ten years and twenty years= 04 More than twenty years = 05	☐☐
15	How many rooms are there in your house?	01-15	☐☐
16	How many rooms do you sleep in?	01-15	☐☐
17	What is the average monthly income for the entire household?	00000-99999 Next to (currency) enter: P = soles; B = reals; H = shillings; S = rand; I = Indian rupees; N = Nepali rupees; R = Pakistani rupees; T = taka	☐☐☐☐☐ ☐ *(currency)*
18	What is the main source of drinking water for members of your household?	Piped into dwelling = 01; Piped to yard/plot = 02 ; Public tap/stand pipe= 03 ; Tube well or borehole = 04 Protected well = 05; Unprotected well = 06; Protected spring = 07 ; Unprotected spring = 08; Rainwater = 09; Tanker truck = 10; Cart with small tank = 11 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 12; Bottled water = 13	☐☐
19	*If piped water:* Is your piped water supply continuous or is it sometimes interrupted?	Continuous = 01; Sometimes interrupted = 02	☐☐
20	*If sometimes interrupted:* How long do these interruptions usually last?	Less than 3 hours = 01; 3 to 7 hours=02; 8 to 11 hours = 03; 12 to 24 hours= 04; More than 24 hours = 05	☐☐
21	What is the main source of water used by your household for other purposes such as cooking and hand-washing?	Piped into dwelling = 01 ; Piped to yard/plot = 02; Public tap/stand pipe = 03; Tube well or borehole = 04; Protected well = 05; Unprotected well = 06; Protected spring = 07; Unprotected spring = 08; Rainwater = 09; Tanker truck = 10; Cart with small tank = 11; Surface water (river / dam /lake /pond /stream/canal/irrigation canal) = 12	☐☐
22	Do you pay or barter for water?	Yes = 01; No = 00	☐☐
23	Where is the water source located?	In own dwelling = 01; In own yard/plot = 02; Elsewhere = 03	☐☐
24	How long does it take to go there, get water and come back in one trip? *If water is located on the premises, response is 000.*	000-200 minutes	☐☐☐

25	Which members of the household ever go to this source to fetch water? *Can enter up to three. Adult is anyone older than 15 years.*	Adult woman = 01; Adult man = 02; Female child under age 15 years = 03; Male child under age 15 years = 04; Other = 05	☐☐ ☐☐ ☐☐
26	Who is the main person in the household who goes to fetch water from this source?	Adult woman = 01; Adult man = 02; Female child under age 15 years = 03; Male child under age 15 years = 04; Other = 05	☐☐
27	Do you treat your water in any way to make it safer to drink?	Yes = 01; No = 00	☐☐
28	What do you usually do to the water to make it safer to drink?	Boil = 01; Add bleach/chlorine = 02; Strain through a cloth = 03; Use water filter (ceramic /sand/ composite/etc.) = 04 ; Solar disinfection = 05; Let it stand and settle = 06; Other = 07	☐☐
29	Do you wash your hands after helping your child defecate?	Always = 01; Sometimes = 02 Rarely = 03; Never = 04	☐☐
30	Do you wash your hands before preparing food?	Always = 01; Sometimes = 02 Rarely = 03; Never = 04	☐☐
31	Do you wash your hands after using the toilet?	Always = 01; Sometimes = 02 Rarely = 03; Never = 04	☐☐
32	Do you use toilet paper?	Always = 01; Sometimes = 02 Rarely = 03; Never = 04	☐☐
33	What kind of toilet facility do members of your household usually use?	Flush to piped sewer system = 01; Flush to septic tank = 02; Flush to pit latrine = 03; Flush to somewhere else = 04; Flush, don't know where = 05; Ventilated improved pit (VIP) latrine= 06; Pit latrine with slab = 07; Pit latrine without slab/open pit = 08; Composting toilet=09; Bucket toilet = 10; No facility/bush/field = 11; Public toilet = 12; Other = 13	☐☐
34	Do you share this toilet facility with other households?	Yes = 01; No = 00	☐☐
35	How many households use this toilet facility?	00-09; 10 or more households = 10	☐☐
36	Does your household ever have electricity?	Yes = 01; No = 00	☐☐
37	*If household ever has electricity:* Is your electricity supply continuous year-round, or is it sometimes interrupted?	Continuous = 01 Sometimes interrupted = 02	☐☐
38	*If sometimes interrupted:* How long do these interruptions usually last?	Less than 3 hours = 01 3 to 7 hours = 02 8 to 11 hours = 03 12 to 24 hours = 04 More than 24 hours = 05	☐☐
39	In case of discontinued power supply, what source does this household usually use?	Generator = 01; Gas/petrol = 02 UPS system = 03; Chargeable lights= 04 Other = 05	☐☐

Now I am going to ask you about whether your household owns a series of items. Please respond yes if you own the item and it is in working form. If you own the item but it is broken or not working, please respond no.			
40	Does your household have a paraffin (kerosene) lamp?	Yes = 01; No = 00	☐☐
41	Does your household have an iron (either charcoal or electric)?	Yes = 01; No = 00	☐☐
42	Does your household have a mattress?	Yes = 01; No = 00	☐☐
43	Does your household have a pressure cooker?	Yes = 01; No = 00	☐☐
44	Does your household have a chair or bench?	Yes = 01; No = 00	☐☐
45	Does your household have a cot or bed?	Yes = 01; No = 00	☐☐
46	Does your household have a sofa?	Yes = 01; No = 00	☐☐
47	Does your household have a cupboard? *Includes cupboards with shutters and open shelves.*	Yes = 01; No = 00	☐☐
48	Does your household have a table?	Yes = 01; No = 00	☐☐
49	Does your household have an electric fan?	Yes = 01; No = 00	☐☐
50	Does your household have a room cooler or air conditioner?	Yes = 01; No = 00	☐☐
51	Does your household have a radio or transistor?	Yes = 01; No = 00	☐☐
52	Does your household have a computer?	Yes = 01; No = 00	☐☐
53	Does your household have a television?	Yes = 01; No = 00	☐☐
54	Does your household have a sewing machine?	Yes = 01; No = 00	☐☐
55	Does your household have a landline telephone?	Yes = 01; No = 00	☐☐
56	Does your household have a mobile telephone?	Yes = 01; No = 00	☐☐
57	Does your household have a refrigerator?	Yes = 01; No = 00	☐☐
58	Does your household have a watch or clock?	Yes = 01; No = 00	☐☐
59	Does your household have a bicycle?	Yes = 01; No = 00	☐☐
60	Does your household have a mototaxi?	Yes = 01; No = 00	☐☐
61	Does your household have an animal-drawn cart?	Yes = 01; No = 00	☐☐
62	Does your household have a tricycle cart?	Yes = 01; No = 00	☐☐
63	Does your household have a motorcycle?	Yes = 01; No = 00	☐☐
64	Does your household have a car or truck?	Yes = 01; No = 00	☐☐
65	Does your household have a boat with a motor?	Yes = 01; No = 00	☐☐

66	Does any member of your household own another form of transportation?	Yes = 01; No = 00	☐☐
67	Does your household have any agricultural tools, such as a thresher, plow, or tractor?	Yes = 01; No = 00	☐☐
68	Does your household have a washing machine?	Yes = 01; No = 00	☐☐
69	Does your household have a camera? *This does not include cell phones with cameras.*	Yes = 01; No = 00	☐☐
70	Does any member of your household own a bank account?	Yes = 01; No = 00	☐☐
71	Does your household have an electric water pump?	Yes = 01; No = 00	☐☐
Which of the following fuels does your household use for cooking? *(can pick more than one)*			
72	Electricity?	Yes = 01; No = 00	☐☐
73	Solar power?	Yes = 01 No = 00	☐☐
74	LPG/Natural gas?	Yes = 01 No = 00	☐☐
75	Biogas?	Yes = 01 No = 00	☐☐
76	Kerosene?	Yes = 01 No = 00	☐☐
77	Coal/lignite?	Yes = 01 No = 00	☐☐
78	Charcoal?	Yes = 01 No = 00	☐☐
79	Wood?	Yes = 01 No = 00	☐☐
80	Straw/shrubs/grass?	Yes = 01 No = 00	☐☐
81	Agricultural crop?	Yes = 01 No = 00	☐☐
82	Animal dung?	Yes = 01 No = 00	☐☐
83	No food cooked in household	Yes = 01; No = 00	☐☐
84	Other	Yes = 01; No = 00	☐☐
85	What is the main source of fuel used for cooking in your household?	Electricity = 01; Solar power = 02; LPG/Natural gas = 03; Biogas = 04; Kerosene = 05; Coal/lignite = 06; Charcoal = 07; Wood = 08; Straw/shrubs/grass = 09; Agricultural crop = 10; Animal dung= 11; No food cooked in household = 12; Other = 13	☐☐
86	Do you ever heat your house? *If no, skip to question 114.*	Yes = 01; No = 00	☐☐
Which of the following fuels do you use to heat your house? *Mark all that apply.*			
87	Electricity?	Yes = 01; No = 00	☐☐
88	LPG/Natural gas?	Yes = 01; No = 00	☐☐
89	Biogas?	Yes = 01; No = 00	☐☐

90	Kerosene?	Yes = 01; No = 00	☐☐
91	Coal/lignite?	Yes = 01; No = 00	☐☐
92	Charcoal?	Yes = 01; No = 00	☐☐
93	Wood?	Yes = 01; No = 00	☐☐
94	Straw/shrubs/grass?	Yes = 01; No = 00	☐☐
95	Agricultural crop?	Yes = 01; No = 00	☐☐
96	Animal dung?	Yes = 01; No = 00	☐☐
97	Do not heat household	Yes = 01; No = 00	☐☐
98	Other	Yes = 01; No = 00	☐☐
99	What is the primary source of fuel used for heating in your household?	Electricity = 01; LPG/Natural gas = 02; Biogas = 03; Kerosene = 04; Coal/lignite = 05; Charcoal = 06; Wood = 07; Straw/shrubs/grass = 08; Agricultural crop = 09; Animal dung=10; No food cooked in household = 11; Other = 12	☐☐
100	What type of cooking stove is mainly used in your house?	Kerosene stove = 01 Gas stove = 02; Open fire = 03 Open fire or stove with chimney or hood = 04 Closed stove with chimney = 05 Electric heaters = 06; Other = 07	☐☐
101	Is cooking done inside the house, outside the house, or both?	Inside the house = 01; Outside the house = 02; Both inside and outside the house = 03	☐☐
102	*Main material of the floor (observation)*	Earth/sand/clay/mud/dung = 01; Wood planks = 02; Parquet or polished wood = 03; Vinyl or asphalt strips = 04; Ceramic tiles = 05; Cement/concrete = 06; Other = 07	☐☐
103	*Main material of the roof (observation)*	No roof = 01 Thatch/palm leaf/reed/grass = 02 Sod/mud and grass mixture = 03 Plastic/polythene sheeting = 04 Rustic mat = 05; Palm/bamboo = 06 Unburnt brick = 07 Loosely packed stone = 08 Metal/GI = 09; Wood = 10 Calamine/cement fiber = 11 Asbestos sheets = 12 RCC/RBC/Cement/Concrete = 13 Roofing shingles = 14; Tiles = 15 Slate = 16; Burnt brick = 17; Other = 18	☐☐
104	*Main material of the exterior walls (observation)*	No walls = 01 Cane/palm/trunks/bamboo = 02 Mud/sand = 03; Bamboo with mud = 04 Stone with mud = 05; Plywood = 06 Cardboard = 07 Raw wood/reused wood = 08	☐☐

		Cement/concrete = 09 Stone with lime/cement = 10; Bricks= 11 Wood planks/shingles = 12 Metal/Asbestos sheets = 13; Other = 14	
105	How many rooms in this household are used for sleeping?	01-10 (rooms)	☐☐
106	Do you have a separate room which is used as a kitchen?	Yes = 01; No = 00	☐☐
107	How many people usually sleep in this household?	01-20 (people)	☐☐
108	Does this household own any agricultural land? *If no, skip to question 124.*	Yes = 01; No = 00	☐☐
109	How much agricultural land does this household own?	00-999 (acres)	☐☐☐
110	Does your household own any livestock?	Yes = 01; No = 00	☐☐
111	Does your household own cows, bulls, or buffaloes?	Yes = 01; No = 00	☐☐
112	Does your household own horses, donkeys, or mules?	Yes = 01; No = 00	☐☐
113	Does your household own any cats?	Yes = 01 ; No = 00	☐☐
114	Does your household own a sheep or goat?	Yes = 01 ; No = 00	☐☐
115	Does your household own any pigs?	Yes = 01 ; No = 00	☐☐
116	Does your household own any dogs?	Yes = 01 ; No = 00	☐☐
117	Does your household own chickens or ducks or other bird?	Yes = 01 ; No = 00	☐☐

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252. Jiang H, Zhang MJ, Liu RC, Tian XY, Gu YX, Zhang JZ. Characteristics of lipo-oligosaccharide loci of *Campylobacter jejuni* isolates associated with Guillain-Barre Syndrome from Hebei, China. Int J Mol Sci. 2010;11(3):1155-61.

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254. Kassem, II, Kehinde O, Kumar A, Rajashekara G. Antimicrobial-Resistant *Campylobacter* in Organically and Conventionally Raised Layer Chickens. Foodborne Pathog Dis. 2017;14(1):29-34.

CURRICULUM VITAE

FRANCESCA SCHIAFFINO SALAZAR

Calle General Borgoño 57, Miraflores, Lima, Peru
fschiaffino@jhu.edu | fschiaffino@gmail.com

EDUCATION

Johns Hopkins Bloomberg School of Public Health **Expected** 2020
PhD in Global Disease Epidemiology and Control, Department of International Health
Certificate in Risk Sciences and Public Policy, Department of Health Policy and Management 2019

Universidad Peruana Cayetano Heredia (UPCH) Lima, Peru 2013
Doctor in Veterinary Medicine (DVM)

San Silvestre School, Lima, Peru 2007
International Baccalaureate Diploma (Bilingual) 2007
International Certificate of Secondary Education (IGCSE's) 2005

PROFESSIONAL & WORK EXPERIENCE

Work

Dept. International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD June 2017 – Present
IRB Research Coordinator for all of Dr. Robert Gilman's studies

International Vaccine Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Data Abstractor, PSERENADE. Supervisor: Kyla Hayford November 2018- Present
Data Abstractor, SISS Project. Supervisor: Kyla Hayford May 2017 – November 2018
Data Abstractor, PRIME Project. Supervisor: Olivia Cohen October 2016 – May 2017

Infectious Diseases Research Laboratory, Universidad Peruana Cayetano Heredia, Lima, Peru July 2014 – Present
Research Assistant
Supervisors: Robert Gilman MD, Maritza Calderón PhD, Manuela Verástegui PhD

Pan American Zoonotic Research and Prevention (PAZ) December 2013 – August 2015
Research Associate
Supervisor: Richard Lerner, DVM, MPH

Groomers Veterinary Clinic, Lima, Peru June – September 2014
Assistant Veterinarian
Veterinary Emergency Medicine
Supervisor: Yanaisa Millares MV

Laboratory of Viral Zoonosis, Instituto Nacional de Salud, Lima, Peru January – June 2014
Professional Rotation
Rabies Diagnosis: Direct Fluorescent Antibody Test, Mouse Inoculation Test, RFFIT, RT-PCR and Sequencing.
Supervisor: Ricardo Lopez, MV, MPVM

Teaching

Faculty of Veterinary Medicine and Animal Management, UPCH, Lima,Peru August 2019 – Present
Adjunct Professor
Veterinary Physiopathology
Veterinary Histology

Department of International Health, JHSPH, Baltimore, MD, USA October 2016 – March 2020
Teaching Assistant
Tackling the Intersectorial Challenge of Antimicrobial Resistance: Problem Solving Seminar
Supervisor: Anthony So, MA
Summer Institute in Tropical Medicine and Public Health
Supervisor: Robert H. Gilman MD, Carlton Evans MD, William Moss MD, David Sullivan PhD
Global Disease Control Programs and Policies
Supervisor: Alain B. Labrique, PhD; Summer Rosenstock, PhD, Anna Kalbarczyk, MPH
Clinical and Epidemiologic Aspects of Tropical Diseases
Supervisor: David Sack, MD; Kawsar Talaat MD.

Environment and Health in Low and Middle Income Countries
Supervisor: Christine Marie George, PhD; Subhra Chakrabortry, PhD
Introduction to International Health
Supervisor: Karen Charron, RN; Anna Kalbarczyk, MPH

International Institute of Field Epidemiology, UPCH, Lima & Tumbes, Peru August 2016
Instructor – Rodent trapping and necropsy
Supervisor: Andres G. Lescano, PhD MHS MHS

Faculty of Veterinary Medicine and Animal Management, UPCH, Lima, Peru September 2014 – July 2015
Academic Tutor March 2015 – July 2015
Teaching Assistant
Avian Pathology March 2015 – July 2015
Large Animal Semiology March 2015 – July 2015
Small Animal Medicine September 2014 – December 2014
Supervisor: Ricardo Grandez, MV, EMAC

Externships

Molecular Biology Laboratory, Faculty of Veterinary Medicine, Universidad Peruana Cayetano Heredia August – September 2013
Supervisor: Armando Hung MV, MSc February - April 2013
Thesis Lab Work

Smithsonian National Zoological Park, Washington DC, USA June - August 2013
Supervisor: Jessica Siegal-Willott, DVM, DACZM
Preceptorship
Extern Project: Case Report: A cerebral tumor in a Nile Hippopotamus. Manuscript in preparation

Zoological Pathology Program, University of Illinois College of Veterinary Medicine, IL, USA May – June 2013
Supervisor: Karen Terio, DVM, PhD, DACVP
Externship
Extern Project: Fort Wayne Children's Zoo Australian Psittacine Cases from 1997 to 2012: A retrospective study

The Marine Mammal Center, Sausalito, CA, USA April – May 2013
Supervisor: Frances Gulland Vet M.B, PhD
Externship (Visitor)

Laboratory of Microbiology, Faculty of Veterinary Medicine and Animal Science, UPCH December – May 2013
Supervisor: Carlos Shiva, MV PhD
Rotation

Laboratory of Preventive Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru November 2012
Supervisor: Dr. Armando Gonzales, DVM PhD February – March 2012
Externship

"El Huayco" Raptor Reproduction and Research Center, Lima, Peru September - October 2012
Supervisor: Lee Schaeffer, Biologist MFC
Externship

Infectious Diseases Research Laboratory, Universidad Peruana Cayetano Heredia, Lima, Peru December - January 2012
Laboratories of Investigation and Development (LID), Universidad Peruana Cayetano Heredia
Supervisor: Dr. Manuela Verástegui, Dr. Robert Gilman MD
Rotation

Cornell University Hospital for Animals, Ithaca, USA July – August 2011
Supervisor: James Morrisey DVM, DABVP (Avian)
Externship in Wildlife and Exotic Animal Service

RESEARCH

"Fecal Contamination and Drug Resistant Campylobacteriosis in the Peruvian Amazon"
2017-2020
Doctoral Dissertation
Supervisors: Dr. Margaret N. Kosek; Pablo Penataro-Yori

Research Project investigating the molecular epidemiology of Campylobacteriosis in the Peruvian Amazon and the molecular determinants of antibiotic resistance. Activities involve sample collection, processing and analysis including techniques such as basic microbiology, qPCR, sequencing and bioinformatic analysis.

"Antibiotic resistance genes exchange between the intestinal microbiota and the community environment of 2014-2015
Pampas, San Juan de Miraflores, Lima, Peru"
Research Assistant
Supervisors: Dr. Robert Gilman MD; Dra. Maritza Calderón PhD; Pedro Tsukayama PhD(c)

Collaborated with Pablo Tsukayama PhD (DANTAS Lab at Washington University in St. Louis) in a study consisting of determining the role of water treatment systems in the dissemination and antibiotic resistant genes in humans and environmental microbial communities using genomics and microbial ecology methods. I was involved in performing regular antibiograms from affluent and effluent sewage samples and the isolation of a variety of coliform bacteria strains such as *Campylobacter*, E. coli, Salmonella and Shiguella for their complete sequencing and resistome analysis.

"Intelligent diagnosis of *Toxoplasma gondii* in immune compromised patients using nanoparticles" 2014-2015
Researcher
Supervisors: Dr. Robert Gilman MD; Dra. Maritza Calderón PhD
Grant awarded by FINCyT, Peruvian Ministry of Production. USD 100,000

Working alongside a multidisciplinary team lead by Dr. Robert H. Gilman head of the Laboratory of Infectious Disease Research at Universidad Peruana Cayetano Heredia, and Professor at Johns Hopkins Bloomberg School of Public Health.
The study involves the standardization of a rapid, easy and sensible diagnostic test using nanoparticles to detect antigens of *Toxoplasma gondii* in immune compromised patients with toxoplasmic encephalitis, given that it is the main cause of encephalitis related deaths in Peru. The potential to impact neglected populations in developing countries is highly valuable.

"Royal College of Veterinary Surgeons TARGET Grant" 2013-2014
Researcher
Supervisors: Richard Lerner DVM, MPH

Target Grant designed to retrospectively evaluate the veterinary caseload in small animal medicine and rank the presenting complaints, diagnosis and interventions by frequency. This will allow a more efficient use of funds and resources for scientific research. This grant was awarded to PAZ, Pan American Zoonotic Research and Prevention, and the target population in Peru was two veterinary health posts at Oasis, Villa El Salvador and Iquitos. Over 2000 cases were evaluated in a 4-month period.

"Presence of *Fusobacterium necrophorum* in feces from free ranging cattle at "El Angolo" game reserve, Piura, Peru" 2012-2013
Co-Researcher – Thesis Dissertation
Supervisor: Roberto Elias MV, DESMAN; Armando Hung MV MSc, Pedro G. Vasquez Ruesta, Ing.

The objective of this investigation was to determine the cause of a white-tailed deer mortality event during 2012 at "El Angolo" game reserve, located in the dry forests of Piura, northern Peru. The main hypothesis was an outbreak of Necrobacillosis from feral cattle in junction with an extreme drought season. Fecal sampling, microbiological assays and conventional PCR were performed. Preventive measures were applied and the population has recuperated gradually since.

"Fort Wayne Childrens Zoo Australian Psittacine Cases from 1997 to 2012: A retrospective study"
2013
Student Research Project
Supervisor: Michael Kinsel, DVM DACVP

Performed a 15-year retrospective study on 212 pathology cases of Australian psittacines at a zoological institution of Illinois. It was determined that the main cause of death was infectious diseases, with bacterial enteritis, tracheitis, mycobacterial diseases and WNV as the main pathologies. This was the research student project performed at the Zoological Pathology Program internship at Chicago, IL.

"Presence of *Cryptococcus neoformans* in feces from urban pigeons located at public parks and plazas in Cercado de 2011-2012
Lima and zonal parks of Lima Met ropolitana, Peru"
Co-Researcher
Supervisor: Roberto Elias, MV, DESMAN

This project involved determining the prescence of Cryptococcus neoformans in feces from urban pigeons from a variety of public parks and plazas in Lima. Itwas performed in collaboration with the Micology Laboratory of the Instituto de Medicina Tropical, Alexander Von Humboldt and the Municipality of Lima. Work involved fecal sampling, seeding and biochemical assays. This disease has public health implications, especially in immune compromised patients.

PUBLICATIONS

Peer-Reviewed Publications

Colston JM, Peñataro Yori P, Moulton LH, Paredes Olortegui M, Kosek PS, Rengifo Trigoso D, Siguas Salas M, Schiaffino F, François R, Fardus-Reid F, Swann JR, Kosek MN. 2019. **Penalized regression models to select biomarkers of environmental enteric dysfunction associated with linear growth acquisition in a Peruvian birth cohort**. PLoS Negl Trop Dis 13(11): e0007851. https://doi.org/10.1371/journal.pntd.0007851

Schiaffino F, Platts-Mills J, Kosek MN. 2019. **A One Health Approach to Prevention, Treatment and Control of Campylobacteriosis.**
Current Opinion in Infectious Diseases. 2019 Oct;32(5):453-460. doi: 10.1097/QCO.0000000000000570

Schiaffino F, Lee GO, Paredes-Olortegui M, Cabrera L, Penataro-Yori P, Gilman RH, Kosek, MN 2018. **Evolution of the Bacillus Calmette-Guerin Scar and Its Association with Birth and Pregnancy Characteristics in a Prospective Cohort of Infants in Iquitos, Peru.** Am J. Perinatol. doi: 10.1055/s-0038-1676614
Schiaffino F, Colston JM, Paredes Olortegui M, Francois R, Pisanic N, Burga R, Penataro Yori P, Kosek MN. 2018. **Antibiotic Resistance of Campylobacter spp. in a Pediatric Cohort Study**. Antimicrob Agents Chemother doi:10.1128/AAC.01911-18.
Steinberg HE, Russo P, Angulo N, Ynocente R, Montoya C, Diestra A, Ferradas C, Schiaffino F, et al. **Toward detection of toxoplasmosis from urine in mice using hydro-gel nanoparticles concentration and parallel reaction monitoring mass spectrometry**. Nanomedicine. 2017;14(2):461-9.
Schiaffino, F: Sander SJ: Pereira ME; Barnes KJ; Walsh T; Murray S. **Short Communication: Cerebellar and Mesencephalon Neoplasia in a Nile Hippopotamus (*Hippopotamus Amphibious*)**. Journal of Zoo and Wild Animal Medicine. 47(4): 1093–1096, 2016.

Conference Presentations

Francesca Schiaffino, Nora Pisanic PhD, Maribel Paredes BSc, Pablo Penataro-Yori RN MPH, Margaret Kosek MD. **Poultry production in the Peruvian Amazon: Implications for Campylobacter infection and control**. Oral Presentation. CHRO 2019. Belfast, Northern Ireland. September 2019.

Francesca Schiaffino, Nora Pisanic, Ruthly Francois, Dixner Renjifo, Mery Siguas, Maribel Paredes Olortegui, Pablo Penataro Yori, Margaret N. Kosek. **Detecting species-specific fecal contamination using microbial source tracking markers: a validation study in the Peruvian Amazon**. Poster Presentation. ASTMH 2018. Chicago, USA, October 2018

F. Schiaffino, D. Renjifo Trigoso, M. Siguas Salas, R. François, N. Pisanic, M. Paredes Olortegui, P. Peñataro Yori, MN. Kosek. **Resistencia antibiótica en cepas de Campylobacter spp. aisladas de pollos de crianza traspatio en Iquitos, Loreto, Peru.** Oral Presenation. National Congress of Veterinary Sciences, Cajamarca, Peru. September 2018.

Francesca Schiaffino Salazar, Maribel Paredes Olortegui, Pablo Penataro Yori, Margaret Kosek. **Antimicrobial Resistance of Campylobacter spp. Isolates in Children: Results from the MAL-ED Cohort Study of Peru**. Poster Presentation. ASM Microbe 2017. New Orleans, USA. June 2017.

Francesca Schiaffino Salazar, Josmel Sevillano, Cusi Ferradas, Noelia Angulo, Margot Faustino, Lilia Cabrera, Maritza Calderón, Pablo Tsukayama, Gautam Dantas, Robert H. Gilman. "**Antibiotic resistance of Enterobacteria in two wastewater treatment plans of two peri-urban communities of Lima, Peru".** Poster Presentation. ASTMH 2015. Philadelphia, USA. October 2015.

Francesca Schiaffino, Janet Acosta, Edith Malaga, Edith Arocutipa, Lenny Sanchez, Luz Agueda Perez, Cusi Ferradas, Noelia Angulo, Jaeson Calla, Mary Kolb, Lilia Cabrera, Robert Gilman, Maritza Calderón**. "Serologic and Molecular Diagnosis of Toxoplasma gondii in domestic cats (Felis catus) of Villa El Salvador, Lima, Peru".** Poster Presentation. XXIV Congress of the Brazilian Society of Parasitology (SBP) and XXIII Latin American Congress of Parasitology (FLAP), Salvador, Brazil. October 2015.

Francesca Schiaffino Salazar, Pedro G. Vasquez Ruesta, Carlos Shiva Rayamoni, Luis Miguel Jara Salazar, Roberto Elias Piperis. **"White Tailed Deer (*Odocoileus Virginianus Peruvianus*) Mortality in a game reserve in Peru. Was the Cow to Blame?".** Poster Presentation. International Conference of the Wildlife Disease Association 2014, New Mexico, USA. August 2014.

HONORS AND AWARDS

UJMT Fogarty Global Health Post-Doctoral Fellowship 2018-2019
Fogarty International Center

Dr. Henry K. and Lola Beye Scholarship
2017
Department of International Health, Johns Hopkins Bloomberg School of Public Health

Center for a Livable Future - Lerner Fellowship 2016-
2017
Johns Hopkins Bloomberg School of Public Health

"International Doctoral Scholarships 2015-I" 2015-
2020
Consejo Nacional de Ciencia y Tecnologia (CONCYTEC), Peru
Full Scholarship awarded to complete doctoral studies at Johns Hopkins Bloomberg School of Public Health

Honors Alumni, 2008-2013: Class Rank - 2nd from a cohort of 50. Dissertation approved with Excellence
2013
Faculty of Veterinary Medicine and Animal Management, Universidad Peruana Cayetano Heredia

Alumni Spokesperson – Faculty Council 2010-
2011
Faculty of Veterinary Medicine and Animal Management, Universidad Peruana Cayetano Heredia

Competitive Fund Award Winner -2000 $US Grant to perform a research study in *Cryptococcus neoformans*
2011
Universidad Peruana Cayetano Heredia

San Silvestre School
2007
Honor Alumni, 2001- 2007: Class Rank - 8th from a cohort of 96
Science Award Winner

PROFESSIONAL ORGANIZATIONS

American Society for Microbiology 2017 –
Present
Student Member

American Society of Tropical Medicine and Hygiene 2015 -
Present
Student Member

Peruvian College of Veterinary Medicine 2014 -
Present
CMVP Nº 8963
Professional / Active Member

Wildlife Disease Association 2012 -
2015
Professional Member 2014 -
2015
Student Member 2012 – 2015,
2017
Student Representative Candidate for the Latin American Section (2011-2013 period)

STUDENT ORGANIZATIONS

International Health Student Group – Johns Hopkins Bloomberg School of Public Health September 2015 – May
2016
Co-chair of Academic Affairs

Conservation Medicine Group – Universidad Peruana Cayetano Heredia Mar 2010 -
Present
Co-Founder

CONTINUING EDUCATION

Short Courses

R for STATA users July 2016
Universidad Peruana Cayetano Heredia, Lima, Peru

International Institute of Field Epidemiology January 2016
Universidad Peruana Cayetano Heredia, Lima; Centro de Salud Global, Tumbes, Peru

Biostatistics (4 credits) April – August, 2015
Postgraduate School, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru

Data Science Specialization (currently completed first four courses) January –April, 2015
Johns Hopkins Bloomberg School of Public Health – by Coursera

Phylogenetics and Bioinformatics Sequence Analysis Training January 2015
Universidad Peruana Cayetano Heredia & NAMRU-6, Lima, Peru
Scholarship awarded

Course / Workshop – Multidisciplinary Approach to Emerging Infectious Diseases: April 2014
From Molecular Biology to National Epidemiologic Surveillance
Universidad Peruana Cayetano Heredia & Instituto Nacional de Salud Pública (México), Lima, Perú
Scholarship Awarded

Managing Infectious Diseases in Conservation Programs October 2013
Durrell Conservation Academy, Durrell Wildlife Conservation Trust, Jersey, UK.
Scholarship Awarded.

1st Field Course: Wildlife Management and Indigenous Communities in the Amazon March 2013
Pucacuro National Reserve, Loreto, Peru - Yavacus & SERNANP
Scholarship Awarded

Regression Models Applied to Research, STATA (v.12) November – December 2012
Universidad Peruana Cayetano Heredia
Faculty of Science and Philosophy, Department of Statistics, Demography, Humanities and Social Sciences

Statistical Analysis with STATA (v.10) July - August 2012
Universidad Peruana Cayetano Heredia
Faculty of Science and Philosophy, Department of Statistics, Demography, Humanities and Social Sciences

Actualization on the Anatomy and Histology of the Ruminant Abdomen May – June 2011
Universidad Peruana Cayetano Heredia, Lima, Peru

Imaging Diagnosis in Companion Animals January – February 2011
Universidad Peruana Cayetano Heredia, Lima, Peru

1st International Course of Wildlife Medicine and Management: Anesthesiology and Immobilization October 2010
CIVEFAS, Universidad Nacional Mayor de San Marcos, Lima, Peru
Claudio Soto, DVM, MSc, PhD (c)

LANGUAGES

Spanish (Native proficiency)
English (Bilingual proficiency)
- Bilingual International Baccalaureate Diploma. Higher level English Literature score: 6/7 (November 2007)
- **TOEFL Score: 113/120** (March 2014)

French (Elementary – conversational proficiency)