Project Title: Understanding the diversity of genotypes of *Giardia intestinalis* in black and gold howler monkeys (*Alouatta caraya*), humans, dogs, and livestock in Corrientes, Argentina

Project Supervisor: Dr. Thomas R. Gillespie, Department of Environmental Sciences, Emory College

Statement of Problem and Proposed Research Question

Recent findings have demonstrated a high burden of *Giardia* infection in developing countries due to inadequate sanitation and increased contact with livestock and wildlife [1, 2]. These animals might share the pathogen and act as potential reservoirs of infection.

Giardia's zoonotic potential has been well documented as the same genotypes have been found in humans and other mammals. *Giardia* is known as the "national parasite" due to its heavy burden throughout Argentina [3]. Humans have a 30% prevalence of *Giardia* infection in Corrientes [4]. Previous research demonstrated that black and gold howler monkeys (*Alouatta caraya*) in Corrientes, Argentina are a reservoir for *Giardia*. However, *Giardia* infection in livestock and dogs and the potential zoonotic transmission of *Giardia* among species in Corrientes is still unexamined. Thus, my project takes a synthetic approach to investigate the host community and circulation of *Giardia* in the region.

Given the phylogenetic relatedness between howler monkeys and humans and the high prevalence of *Giardia* in black and gold howler monkeys (*Alouatta caraya*) in Argentina [5], howler monkeys will be sampled as a wildlife proxy for zoonotic *Giardia* sharing. Further, humans share their habitats with dogs and livestock in Corrientes, so dogs and livestock will also be sampled to assess if niche interaction plays a role in zoonotic *Giardia* sharing.

This project investigates two research questions. First, what is the distribution of genotypes and sub-genotypes of *Giardia intestinalis* found among humans, black and gold howler monkeys, dogs, and livestock within Corrientes? My research will occur at three sampling sites that vary in human population densities and degree of species range overlaps: village, rural, and remote. This establishes a natural experiment to test my second question, what is the genotypic variability among the three types of sampling sites. Overall, my research intends to understand how proximity to and contact with monkeys and domesticated animals affects genotypic variation of *Giardia* in humans.

I hypothesize that *Giardia* genotypes will be shared among species in sample sites with higher human population densities and a higher degree of human-animal range overlaps due to increased contact and interaction among species. Similarly, *Giardia* genotypes will be distinct among species in sample sites with low human population densities and a low degree of human-animal range overlaps. I predict that genotypes A and B are more likely to be shared across all species in village sites due to their potentially zoonotic nature. Further, I hypothesize that proximity to and contact with howler monkeys and domesticated animals affects zoonotic transmission of *Giardia* genotypes in humans.

Background

Human population growth and expansion of land-use have led to greater contact among species and have exacerbated the transmission of pathogens between humans and other animals, presenting challenges for global human health and conservation. *Giardia intestinalis* is associated with up to 30% of enteric protozoa infections worldwide [6].

Giardia is a protozoan flagellate that lives in the intestinal tract of hosts and causes the diarrheal disease, giardiasis [7, 8]. *Giardia* is most commonly transmitted by ingestion of contaminated water. However, humans can also become infected through contact with cysts from animals [9].

Eight genotypes of *Giardia intestinalis* are described in mammals. Genotypes A and B are found in multiple hosts, but genotypes C, D, and E are host-specific [10]. Humans and howler monkeys have been characterized as genotype A or B [10, 11, 12], whereas dogs and livestock can be characterized as their host-specific genotypes – C and D for dogs and E for livestock – or as potentially zoonotic genotypes A and B [10]. Genotypes A and B separate into sub-genotypes AI, AII, BIII, and BIV [11].

The black and gold howler monkeys, *Alouatta caraya*, are hosts to a variety of parasites and are considered to be sentinels of ecosystem health [13, 5]. Howler monkeys survive in fragmented forests and cross terrestrially from patch to patch, allowing more contact with humans [5]. They also drink water from streams and lagoons [5], increasing their potential to acquire *Giardia*.

Giardia is known as the "national parasite" due to its heavy burden throughout Argentina [3]. Humans have a 30% prevalence of *Giardia* infection in Corrientes [4]. The system is located at 27° 30' S and 58° 41' W in Corrientes, Argentina. The semi-deciduous forest of the ecosystem is prone to flooding, leading to fecal matter contamination in water sources.

The health significance and relative infectivity of *Giardia* is high [14]. Incidence of infection and risk patterns of *Giardia* in Corrientes are unexplained, and this proposal will be the first crucial contribution to understanding *Giardia* infectivity in the region.

Methods

Fecal samples will be collected in Corrientes, Argentina during Summer 2017, and I will stay at the field station Estacion Biologica de Corrientes (EBCo). I will sample humans, dogs, livestock (cows and sheep), and howler monkeys to evaluate zoonotic potential.

Three types of sites differing in levels of human population densities and degree of species range overlap will be sampled: village (high population density and degree of species overlap), rural (medium population density and degree of species overlap), and remote (low population density and species degree of overlap). I will sample ten sites of each type, totaling in 30 sites.

I have already collected viable samples from remote sites during my pilot season in Summer 2016. During Summer 2017, I will collect samples in village and rural sites. I will follow the Gillespie 2006 [15] recommendations and assume a 5% prevalence of *Giardia* across all species; so I will collect 60 independent fecal samples of each species – humans, monkeys, dogs, cows, and sheep – in each type of sampling site. My expected sample size across all species is 300 per sampling site to ensure a statistically viable sampling frame.

I will rely on local forest rangers to locate the monkeys and help me communicate with townspeople. Fresh animal fecal samples will be collected in the morning when animals are most active, and samples will be collected using the Gillespie 2006 [15] non-invasive standard methodology. Once an animal defecates, its fecal sample will be identified by sex, relative age, and group name or owner, and one gram of uncontaminated sample taken from the middle of the fecal matter will be scooped into one milliliter of RNAlater Stabilization Solution.

Dogs and livestock will be opportunistically sampled after owner consent is given. I will ask for both oral and written consent in Spanish from people I met in the 2016 pilot study to systematically sample households in each type of site. A brief questionnaire will also be administered to document sex, age, and demographic information of each individual and to assess their source of drinking water, frequency of contact with animals, and personal hygiene.

Molecular analysis will identify the genotypic variability of *Giardia* in all samples. I will extract DNA from the RNAlater-preserved fecal samples using the FastDNA Spin Kit for Soil from MP Biomedicals LLC. Next, I will amplify multi-locus regions using a nested Polymerase Chain Reaction (PCR) method described in Roellig et al. 2015 [16]. This protocol was developed through coordination with Dr. Gillespie's collaborators from the parasitic disease group at the Centers for Disease Control (CDC), considered to be the leading research group on *Giardia* in the United States, and has been optimized. Further, Dr. Dawn Roellig from CDC's parasitic disease group has agreed to provide guidance as needed.

The genes amplified will be glutamate dehydrogenase, triosephosphate isomerase, and beta-giardin and are known amplification targets for *Giardia* genotype identification [17]. For each locus that is amplified, initial forward and reverse primers as well as secondary forward and reverse primers will make up the nested PCR process. The *Giardia*-positive PCR products will be purified and submitted for sequencing at Genewiz LLC. Sequences generated will be compared to published references using Genbank's BLASTn tool.

Data from this cross-sectional study will be statistically analyzed using linear regression models, where genotype will respond to type of site as a proxy for human population density as a predictor variable. A logistic regression will calculate odds ratio to assess risk factors for *Giardia* based on survey questions. Finally, a phylogenetic analysis will be conducted to evaluate the shared genotypes between species.

Timeline

Month	Task
June – July	Collect samples in Argentina
Weeks 1-2	Village Sites 1-6
Weeks 3-4	Rural Sites 1-6
Week 5	Village Sites 7-10
Week 6	Rural Sites 7-10
Week 7	Prepare samples for shipment
August	Ship samples to United States
September	Molecular analysis – PCR

October	Molecular analysis – PCR
November	Sequencing
February	Complete thesis
July	Publish manuscript

Goals

This research is a keystone to my academic development and is a necessary follow-up to the pilot study of Summer 2016. The data from both field seasons will serve as the basis for my Master's thesis for the Environmental Science 4+1 BS/MS program at Emory University. This project will identify all circulating genotypes and sub-genotypes of *Giardia* and determine the potential for sharing *Giardia* among people, monkeys, dogs, and livestock in Corrientes, Argentina.

I hope to gain crucial field skills in tracking and identifying animals, collecting fecal samples from a myriad of species, and surveying people. I will be able to further cultivate my microscopy and molecular analysis skills and develop a basic understanding of sequencing the *Giardia* phylogeny. I plan to work in water quality and disease transmission so this project is a crucial hands-on experience that will help build my career portfolio. An understanding of the dynamics of waterborne parasites will be integral in my career goals. Further, the experience of working within a collaborative team will introduce me to diverse perspectives and enable me to comprehend the linkages of multidisciplinary research.

Diarrheal disease is an extremely important health concern in Northern Argentina. My research will give insight on the health of study subjects and overall community human health in Corrientes. My project will expand upon the Gillespie-EBCo collaborative long-term study on howler monkey disease dynamics and will focus on human health, an aspect of the study that has previously received less attention.

All data will be shared with the local community to increase awareness of *Giardia* prevalence and risk factors. The results from this project can be used to educate individuals as well as inform health care practices and interventions. It is our hope that by gaining a greater understanding of the ecological and epidemiological drivers of Giardiasis, we can form strategic and informed solutions that will ultimately reduce the burden of diarrheal disease within the region.

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*prices do not account for Emory University's discount

Field Season Budget

Item	Description	Cost per Unit	Number of Units	Total	Source
Round trip flight from Atlanta to Buenos Aires	N/A	$1,658.00	1	$1,658.00	Air Canada
Round trip flight from Buenos Aires to Corrientes	N/A	$323.00	1	$323.00	Aerolineas Argentinas
Vehicle rental	Vehicle for traveling to field sites	$100.00	1	$100.00	EBCo
Vehicle gas	N/A	$50.00	1	$50.00	EBCo
Food and Lodging	I will buy food from the local supermarket and stay at the field station, EBCo	$100.00	1	$100.00	EBCo
Garmin GPS Map64s	Each sample will be associated with a geographical data point	$250.00	1	$250.00	Garmin on Amazon
Latex Gloves	Safety measure when collecting samples	$37.00	1	$37.00	Fisher Scientific
Cryovials (100)	Will contain the samples	$60.78	3	$182.34	Fisher Scientific
RNAlater Stabilization Solution (500mL)	Removes infectivity of pathogens while stabilizing cellular RNA of the sample	$263.00	1	$263.00	Fisher Scientific
Total				$2,963.34	

DNA Extraction Budget

Item	Cost per Unit	Number of Units	Total	Source

MP Biomedical FastDNA SPIN Kit for Soil (50 preps)	$349.86	2	$699.72	Fisher Scientific #MP116560200
Ethanol (500mL)	$71.96	1	$71.96	Fisher Scientific ##BP2818500
Latex Gloves	$37.00	1	$37.00	Fisher Scientific
15mL Falcon Tubes (500)	$264.97	1	$264.97	Fisher Scientific
Microcentrifuge tubes (500)	$14.20	1	$14.20	usa scientific #1615-5500
100-1000uL Pipette tips (960)	$73.95	1	$73.95	usa scientific #1126-7810
Total			$1,161.80	

PCR Analysis and Sequencing Budget

Item	Cost per Unit	Number of Units	Total	Source
8-Well Strips + Lids (125)	$128.35	2	$256.70	Axygen/Genesee Scientific
Taq PCR Master Mix Kit (250)	$182.00	2	$364.00	Qiagen #201443
20uL Pipette tips (960)	$47.22	1	$47.22	Rainin
200uL Pipette tips (960)	$47.22	1	$47.22	Rainin
TBE Buffer 10x	$60.00	1	$60.00	Life Technologies
PCR Ladder	$193.37	1	$193.37	Fisher Scientific # BP2571100
Load Dye (4mL)	$42.00	1	$42.00	Fisher Scientific #50-591-186
Sybr Green	$297.00	1	$297.00	Thermo Fisher #S7563
GDH Primers	$10.00	4	$40.00	Thermo Fisher
TPI Primers	$10.00	4	$40.00	Thermo Fisher
BG Primers	$10.00	4	$40.00	Thermo Fisher
Sequencing by Genewiz	$200.00	1	$200.00	Genewiz LLC
Total			$1,627.51	

Total Budget: $5,752.65

Pending Awards: National Geographic Young Explorers and Emory Global Health Institute