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

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Introduction

Diarrheal diseases are a major cause of global morbidity and mortality in low- and middle-income countries (LMIC). Children are particularly susceptible to negative health outcomes from diarrheal diseases due to their underdeveloped response systems, the risk of physical stunting, and possibility of stagnant mental development[1]. In 2010, diarrhea accounted for over 750,000 global deaths in children[2]. In Madagascar, 23% of deaths in children five years of age and younger are attributable to enteric diseases[3]. The villages surrounding Madagascar's Ranomafana National Park are at an increased risk of diarrheal disease due to their proximity to forestry, their reliance on subsistence farming, the lack of adequate healthcare, and household hygiene practices.

Campylobacter is a leading cause of global enteric diseases and causes varying forms of diarrhea, abdominal cramping, and fever. Historically, *Campylobacter* was considered an animal disease that caused diarrhea in cattle, sheep, and chickens[4]. Human infection of *Campylobacter* typically occurs through the ingestion of food, animal products, or drinking water contaminated with the bacteria[5]. Thus, campylobacteriosis is considered both a foodborne illness and a zoonotic disease.

Project Objectives

1. Conduct a literature review to understand certain vulnerabilities to ecologic-associated diarrheal diseases and to determine relevant risk factors associated with enteric infections
2. Develop a Directed Acyclic Graph (DAG) to display a qualitative representation of identified variables between exposures and outcome
3. Formulate three epidemiologic models that estimate the prevalence ratios between household level exposures of owning a sick domestic animal, encountering a wild lemur in the last four weeks, and ingesting wildlife meat in the last four weeks with *Campylobacter* infection
4. Produce a report that documents the analysis conducted, an interpretation of the results, and future implications of these findings that is shared with the research team, Principal Investigator, and involved non-governmental organizations.

Data Source

In 2017, Dr. Thomas Gillespie, his research team, and partners at the Center ValBio (CVB) conducted a population-wide, cross-sectional study to determine the risks associated with diarrheal disease in the Ifanadiana District of Madagascar. The study consisted of a comprehensive survey, human fecal sampling, and domestic animal fecal sampling, which were all administered by CVB's mobile health team. PCR was conducted on the fecal samples to detect pathogens. Over the course of six months, the CVB team visited 20 villages and collected information from 211 households.

Methods

- **Literature Review**: Conducted using PubMed to grasp the burden of diarrheal diseases in LMIC and to identify risk factors prioritized by disease ecologists (Table 1).
- **Directed Acyclic Graph (DAG)**: Built using the variables found during the literature review to visualize the relationship between exposure and outcome while incorporating confounding variables using subject-matter knowledge and *a priori* assumptions (Figure 1).
- **Epidemiologic Modeling**: Constructed 3 Generalized Linear Mixed Effects Models, evaluated collinearity using the Variance Inflation Factor (VIF) with a threshold of 5, and assessed confounding using the 10% rule by comparing the prevalence ratio (PR) of the Gold Standard Model to models that include confounding variables (Table 3).

Supporting Tables and Figures

Table 1. PubMed Literature Search Strings and Results.

Search String	*Results*
((("Zoonoses"[Mesh]) AND "Madagascar"[Mesh]) AND "Epidemiological Models"[Mesh])	0 articles
(("Zoonoses"[Mesh]) AND "Epidemiological Models"[Mesh]) OR "Zoonoses/epidemiology"[MeSH]	4,846 articles
(("Zoonoses"[Mesh]) OR "Bacterial Zoonoses"[Mesh]) AND "Regression Analysis"[Mesh]	157 articles
((("Zoonoses"[Mesh]) OR "Bacterial Zoonoses"[Mesh]) OR "Stomach Diseases"[Mesh]) AND "Regression Analysis"[Mesh]	3,871 articles
((((("Zoonoses"[Mesh]) OR "Bacterial Zoonoses"[Mesh]) OR "Dysentery"[Mesh]) OR "Waterborne Diseases"[Mesh]) AND "Regression Analysis"[Mesh]	241 articles

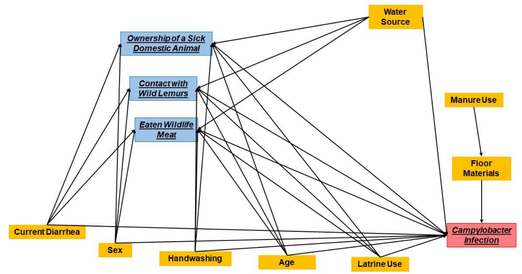

Figure 1. Visual display of the relationship between Ownership of a Sick Domestic Animal, Contact with Wild Lemurs, Eaten Wildlife Meat, and *Campylobacter* Infection. Blue represents exposures, yellow represents confounders, and red represents the outcome.

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)
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%)
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%)
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%)
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%)

Model 1: $Campylobacter\ infection = \alpha + \beta_1 Owns\ Sick\ Domestic\ Animal + \gamma_1 Sex + \gamma_2 Age + \gamma_3 Current\ Diarrhea + \gamma_4 Latrine\ Use + (1|HouseholdID)$

Model 2: $Campylobacter\ infection = \alpha + \beta_1 Contact\ with\ Wild\ Lemurs + \gamma_1 Sex + + \gamma_2 Current\ Diarrhea + \gamma_3 Latrine\ Use + (1|HouseholdID)$

Model 3: $Campylobacter\ infection = \alpha + \beta_1 Eaten\ Wildlife\ Meat + \gamma_1 Sex + \gamma_2 Age + \gamma_3 Current\ Diarrhea + \gamma_4 Latrine\ Use + (1|HouseholdID)$

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

Exposure	N (%)	aPR	95% CI
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)

Results

168 children five years old and younger participated in the survey and fecal sampling. Approximately 45% of children (75 out of 168) tested positive for *Campylobacter* infection; however, only 6.5% (11 out of 168) were currently experiencing diarrhea. 23.2% of children lived in a household that owned a sick domestic animal, 14.9% lived in a household that encountered a wild lemur in the last four weeks, and 3.6% lived in a household where someone ate wildlife meat.

Children who lived in households that own sick domestic animals had 2.6 times the prevalence of *Campylobacter* compared to children who lived in households that did not, controlling for sex, age, current diarrhea, and latrine use (95% CI: 0.96 – 7.04).

Children who lived in households where someone encountered a wild lemur in the last 4 weeks had 0.98 times the prevalence of *Campylobacter* compared to children who lived in households that did not, controlling for sex, current diarrhea, and latrine use (95% CI: 0.35 – 2.76).

Children who lived in households where someone consumed wildlife meat in the last 4 weeks had 1.16 times the prevalence of *Campylobacter* compared to children who lived in households that did not, controlling for sex, age, current diarrhea, and latrine use (95% CI: 0.19 – 6.93).

Discussion

This project highlights diarrheal disease prevalence in a rural, understudied population. The results of this project's estimations call for additional research of ecological exposures on diarrheal diseases. Although the findings were not statistically significant, other scientific research identifies exposure to sick domestic animals as a risk factor for enteric diseases. However, contact with wild lemurs and ingestion of wildlife meat are not well studied in the context of diarrheal diseases.

In resource limited settings, I advise Madagascar's Ministry of Health (MoH) to focus its intervention efforts on protecting the public from sick domestic animals, understanding the impacts of asymptomatic *Campylobacter* infection, and incorporating the expertise of environmental, animal, and public health professionals through a One Health approach. In addition, MoH should continue to support organizations that provide healthcare to these communities, such as CVB and Pivot.

References

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6. Figures created with BioRender.com.

Acknowledgements

Stephen – Thank you for your endless mentorship and support in making this a successful capstone project!

Dr. Gillespie – Thank you for sharing your knowledge and for welcoming me into your research.

Tim – Thank you for being my rubber ducky and supporting me unconditionally throughout graduate school.