Socio-Ecological Risk Factors for *Campylobacter* Infection in Rural Malagasy Children

Marisa Wong
MPH Candidate, Environmental Health

Capstone Mentors: Thomas R. Gillespie, PhD and Stephen G. Mugel, MS

Emory University
Department of Environmental Sciences &
Gangarosa Department of Environmental Health

Acknowledgements

I would like to express my sincerest gratitude to both Dr. Gillespie and Stephen for their invaluable support and encouragement. I am so appreciative of Dr. Gillespie for trusting me with his research, and I am so grateful for all of Stephen's willingness to share his knowledge and expertise in academic research and epidemiology.

I would also like to thank my classmates and cohort members, especially those in my capstone accountability group, for their review and reassurance.

Lastly, I want to recognize my partner, Tim, for all his unconditional support throughout graduate school which ranged from debugging my code to listening to me rehearse presentations to working late nights with me while I finished my schoolwork. His belief in me has pushed me to excel and pursue my dreams. I would also like to thank my two cats, Abby and Steve, for their never-ending comfort and companionship through my graduate education.

Table of Contents

1. Abstract

Diarrheal disease is a major cause of global morbidity and mortality in low and middle income countries, and one of the leading causes of enteric disease is *Campylobacter* infections. *Campylobacter* is commonly transmitted to humans through the ingestion of contaminated food and water; however, further evidence suggests animal encounters may play a substantial role in transmitting the pathogen. This project utilizes the dataset from Dr. Thomas Gillespie's 2017 Eco-Epidemiology of Diarrheal Disease study of rural Madagascar villages to estimate the associations between three household level ecological exposures (ownership of a sick domestic animal, contact with a wild lemur, and ingestion of wildlife meat) and *Campylobacter* infection in children five years of age and younger. This was accomplished by applying epidemiologic tools including a literature review to identify relevant risk factors, the creation of a directed acyclic graph to display the relationship between all included variables, and the generation of three generalized linear mixed effects models to estimate three distinct prevalence ratios. Overall, 168 children were included in the analysis, where 45% tested positive for *Campylobacter* infection. The prevalence of *Campylobacter* infection was positively associated with ownership of sick domestic animals (PR: 2.6, 95% CI: 0.96-7.04) and ingestion of wildlife meat (PR: 1.16, 95% CI: 0.19-6.93), though not statistically significant. The prevalence of *Campylobacter* infection was not associated with contact with wild lemurs (PR: 0.98, 95% CI: 0.39-2.47). These findings suggest that in resource limited settings, interventions should be directed at caring for animal health as a preventive measure for enteric diseases in humans. This work also calls on researchers to continue investigating the links between animal encounters and diarrheal diseases. The efforts of Centre ValBio and Pivot in serving rural Madagascar are recognized and shall be continually supported in improving health in Malagasy villages. The One

Health approach shows promise in applying expertise from human, animal, and environmental health specialists in collaboration towards the elimination of *Campylobacter* in rural Madagascar.

2. Introduction

2.1 Background

Although remarkable improvements have been made to address the global burden of diarrheal diseases, diarrhea remains a concerning health outcome that requires additional scientific research and intervention measures in low- and middle-income countries. In 2016, approximately 1.6 million deaths were attributed to enteric diseases[1]. Children are particularly susceptible to negative health outcomes caused by diarrheal disease. Children two years of age and younger are most vulnerable to serious enteric disease due to the body's underdeveloped response system and due to the risk of physical stunting and stagnant mental development[2]. In 2010, diarrhea accounted for over 750,000 global deaths in children under five years of age[3]. With children at-risk in resource-limited settings, public health researchers need to focus their efforts on identifying etiologies, understanding routes of transmission, and intervening on the global burden of enteric diseases.

Campylobacter is one of the leading causes of global diarrheal and enteric diseases. The primary, non-specific symptoms of *Campylobacter* infection include varying forms of diarrhea, abdominal cramping, and fever[4]. In severe cases, the globally dominant strain causing *Campylobacter*, *C. jejuni*, can be associated with the development of autoimmune diseases and colorectal cancer[5]. Human infection from *Campylobacter* typically occurs through the ingestion of food, animal products, or drinking water contaminated with the bacteria[6]. Historically, *Campylobacter* was considered an animal disease, as it caused diarrhea in cattle and sheep[4].

More recently, *Campylobacter* has been found in the digestive tracks of chickens[6]. With the potential of infection through the fecal-oral route and confirmed identification in domestic animals and humans, campylobacteriosis is considered both a foodborne illness and a zoonotic disease.

Epidemiologic data confirms that *Campylobacter* remains a persistent, global issue. In many developed countries, *Campylobacter* cases have risen in recent years, whereas underdeveloped countries retain endemic rates of infection[5]. With limited healthcare infrastructure capacity and predominantly rural populations, Madagascar endures a high burden of diarrhea, attributing to approximately 11% of total deaths and approximately 23% of deaths for children under five years of age[7]. In a study conducted in Moramanga, Madagascar, *Campylobacter* was found in 9.3% of the stool samples collected[8]. Rural Malagasy people are particularly vulnerable to diarrheal diseases and *Campylobacter* infection due to cultural beliefs[9], disdain for latrine use, and lack of available resources to diagnose (and therefore treat) the specific pathogens causing diarrhea[10]. The distant location proximal to forestry, lack of adequate healthcare, and household hygiene practices in villages surrounding Madagascar's Ranomafana National Park put families at an increased risk of infection from *Campylobacter* and risk of developing of diarrheal disease.

The Centre ValBio (CVB) serves as a research center that is housed in Ranomafana, Madagascar and is overseen by Stony Brook University. From its conception, CVB established and implemented a mobile health team that has been facilitating health care services to communities and villages bordering the Ranomafana forest[11]. In conjunction with CVB's work, Pivot acts as a non-governmental organization aimed at improving the Ifanadiana District's public health system. Pivot created a regional model that includes a hospital and multiple health

and community centers trained in rural health with the goal of providing universal health coverage in Ifanadiana, Madagascar[12]. Pivot partners with the local government and village leaders to institute their model while also building capacity to respond to Ifanadiana District's maternal and child mortality rates. Even with organizations like CVB and PIVOT making great strides in improving healthcare and health outcomes in rural Madagascar, *Campylobacter* infection remains a concern for Malagasy children five years of age and younger.

2.2 Project Objectives

Biological and physiological transmission of *Campylobacter* from animal to human hosts through food consumption are well studied; however, few studies analyze this transmission through epidemiologic modeling principles. Epidemiologic methods use a vast toolkit of statistical analyses to determine measures of frequency and measures of association between variables, which are often not employed by disease ecologists. Thus, this project will serve as an example of applying an epidemiologic modeling strategy to a cross-sectional dataset using a set of well-defined variables with the goal of elucidating risk factors associated with *Campylobacter* infection in rural Madagascar. To achieve this, my project will address the following aims:

1. Conduct a literature review to understand certain vulnerabilities to ecologic-associated diarrheal diseases and to determine relevant risk factors associated with enteric infections (Table 1).

2. Develop a Directed Acyclic Graph (DAG) to display a qualitative representation of identified variables between exposure and outcome (Figure 1).

3. Formulate three Generalized Linear Mixed Effects Models to estimate the prevalence ratio between exposures of sick domestic animal ownership, encounters with wild lemurs, and ingestion of wildlife meat with *Campylobacter* infection (Table 3).

4. Produce a report that documents the analysis conducted, an interpretation of the results, and future implications of these findings that is to be shared with the research team, Principal Investigator, CVB, and Pivot (Appendix B).

The results of this work should add knowledge to the field of disease ecology regarding the association of animal encounters on *Campylobacter* infection, inform public health intervention strategies for Madagascar's Ministry of Public Health in collaboration with CVB and PIVOT, and advocate for the incorporation of One Health collaboration for *Campylobacter* and enteric disease control in Madagascar.

3. Methods

3.1 Study Design & Data Collection

In 2017, Dr. Thomas Gillespie, his research team, and partners at the CVB conducted a population-wide, cross-sectional study to determine the risks associated with diarrheal disease in the Ifanadiana District of Madagascar, called The Ecology and Epidemiology of Diarrheal Disease in Rural Madagascar (Eco-Epidemiology of Diarrheal Disease). This study consisted of a comprehensive demographic survey questionnaire, human fecal sample collection, and domestic animal fecal sample collection, which were all administered by the CVB's mobile health team. DNA was extracted from human and animal fecal samples through bead-beating, purified through the use of QiaAmp DNA Stool Kit, and quantified with Nanodrop which screened for target enteric pathogens (i.e., *Shigella flexneri*, *Salmonella*, *Campylobacter*, or *Entamoeba histolytica*) via Polymerase Chain Reaction (PCR). Diagnostic results were then considered in relation to survey data which was coded in REDCap. Over the course of six months, the CVB team visited 20 villages, collected information from 211 households, and gathered data pertaining to 978 individuals. Of the participants from the Eco-Epidemiology of

Diarrheal Disease study, there were 168 children five years of age and younger were represented from 8 villages and 116 households.

3.2 Literature Retrieval and Review

To address the first aim, a literature review was conducted in December 2022 using the PubMed database to identify risk factors prioritized by disease ecologists and to understand the burden of diarrheal diseases in low- and middle-income countries. Literature searches included a combination of terms found in Table 1. A total of 9,115 peer-reviewed journal articles resulted from this search, and 17 of these publications were relevant to this project in determining potential covariates for statistical analyses. Articles were assessed for their relevance in title, abstract, and methods screening. Articles were excluded if they did not perform regression modeling and if the context of enteric diseases occurred in an industrial food processing setting. Articles that were included provided a basis for variable specification, focused on a low-and-middle income country, and assessed an enteric or zoonotic disease. Pertinent articles were organized in an Excel document, where the publication title, author, study location, outcome, exposure(s), confounder(s), effect modifier(s), and other information were documented. Many ecological studies did not differentiate between exposure(s), confounder(s), and effect modifier(s). Instead, these studies categorized these factors as potential exposure(s) and utilized a cutoff value of $p < 0.25$ when using stepwise identification of factors included in the analysis.

Table 1. Literature Search Combinations and Results.

Database	Search String	Results
PubMed	(((("Zoonoses"[Mesh]) AND "Madagascar"[Mesh]) AND "Epidemiological Models"[Mesh])	Zero articles
PubMed	(("Zoonoses"[Mesh]) AND "Epidemiological Models"[Mesh]) OR "Zoonoses/epidemiology"[MeSH]	4,846 articles
PubMed	(("Zoonoses"[Mesh]) OR "Bacterial Zoonoses"[Mesh]) AND "Regression Analysis"[Mesh]	157 articles
PubMed	(((("Zoonoses"[Mesh]) OR "Bacterial Zoonoses"[Mesh]) OR "Stomach Diseases"[Mesh]) AND "Regression Analysis"[Mesh]	3,871 articles

PubMed	((((("Zoonoses"[Mesh]) OR "Bacterial Zoonoses"[Mesh]) OR "Dysentery"[Mesh]) OR "Waterborne Diseases"[Mesh]) AND "Regression Analysis"[Mesh]	241 articles

The compiled list of exposures, confounders, and effect modifiers were utilized in variable specification as part of the modeling process. The most common demographic data used in statistical analyses comprised of age, sex, occupation, education level, location, and household income. Variables related to individual health and behavior consisted of sanitation and hygiene practices, current symptoms, vaccination status, pre-existing health conditions, and symptoms experienced by other household members. Other noteworthy factors relating to zoonotic exposure potential were animal contact, animal ownership, animal vaccination, animal water source, illness amongst agricultural animals, type of meat consumed, animal residence within the home, and use of animal manure. These factors were compared to the available variables in the Eco-Epidemiology of Diarrheal Disease dataset and considered in the generation of a DAG.

3.3 Directed Acyclic Graph (DAG) Generation

A DAG was built in Microsoft PowerPoint using the variables identified from the literature review and variables that were collected as part of the Eco-Epidemiology of Diarrheal Disease survey questionnaire. This DAG provides a visualization of the causal relationship between exposure and outcome while also incorporating confounding variables using subject-matter knowledge and *a priori* assumptions[13]. In addition to visualization, the DAG served to elucidate underlying biases and to represent the results of the literature search. The factors identified from variable specification were denoted as the exposure variables in blue, the outcome variable in red, and the confounding variables in yellow (Figure 1).
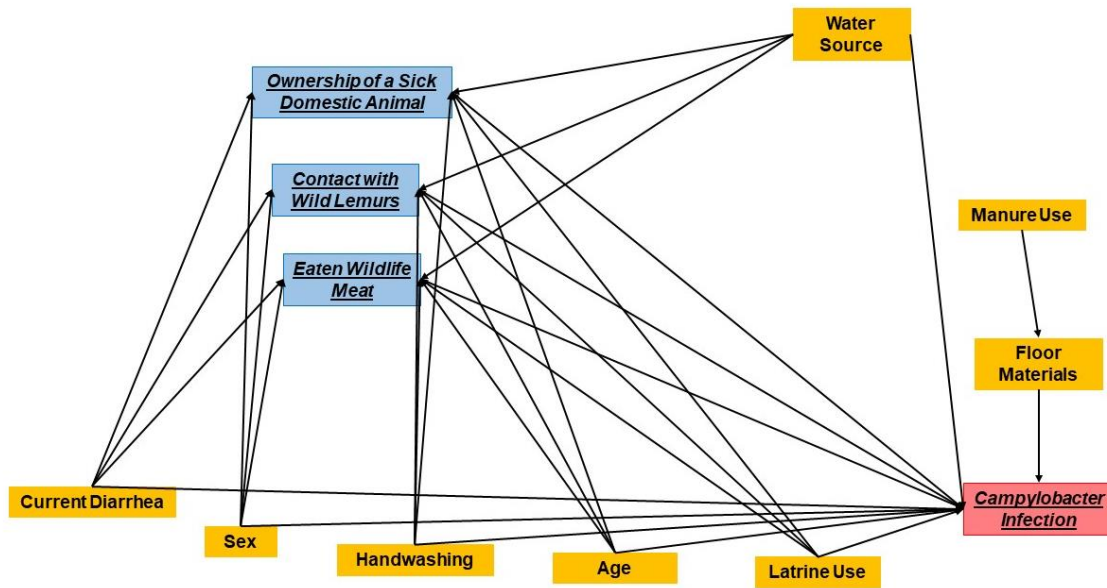
Figure 1. Visual display of the relationship between Ownership of a Sick Domestic Animal, Contact with Wild Lemurs, Eaten Wildlife Meat, and *Campylobacter* Infection.

3.4 Data Management

Next, the data were cleaned and reformatted using RStudio[14]. The original dataset

consisted of 211 observations and 1,080 variables. Most of the variables were data collected

from the survey questionnaire and were primarily categorized in binary form, though some

categorical and continuous variables were also present. The `tidyverse` library was used for

data management[15]. The dataset was presented in a wide format, where each row represented a

household with up to twenty columns for individual level data from that household. First, the

data was pivoted into a long format, allowing each row to now represent an individual and

linking this individual to the household in which they belonged. New columns were created, and

a "for loop" based on column number was implemented to move the individual-level data from

the wide format to the long format. This transformation was applied to seven demographic

variables, three employment and education variables, nine variables describing symptomatic

effects, and five variables related to pathogen testing. Variables of interest were renamed from

11

the question number and survey question to an abbreviated, representative title. Multiple binary

variables surrounding the same topic were collapsed into three respective nominal variables.

Variables of interest with "Yes" or "No" outcomes were recoded with one representing yes and

zero representing no. Since *Campylobacter* infections were only tested in children 5 years of age

and younger, the dataset was filtered to only include participants who reported their age in this

range.

3.5 Epidemiologic Modeling & Statistical Analyses

     To address my third aim, the variables of interest from this dataset were modeled in a

generalized linear mixed effects regression using RStudio. Following the same modeling

process, three models were created, one for each exposure of interest: ownership of a sick

domestic animal, contact with wild lemurs, and ingestion of wildlife meat. The models were built

using the Generalized Linear Mixed Models (`glmer`) function to assign all variables while

considering the random effect of household clustering, represented by household identification

number[16]. Village level clustering was also considered; however, the inclusion of this variable

hindered the model's convergence. The `tidy` function from the `broom.mixed` package

exponentiated estimates and provided confidence intervals[17]. Then, a collinearity assessment

using the Variance Inflation Factor (`VIF`) function identified highly-correlated variables with

each other[18]. The correlation threshold for VIF was set to five, meaning the model presents a

multicollinearity issue for variables showcasing VIF greater than five. Any variables identified

as causing this issue were singularly dropped while the model was refit until no collinearity

issues were present. No interaction terms were considered for any of the three models.

     The models that emerged from the collinearity assessment were considered the Gold

Standard model for my research question. Confounding was assessed for sex, age, current

diarrhea, and latrine use (defined as no latrine use, latrine use, or shared latrine use) for all three models. A confounding assessment was conducted on the Gold Standard Model, which identified any confounding variable or combination of variables that altered the prevalence ratio (PR) more than 10% compared to the Gold Standard Model's PR. Variables that were deemed as confounding factors remained in the model, while variables that did not have a confounding effect were dropped. Adjusted PRs were obtained for the finalized version of the model. This process was repeated for the three exposure variables and their respective covariates.

Although type of water source emerged as a variable of interest from the literature review and DAG, there was no variation in the data collected for this variable. All children included in this study reported that their households source water from nearby rivers or streams, which is defined as an unimproved water source. In addition, the observance of a handwashing area was also considered, but due to the lack of variability, the inclusion of this variable prevented the models from converging.

4. Results

4.1 Descriptive Statistics

In the Eco-Epidemiology of Diarrheal Disease dataset, 168 children five years old and younger participated in the survey response and fecal sampling. The average age of children in this study was about 3 years old. These children accounted for 17% of study participants from the original dataset. Approximately 45% of children (75 out of 168) tested positive for *Campylobacter* infection; however, only 6.5% of children (11 out of 168) were currently experiencing diarrhea. These children belonged to 30.3% of households involved in this study. Less than one quarter of children (23.2%) reported that their household owned a sick domestic

animal, while 14.9% reported household level contact with wild lemurs and 3.6% reported

household level ingestion of wildlife meat in the last four weeks (Table 2).

Table 2. Participant Characteristics from the Eco-Epidemiology of Diarrheal Disease Study (2017), Stratified by Ownership of a Sick Domestic Animal.

	Owns a Sick Domestic Animal (N=39)	Does Not Own a Sick Domestic Animal (N=89)	Missing (N=40)	Overall (N=168)
Campylobacter PCR Result				
Positive	25 (64.1%)	34 (38.2%)	16 (40.0%)	75 (44.6%)
Negative	14 (35.9%)	55 (61.8%)	24 (60.0%)	93 (55.4%)
Age				
Mean (SD)	2.98 (1.34)	3.09 (1.50)	3.18 (1.24)	3.09 (1.40)
Median [Min, Max]	3.00 [0.500, 5.00]	3.00 [0.300, 5.00]	3.00 [1.00, 5.00]	3.00 [0.300, 5.00]
Sex				
Female	20 (51.3%)	44 (49.4%)	19 (47.5%)	83 (49.4%)
Male	18 (46.2%)	45 (50.6%)	21 (52.5%)	84 (50.0%)
Missing	1 (2.6%)	0 (0%)	0 (0%)	1 (0.6%)
Available Water Source				
Unimproved	39 (100%)	89 (100%)	40 (100%)	168 (100%)
Latrine Use				
No Latrine Use	14 (35.9%)	49 (55.1%)	24 (60.0%)	87 (51.8%)
Latrine Use	4 (10.3%)	14 (15.7%)	5 (12.5%)	23 (13.7%)
Shared Latrine Use	21 (53.8%)	26 (29.2%)	11 (27.5%)	58 (34.5%)
Currently Experiencing Diarrhea				
Yes	6 (15.4%)	4 (4.5%)	1 (2.5%)	11 (6.5%)
No	33 (84.6%)	85 (95.5%)	39 (97.5%)	157 (93.5%)
Has access to a Handwashing Area				
Yes	0 (0%)	4 (4.5%)	0 (0%)	4 (2.4%)
No	39 (100%)	85 (95.5%)	40 (100%)	164 (97.6%)
Encountered Wild Lemurs in the last 4 weeks				
Contact with Lemurs	7 (17.9%)	16 (18.0%)	2 (5.0%)	25 (14.9%)
No Contact with Lemurs	32 (82.1%)	68 (76.4%)	37 (92.5%)	137 (81.5%)
Missing	0 (0%)	5 (5.6%)	1 (2.5%)	6 (3.6%)

Table 2. Participant Characteristics from the Eco-Epidemiology of Diarrheal Disease Study (2017), Stratified by Ownership of a Sick Domestic Animal.

	Owns a Sick Domestic Animal (N=39)	Does Not Own a Sick Domestic Animal (N=89)	Missing (N=40)	Overall (N=168)
Eaten Wildlife Meat in the last 4 weeks				
Has Eaten Wildlife Meat	3 (7.7%)	1 (1.1%)	2 (5.0%)	6 (3.6%)
Has Not Eaten Wildlife Meat	36 (92.3%)	83 (93.3%)	38 (95.0%)	157 (93.5%)
Missing	0 (0%)	5 (5.6%)	0 (0%)	5 (3.0%)

Of those children who tested positive for *Campylobacter* infection, the average age was younger than those who tested negative for a *Campylobacter* infection (2.7 vs. 3.4 years old). More female children tested positive for *Campylobacter* infection, while more male children tested negative. Eighty-seven children reported not using the latrine; however, only thirty-four children showed a positive *Campylobacter* isolation. The four children that lived in households with an observed handwashing area were all negative for a *Campylobacter* infection (Appendix A).

4.2 Epidemiologic Modeling & Statistical Analyses

Three generalized linear mixed effects models were formulated to estimate the PR between each of the exposure variables and *Campylobacter* infection. Models focused on children who used unimproved water sources, defined as unprotected wells and springs, untreated surface water, vendor-provided water, and bottled water[19]. Most of these children did not have access to handwashing areas.

One hundred and twenty-eight non-missing observations were used in building model one, which assessed the association between household ownership of a sick domestic animal and *Campylobacter* infection. Thirty-nine (23.2%) children reported their household owning a sick

animal (Table 3). Children who lived in households that own sick domestic animals had 2.6 times the prevalence of a positive *Campylobacter* infection in their stool sample compared to children who live in households that did not own sick domestic animals, controlling for sex, age, current diarrhea, and latrine use (95% CI: 0.96 – 7.04). Although this result is not statistically significant, living in a household that owned sick domestic animals was positively associated with *Campylobacter* infection.

One hundred and sixty-two non-missing observations were used in building model two, which assessed the relationship between lemur contact and *Campylobacter* prevalence. Twenty-five or 14.9% of children reported someone in their household encountering a lemur in the last four weeks (Table 3). Children who lived in households where an individual encountered a wild lemur in the last four weeks had 0.98 times the prevalence of a positive *Campylobacter* infection compared to children who live in households without any wild lemur contact in the past four weeks, controlling for sex, current diarrhea, and latrine use (95% CI: 0.35 – 2.76). Although this result is not statistically significant, living in a household where a member had contact with a wild lemur in the last four weeks was not associated with *Campylobacter* infection.

One hundred and sixty-three non-missing observations were used in building model three, which assessed the relationship between eating wildlife meat and *Campylobacter* infection. Only 3.6% of children reported someone in their household consumed wildlife meat in the last four weeks (Table 3). Children who lived in households where an individual consumed wildlife meat in the last four weeks had 1.16 times the prevalence of *Campylobacter* in their stool compared to children who lived in households where no individuals consumed wildlife meat in the last four weeks, controlling for sex, age, current diarrhea, and latrine use (95% CI: 0.19 – 6.93). Although this result was not statistically significant, living in a household where a

16

member had eaten wildlife meat in the last four weeks was positively associated with

Campylobacter infection.

Table 3. Adjusted Prevalence Ratios of Ecological Exposures among Children under 5 years old and younger in the Eco-Epidemiology of Diarrheal Disease Study (2017).

Exposure	Exposed N (%)	Adjusted Prevalence Ratio	95% Confidence Interval
Ownership of Sick Domestic Animals	39 (23.2)	2.60	(0.96 – 7.04)
Encountered Wild Lemurs in the last 4 weeks	25 (14.9)	0.98	(0.39 – 2.47)
Ate Wildlife Meat in the last 4 weeks	6 (3.6)	1.16	(0.19 – 6.93)

5. Discussion

5.1 Research Questions and Objectives

This work aimed to estimate the association between ownership of a sick domestic animal, contact with a wild lemur, and eating wildlife meat with *Campylobacter* infection amongst children five years of age and younger in rural Madagascar. To accomplish this, a review of PubMed's peer-reviewed literature that involved modeling a zoonotic disease was conducted, a DAG was developed in response to the variables collected from the literature review, and three distinct generalized linear mixed effects model were constructed. While I hypothesized that all three exposures would be positively associated with *Campylobacter* infection, this analysis found ownership of a sick domestic animal and eating wildlife meat in the last four weeks to be positively associated, while encountering a lemur appeared to be unassociated.

5.2 Context of Findings in Published Literature

The results of this analysis add to the vast knowledge of *Campylobacter* transmission among households practicing animal husbandry. Although this analysis did not specify exposure to animal species, it is well known that *Campylobacter* is found in agricultural animals and pets[20]. In low- and middle-income countries, *Campylobacter* is particularly persistent in poultry and can

be transmitted to humans via caretaking and cleaning activities. A Peruvian study found that the rate of *Campylobacter*-induced diarrhea among children living in households exposed to free-roaming chickens was higher than the rate of children not exposed[21]. Exposures to sick domestic animals are further supported as a risk factor in developing *Campylobacter* infection. A study in Egypt found that households with *Campylobacter* infected chickens had almost 4 times higher odds of their children testing positive for *Campylobacter*[22]. Other meta-analyses and systematic reviews support the idea of *Campylobacter* transmission occurring from children who are exposed to animal fecal matter[20]. The scientific community studying the ecological origins of *Campylobacter* infection has strong evidence linking the ownership of animals, both sick and healthy, to childhood diarrheal diseases.

The inclusion of social and behavioral factors contributing to *Campylobacter* infection is a common practice amongst other published literature. Other studies confirmed that social factors (such as age and sex) and history of animal contact prove to be strong predictors of enteric diseases[23]. However, some studies emphasized the health status and illness in poultry, rather than a focus on social and behavioral variables, as significant predictors of campylobacteriosis[24]. Other publications utilized different study designs to estimate associations between animal-related factors and diarrheal diseases and highlighted more specific animal-related behaviors occurring in the household (such as animal presence in the cooking area, use of animal excreta, animal sleeping areas)[25]. Although relevant variables differ amongst each of these studies, additional research with thorough and complete data is still needed to determine the effect of the many diverse social factors and animal encounters on *Campylobacter* infection.

While the ownership of sick domestic animals is well studied in *Campylobacter* transmission, contact with wild animals and ingestion of wildlife meat as primary risk factors are

18

not. However, studies focused on other zoonotic pathogens have investigated this relationship. Specifically, Bratcher et al. found that contact with other non-human primates act as a protective feature against the development of zoonotic Ebola and ingestion of non-human primate meat reduced the odds of Ebola amongst populations in the Democratic Republic of the Congo[26]. This lack of research on wild animal contact and ingestion of wildlife meat calls on the scientific community to continue studying their relationship with other enteric diseases.

5.3 Strengths and Weaknesses

This project featured a wide range of strengths, from novel methodologies to research in underserved populations. This work provided a great opportunity to apply an epidemiologic modeling strategy to a real-world dataset that included social factors and ecological encounters as they relate to *Campylobacter* infection. Underserved populations living at the border of the Ranomafana forest in rural Madagascar were highlighted in this analysis, and this work encourages continued research efforts in understanding the unique health-related risks and outcomes that tropical forest-associated communities endure through culturally appropriate and respectful methods. Although this study cannot determine temporality between ecological encounters and *Campylobacter*, prospective studies can utilize this background knowledge to inform their collection of temporal risk factors in a succinct and well-defined manner.

While this work followed robust, rigorous, and methodical epidemiological analyses, there were limitations. Due to the cross-sectional nature of the original dataset, the results of this project can only estimate and interpret the difference in prevalence of *Campylobacter* at the time of data collection. Thus, we cannot determine population-wide causal inferences nor estimate the prevalence ratio from any other period using this dataset. While these findings are applicable to the villages studied in 2017, they cannot be generalized to populations outside of these rural

communities. Some social and behavioral factors lacked diversity amongst responses and were removed from analysis when other publications showed clear evidence of a confounding effect when controlling these variables. Although the sample sizes varied for the different exposures and their corresponding models, the limited number of observations contributes to the lack of diversity amongst social and behavioral practices and contributes to the wide confidence intervals produced. The lack of statistical significance tied to the estimated prevalence ratios provides an opportunity to study the social and ecological risk factors of *Campylobacter* in Madagascar and other predominantly rural areas.

5.4 Next Steps

Some of the limitations identified in this project call for additional research in distinct areas related to *Campylobacter* infection in rural Madagascar, specifically investigating all ages of *Campylobacter* infection, including individual level exposures, and exploring asymptomatic *Campylobacter* infections. Although it is clear that children are uniquely vulnerable to *Campylobacter*, studies lack the inclusion of adults. Adults as caretakers of sick children and sick animals introduce the opportunity for pathogens to spread. People of all ages are at-risk of *Campylobacter* infection and should be included in future analyses. This research analyzed the exposures at the household level; however, individual level exposures are also important to study. Household level exposures introduce potential recall biases and overgeneralization to all household members. By investigating individual level exposures, researchers may reduce potential biases and gain a better understanding of the direct relationship between ecological exposure and diarrheal disease outcome. Lastly, asymptomatic *Campylobacter* infection was common amongst participants in this study. If rural communities experience high rates of asymptomatic infections, then symptomatic screening would be insufficient in detecting enteric pathogens. If this pattern

holds true for animals, then identifying sick animals may not be useful in preventing the spread of *Campylobacter*. The etiology behind asymptomatic *Campylobacter* infection needs to be better understood to prevent uninfected individuals from experiencing severe outcomes. With immense gains in understanding *Campylobacter* transmission, more opportunities present themselves to research niche areas related to *Campylobacter*.

5.5 Recommendations

With *Campylobacter* remaining a prevalent cause of diarrheal diseases, innovative measures for controlling and eliminating *Campylobacter* are needed, especially for those living in rural areas that lack access to healthcare resources. An innovative approach that has shown great promise is the implementation of One Health collaborations between human, animal, and environmental health experts in active surveillance of diarrheal disease outbreaks. Through connections, awareness, and collaboration, the One Health approach has potential to prevent major disease outbreaks of zoonotic origin while protecting human and animal health[27].

I recommend Madagascar's Ministry of Health enlist the expertise of animal and environmental health workers when developing surveillance strategies or interventions for its rural communities, while also continuing to support the work of non-governmental organization partners. CVB and Pivot's trusted relationships with and passion for work in Malagasy villages provide a prime opportunity for collaboration with the Ministry of Health for incorporation of effective control measures. This work draws attention to the lack of safe water, sanitation, and hygiene resources available for rural communities. This work also provides evidence that ownership of sick domestic animals is more associated to diseases in humans compared to contact with wild lemurs or ingestion of wildlife meat. Thus, in resource limited settings, interventions

focused on caring for the health of animals should be prioritized as it protects the human health

and can greatly improve the quality of life amongst communities in rural Madagascar.

References

1. Troeger C, Blacker BF, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases*. 2018;18(11):1211-1228. doi:10.1016/S1473-3099(18)30362-1

2. Mokomane M, Kasvosve I, de Melo E, Pernica JM, Goldfarb DM. The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. *Ther Adv Infect Dis*. 2018;5(1):29-43. doi:10.1177/2049936117744429

3. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet*. 2012;379(9832):2151-2161. doi:10.1016/S0140-6736(12)60560-1

4. Moore JE, Corcoran D, Dooley JSG, et al. *Campylobacter*. *Vet Res*. 2005;36(3):351-382. doi:10.1051/vetres:2005012

5. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global Epidemiology of Campylobacter Infection. *Clinical Microbiology Reviews*. 2015;28(3):687-720. doi:10.1128/CMR.00006-15

6. Young KT, Davis LM, DiRita VJ. Campylobacter jejuni: molecular biology and pathogenesis. *Nat Rev Microbiol*. 2007;5(9):665-679. doi:10.1038/nrmicro1718

7. GBD Compare | IHME Viz Hub. Accessed September 25, 2022. http://vizhub.healthdata.org/gbd-compare

8. Randremanana RV, Randrianirina F, Sabatier P, et al. Campylobacter infection in a cohort of rural children in Moramanga, Madagascar. *BMC Infect Dis*. 2014;14:372. doi:10.1186/1471-2334-14-372

9. Project B. The Fatal 4: Top Diseases in Madagascar. The Borgen Project. Published August 15, 2016. Accessed September 25, 2022. https://borgenproject.org/the-fatal-4-top-diseases-in-madagascar/

10. Randremanana R, Randrianirina F, Gousseff M, et al. Case-Control Study of the Etiology of Infant Diarrheal Disease in 14 Districts in Madagascar. *PLOS ONE*. 2012;7(9):e44533. doi:10.1371/journal.pone.0044533

11. History | Centre ValBio. Accessed April 7, 2023. https://www.stonybrook.edu/commcms/centre-valbio/valbio/history.php

12. Pivot | PIVOT HEALTH. Accessed September 25, 2022. https://www.pivotworks.org/health/

13. Tennant PWG, Murray EJ, Arnold KF, et al. Use of directed acyclic graphs (DAGs) to identify confounders in applied health research: review and recommendations. *International Journal of Epidemiology*. 2021;50(2):620-632. doi:10.1093/ije/dyaa213

14. RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

15. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). "Welcome to the tidyverse." Journal of Open Source Software, 4(43), 1686. doi:10.21105/joss.01686.

16. Bates D, Mächler M, Bolker B, Walker S (2015). "Fitting Linear Mixed-Effects Models Using lme4." Journal of Statistical Software, 67(1), 1–48. doi:10.18637/jss.v067.i01.

17. Bolker B, Robinson D, Menne D, et al. broom.mixed: Tidying Methods for Mixed Models. Published online April 17, 2022. Accessed March 12, 2023. https://CRAN.R-project.org/package=broom.mixed

18. Fox J, Weisberg S (2019). An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

19. Improved sanitation facilities and drinking-water sources. Accessed March 15, 2023. https://www.who.int/data/nutrition/nlis/info/improved-sanitation-facilities-and-drinking-water-sources

20. Delahoy MJ, Wodnik B, McAliley L, et al. Pathogens transmitted in animal feces in low- and middle-income countries. *International Journal of Hygiene and Environmental Health*. 2018;221(4):661-676. doi:10.1016/j.ijheh.2018.03.005

21. Oberhelman RA, Gilman RH, Sheen P, et al. An intervention-control study of corralling of free-ranging chickens to control Campylobacter infections among children in a Peruvian periurban shantytown. *Am J Trop Med Hyg*. 2006;74(6):1054-1059.

22. El-Tras WF, Holt HR, Tayel AA, El-Kady NN. Campylobacter infections in children exposed to infected backyard poultry in Egypt. *Epidemiology & Infection*. 2015;143(2):308-315. doi:10.1017/S095026881400096X

23. Das R, Haque MA, Chisti MJ, Ahmed T, Faruque ASG. Nontyphoidal Salmonella among Children under 5 Years Old in Sub-Saharan Africa and South Asia in the Global Enteric Multicenter Study. *The American Journal of Tropical Medicine and Hygiene*. 2022;106(2):504-512. doi:10.4269/ajtmh.21-0762

24. Singer RS, Cox LA, Dickson JS, Hurd HS, Phillips I, Miller GY. Modeling the relationship between food animal health and human foodborne illness. *Preventive Veterinary Medicine*. 2007;79(2):186-203. doi:10.1016/j.prevetmed.2006.12.003

25. Conan A, O'Reilly CE, Ogola E, et al. Animal-related factors associated with moderate-to-severe diarrhea in children younger than five years in western Kenya: A matched case-control study. *PLOS Neglected Tropical Diseases*. 2017;11(8):e0005795. doi:10.1371/journal.pntd.0005795

26. Bratcher A, Hoff NA, Doshi RH, et al. Zoonotic risk factors associated with seroprevalence of Ebola virus GP antibodies in the absence of diagnosed Ebola virus disease in the Democratic Republic of Congo. *PLoS Negl Trop Dis*. 2021;15(8):e0009566. doi:10.1371/journal.pntd.0009566

27. One Health Basics | One Health | CDC. Published November 8, 2022. Accessed March 16, 2023. https://www.cdc.gov/onehealth/basics/index.html

Table 4. Participant Characteristics from the Eco-Epidemiology of Diarrheal Disease Study (2017), Stratified by *Campylobacter* Infection.

	Positive (N=75)	Negative (N=93)	Overall (N=168)
Age			
Mean (SD)	2.70 (1.05)	3.40 (1.56)	3.09 (1.40)
Median [Min, Max]	3.00 [0.500, 4.00]	4.00 [0.300, 5.00]	3.00 [0.300, 5.00]
Sex			
Female	39 (52.0%)	44 (47.3%)	83 (49.4%)
Male	35 (46.7%)	49 (52.7%)	84 (50.0%)
Missing	0 (0%)	0 (0%)	0 (0%)
Available Water Source			
Unimproved	75 (100%)	93 (100%)	168 (100%)
Latrine Use			
No Latrine Use	34 (45.3%)	53 (57.0%)	87 (51.8%)
Latrine Use	7 (9.3%)	16 (17.2%)	23 (13.7%)
Shared Latrine Use	34 (45.3%)	24 (25.8%)	58 (34.5%)
Currently Experiencing Diarrhea			
Yes	6 (8.0%)	5 (5.4%)	11 (6.5%)
No	69 (92.0%)	88 (94.6%)	157 (93.5%)
Has access to a Handwashing Area			
Yes	0 (0%)	4 (4.3%)	4 (2.4%)
No	75 (100%)	89 (95.7%)	164 (97.6%)
Owns a Sick Animal			
Owns a Sick Domestic Animal	25 (33.3%)	14 (15.1%)	39 (23.2%)
Does Not Own a Sick Domestic Animal	34 (45.3%)	55 (59.1%)	89 (53.0%)
Missing	16 (21.3%)	24 (25.8%)	40 (23.8%)
Encountered Wild Lemurs in the last 4 weeks			
Contact with Lemurs	11 (14.7%)	14 (15.1%)	25 (14.9%)
No Contact with Lemurs	62 (82.7%)	75 (80.6%)	137 (81.5%)
Missing	2 (2.7%)	4 (4.3%)	6 (3.6%)
Eaten Wildlife Meat in the last 4 weeks			
Has Eaten Wildlife Meat	3 (4.0%)	3 (3.2%)	6 (3.6%)

Table 4. Participant Characteristics from the Eco-Epidemiology of Diarrheal Disease Study (2017), Stratified by *Campylobacter* Infection.

	Positive (N=75)	Negative (N=93)	Overall (N=168)
Has Not Eaten Wildlife Meat	71 (94.7%)	86 (92.5%)	157 (93.5%)
Missing	1 (1.3%)	4 (4.3%)	5 (3.0%)

Campylobacter and Diarrhea in Rural Madagascar Children
Marisa Wong, MPH Candidate
Capstone Mentors: **Thomas R. Gillespie, PhD** and **Stephen G. Mugel, MS**

Background

Diarrhea consists of loose or watery poop multiple times a day[1] and is a common illness in children. Children are especially vulnerable to diarrhea because of the risk it poses to their growth[2]. *Campylobacter* is a germ that can cause severe diarrhea and even death. People become infected with *Campylobacter* by eating food or drinking water contaminated with the germ. Studies have also found this germ present in cattle, sheep, and chickens[3,4]. The people of Madagascar are at a high risk of diarrhea from *Campylobacter* because of their rural settings, agricultural livestock, and lack of healthcare services. However, Centre ValBio and Pivot are two organizations working to combat that.

In 2017, Dr. Gillespie ran a study that examined the characteristics of people who have diarrhea in villages that live near Madagascar's Ranomafana forest. This study included an in-depth survey, human stool samples, and animal stool samples to determine people's habits and whether they were infected with any diarrheal bacteria.

Methods

My project analyzed the data from Dr. Gillespie's 2017 study to see if animal-related exposures had any links to *Campylobacter* infection. Specifically, I was interested in households that owned a sick domestic animal, households that encountered a wild lemur in the last four weeks, and households that ate any wild meat in the last four weeks. To test my hypothesis, I researched previous scientific studies that investigated the association between animal contact and diarrhea to see which human and animal traits they considered important in their studies. Then, I gathered those traits and displayed them on a visual map that showed the relationship between all the identified traits. Finally, I calculated three ratios of *Campylobacter* infections for children who lived in households that owned a sick animal compared to children who did not, children who lived in households that encountered a wild lemur in the last month to children who did not, and children who lived in households that ate wild meat in the last month compared to children who did not.

Results

The results from Dr. Gillespie's 2017 study showed that 168 children participated, 45% of children were infected with *Campylobacter,* and 6.5% of children were having diarrhea. My analysis showed that children who lived in households that owned sick animals had *Campylobacter* 2.6 times more than children who did not, children who lived in households that contacted wild lemurs had no difference in *Campylobacter* infections compared to children who did not, and children who lived in households that ate wild meat had *Campylobacter* 1.2 times more than children who did not. Although I calculated these values, none of the ratios were vastly different to their comparison group.

Discussion

The results of my work both contribute to understanding the link between animal encounters and *Campylobacter* infection and call for more research dedicated to this topic. Many studies have determined that agricultural animals and pets are sources of *Campylobacter*, which can lead to human illness, and owning sick animals is highly related to getting people sick. However, contacting a lemur and eating wild meat are not well studied.

To continue serving the unique needs of their rural villages, I recommend the Madagascar Ministry of Health involve animal health and environmental health experts in their efforts to reduce *Campylobacter* and diarrhea. The combination of these experts is called the One Health approach and has been recommended for use when human health is impacted by outside forces[5]. This method is supported by many global health institutions, including the World Health Organization (WHO). I also recommend the Malagasy Ministry of Health continue aiding organizations like Center ValBio and Pivot in their efforts of improving rural health. Lastly, I encourage health teams to explore *Campylobacter* infections that do not lead to diarrhea by assessing the impacts of these infections to the rest of the household, by using new testing methods that do not rely on diarrheal symptoms, and by considering the possibility of re-infection from *Campylobacter*.

References

1. Definition & Facts for Diarrhea - NIDDK. National Institute of Diabetes and Digestive and Kidney Diseases. Accessed April 27, 2023. https://www.niddk.nih.gov/health-information/digestive-diseases/diarrhea/definition-facts
2. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet*. 2012;379(9832):2151-2161. doi:10.1016/S0140-6736(12)60560-1
3. Moore JE, Corcoran D, Dooley JSG, et al. *Campylobacter*. *Vet Res*. 2005;36(3):351-382. doi:10.1051/vetres:2005012
4. Young KT, Davis LM, DiRita VJ. Campylobacter jejuni: molecular biology and pathogenesis. *Nat Rev Microbiol*. 2007;5(9):665-679. doi:10.1038/nrmicro1718
5. One Health Basics | One Health | CDC. Published November 8, 2022. Accessed March 16, 2023. https://www.cdc.gov/onehealth/basics/index.html