Risk factors associated with endoparasitism in two rural Ecuadorian communities

William D. Helenbrook

SUNY-ESF, State University of New York College of Environmental Science and Forestry, Environmental and Forest Biology, 1 Forestry Drive, Syracuse, New York 13210.

Correspondence: William D. Helenbrook, 1 Forestry Drive, Syracuse, New York 13210.

Abstract

Gastrointestinal parasites affect nearly half a billion people worldwide, significantly impairing their ability to live healthy, productive lives. Ideally, access to clean water, proper sanitation, and anti-parasitic drugs would limit infectious disease; however, the resources aren't always available, especially in poor, rural communities. This study investigated 54 individuals (44% of all inhabitants) in two Ecuadorian communities, and the risk factors associated with gastrointestinal parasitism. Six gastrointestinal parasite species were identified. Parasite species richness per individual was significantly associated with boiling water before consumption. Presence of *Capillaria* sp. was negatively associated with age of participants, boiling water, and treatment with anti-parasitic medication within the previous year, while larger family size was positively associated with infection. *Blastocystis* sp. was also significantly associated with boiling water treatment. The results of this study demonstrate that people who don't boil their water, are relatively young, live in larger families, and haven't been treated with some form of chemotherapy or anti-helminthic drug are at increased risk of acquiring certain gastrointestinal parasites. Ideally, a comprehensive strategy to control these parasites would couple large scale infrastructure development and mass drug administration of preventative chemotherapy treatment with hygiene and water treatment education.

Keywords

Ecuador, gastrointestinal parasitism, epidemiology, human health

1. Introduction

Chronic gastrointestinal parasitosis is a common problem affecting hundreds of millions of people living in rural, relatively poor, communities with limited access to medical care, proper sanitation, and clean water [1], [2], [3], [4], [5], and [6]. In particular, infants, children, and pregnant women living in developing countries show an increased risk of infection [7] and [8]. Areas with a confluence of inadequate water supplies, insufficient sanitation, and people living in close contact with wild and domesticated animals are hotbeds for gastrointestinal parasitic diseases [9].

The impact of parasites and their associated diseases is particularly problematic in tropical climates of the developing world where some of the most prolific and neglected gastrointestinal parasites are found, including the following species (approximate number of cases in parentheses): *Taenia* spp. (50 million), *Ascaris lumbricoides* (1.2 billion), *Trichuris trichiura* (795 million), *Ancylostoma duodenale* (1.2 billion), *Necator americanus* (740 million), *Strongyloides* spp. (30-100 million), *Giardia intestinalis* (2.8 million), *Entamoeba histolytica* (50 million), and *Cryptosporidium* spp. (250-500 million) [3], [4], [7], [10], [11], [12], [13], [14], and [15]. The impact these infections have on tropical communities has been well documented (Table 1). Helminth infections may impair appetite, leading to varying degrees of malnourishment [24] and [25]. Gastrointestinal malabsorption may arise from *Ascaris* species infections and hookworms [26]. Decline in cognitive function has been associated with hookworm infection [22]. Research in a Colombian community found sixty-three percent of boys infected with any of 6 parasites studied (*N. americanus*, *A. lumbricoides*, *E. histolytica*, *T.*

trichiura, *Giardia* spp., *Enterobius vermicularis*) experienced some form of growth impairment such as lower weight and stature [2]. Results from an Ecuadorian community suggest that *A. lumbricoides* infections were correlated with verbal impairment [27]. When anti-helmintic treatments are implemented, a positive association with weight gain, height and other anthropometric measurements has been found [18].

Numerous studies and agencies have investigated the effect of control and treatment options including chemotherapy, broad-spectrum anti-helminthics, vaccines, improved water treatment and sanitation, health education, and overall improved economic development [3], [8], [9], [28], [29], and [30]. Chemotherapy in particular is a cost-effective treatment for school-aged children, as programs can be easily set-up through schools and administered by teachers [3] and [31]. However, long-term control of parasitic infections is highly dependent on routine behavioral changes (i.e. hygiene education) [31]. The World Health Organization (WHO) has encouraged countries to ensure access of anti-helminthic drugs at all levels of the health care system, while also introducing two primary health components involving education and sanitation [4] and [33].

Understanding inherent characteristics and behaviors that predispose people to particular infections would allow both education programs and public health strategies to be specifically tailored to at-risk communities. Here we investigate the association of several demographic, behavioral and geographic factors on gastrointestinal species richness and prevalence obtained from morphological analysis. Based on earlier studies, it is expected that age, gender, family size, recent gastrointestinal parasitic treatment, proximity of wildlife and rainforest, method of obtaining water, and hunting practices may be associated with differences in gastrointestinal parasite communities.

2. Materials and Methods

Two local communities surround the Bilsa Biological Station (00°21'33"N 79°42'02"W) including La Yacita (19 families and 64 people) and Dogola (20 families and 63 people) (Figure 1). Staff from the reserve assisted the Principal Investigator in meeting with local communities to discuss the project in the summer of 2010. Recruitment for participants was done through primary schools in which information about a community meeting was announced in the classroom. A meeting was conducted at a local school in each community and information on the entire project was explained. Interested individuals were then provided written details of the project, given information on the risks and benefits of participating, and provided with details on safeguarding their information and confidentiality. Written and oral consent was obtained from all participants over 18 years of age, and from the parents of participating children. In addition, verbal assent was obtained from all children. All research was non-invasive and approved in-country according to guidelines and permit No 033-FAU-DPE-MA from the Ministerio del Ambiente in Quito, Ecuador.

A total of fifty-four people from both La Yacita and Dogola participated in the study. Each person was administered a questionnaire and sealed fecal samples were collected within 3 hours or less of defecation. Questionnaires included contact information, age, gender, family size, previous anti-parasitic treatments, and information relevant to water treatment, wildlife proximity, and distance to forest. Participants manipulated fecal samples into 50 ml tubes using sterile surgical gloves to minimize cross-contamination. Upon collection fecal samples were divided into two separate preservation solutions in 50 ml conical tubes. Zinc polyvinyl alcohol (Zn-PVA) was used for fecal smears, flotations and sedimentations. RNAlater (Qiagen Inc.,

Valencia, California) was used to preserve parasite DNA. Researchers visited each home to collect GPS data points that could be coupled with satellite imagery to estimate distance of homes to forest.

Fecal samples were examined for parasites at the Fish and Wildlife Disease Laboratory at SUNY-ESF, Syracuse, New York for helminth eggs and larvae, and protozoan cysts using trichrome stain on fecal smears, centrifugal flotations, and sedimentations (single slide each) as described by [35] and [36] with the following modifications. A $NaNO_3$ solution (SG 1.2) was used for optimal retrieval of parasite eggs in flotations [36]. Cover slips were placed on tubes for 10 min following centrifugation as opposed to before centrifugation. Flotations are optimal for retrieving nematode eggs and protozoan cysts and sedimentations for obtaining trematodes which are too heavy to be retrieved from flotations [36]. We also used smears which are useful for obtaining protozoan parasites [35]. One gram of Zn-PVA preserved sample was used in the fecal flotation and the remaining pellet was used in the sedimentation. Results from fecal smears, flotations, and sedimentation were combined to confirm presence or absence, and are subsequently reported as a single value for all calculations. Slides were scanned at 20x objective lens using a Nikon 80i compound microscope with Nomarski and phase objectives. Images were captured at 40x objective lens with a 3MP IDEA digital camera and analyzed with photomicrography software (Diagnostic Instruments, Inc. Spot RT Software 4.6 Sterling Heights, Michigan). Identification was based on size, shape, color, and interior structure.

PCR-based detection was used to identify *Blastocystis* sp. since identification of cysts alone is difficult [37]. Parasite DNA was extracted from approximately 200 mg of feces using the QIAamp DNA Stool Mini Kit following the manufacturer's instructions. *Blastocystis* species were amplified using primers BH1F and BHRDr [38] and BLF and BLR primers [39]. Because

finding a *Capillaria* species was unexpected, we amplified small subunit ribosomal DNA sequence using primer combinations F-573M (5'-CGCGGTAATYCCAGCTCCA-3') and 18S1330R (5'-GTACGCGCCGTCACTATTTA-3') to confirm our morphological assessment. Both PCR-based regimes, coupled with single strand conformation polymorphism (SSCP) and DNA sequencing utilized the same protocols previously reported by [34].

Descriptive data were reported on parasite species richness and prevalence across all individuals and for each potential risk factor. A Mann-Whitney U test was used to analyze univariate association of each potential risk factor with the presence or absence of each described parasite species and individual parasite species richness. A Kruskal-Wallis test was used for variables with more than two categorical responses. The relationship between individual species richness and continuous risk factors was calculated using Pearson correlation coefficient. Odds ratios and 95% confidence intervals were computed with logistic regression analysis. Abundance and intensity data were not used in statistical analyses due to the many factors that can influence parasite counts [40]. Effects were considered significant if $p < 0.05$. All statistical analyses were done with STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, USA).

3. Results

Six gastrointestinal parasite species were found in fecal samples from 54 humans: *Blastocystis* sp. (81.5%), *Capillaria* sp. (20.4%), *Entamoeba* spp. (14.8%), Ascarididae gen. sp. (13.0%), *Balantidium* sp. (1.9%), and *Strongyloides* spp. (1.9%). The majority of species encountered were identifiable to genus with the exception of the ascarid species. These eggs were mammillated (48-57 µm long), consistent with an *Ascaris* sp., but we took a conservative approach and categorized this to family rank as Ascarididae gen. sp. Our taxonomic

classification of *Capillaria* sp. was confirmed using PCR-based amplification of small subunit ribosomal DNA sequence. The closest related species in GenBank was *Capillaria xenopi* (92% similarity across >705bp).

Eighty-five percent of individuals were infected with at least 1 parasite. Twenty-five individuals (46.3%) harbored a single parasite, 16 individuals (29.6%) had two parasite species, and five individuals (9.3%) had three parasite species, while eight individuals had no observed parasitic infections (14.8%). An average of 1.3 parasite species was found per individual. There was no difference in prevalence of infections between males and females (p=0.09). All but two females were infected with at least one parasite (27/29, 93.1%), while 76% of males (19/25) were infected. The only reported risk factor significantly associated with parasite species richness was boiling water (p=0.004). Those that reported boiling their water before consumption harbored far fewer parasites species on average (*M*=0.6) than those who didn't boil their water (*M*=1.5). Species richness was not associated with age, gender, recent gastrointestinal parasite treatment, distance to house or farm, or hunting; however, larger families did tend to have more parasite species, but not significantly so (r=0.26, p=0.06).

When individual parasite species were considered, additional associations were identified (Table 2). Age of participants was inversely associated with the presence of *Capillaria* sp. (p=0.004). Those positive for *Capillaria* sp. averaged 13.7 years of age and were significantly more likely to be infected, while uninfected participants averaged 31.2 years (OR=9.2; 95% CI: 2.0-40.0; Table 3). Individuals that boiled their water were significantly less likely to harbor *Capillaria* sp. (p=0.05), and *Blastocystis* sp. (p=0.002). Those who boiled their water were never positive for *Capillaria* sp. (0/9), while 31.3% of those who did not boil their water were positive (10/32). Ninety four percent (93.8%) of those that didn't boil their water were found to have

Blastocystis sp. versus 44.4% of those who did boil before consumption. This equates to an increased likelihood of becoming infected with *Blastocystis* sp. if individuals don't boil their water (OR=12.0; 95% CI: 1.7; 83.8; Table 3).

Individuals with the ascarid species averaged 11.0 family members, but individuals without averaged 7.5 members (p=0.000). Those who described themselves as living within 1 km of monkeys had lower ascarid prevalence (4.4%) than those further from monkeys (75% prevalence; p=0.00). Treatment with anti-parasitic medication within the previous year was negatively associated with the presence of *Capillaria* sp. (F=3.175, p=0.05). No effect of treatment on other parasite species was found. Individuals positive for *Capillaria* sp. averaged 10.6 family members while uninfected individuals averaged 7.2 family members (p=0.04).

4. Discussion

This study identified the following risk factors associated with gastrointestinal parasitism in people living in rural Ecuadorian communities: not boiling water before consumption, age, family size, proximity of monkeys to people and farms, and no previous administration of anti-parasitic drugs. Six gastrointestinal parasite species were found throughout the sampled communities, including *Entamoeba* spp., *Blastocystis* sp., Ascarididae gen. sp., *Balantidium* sp., *Capillaria* sp. and *Strongyloides* sp. All have been previously described in Ecuadorian communities with the exception of *Capillaria* sp. (Table 2; [41], [42], [43], and [44]. No cases of *Capillaria* sp. have been described in South American communities, though numerous studies have described *Trichuris* spp. infections [42] and [43]. For this reason we amplified and sequenced a portion of the small unit ribosomal DNA for analysis and found that the closest genetically related species was *Capillaria xenopi* (91% similarity) versus 73.6% sequence

similarity to *Trichuris trichuria*. At this time, I cannot determine if this is a unique species of *Capillaria* limited to this area as we only have observations on eggs and only 8 nominal species have DNA sequence in GenBank. Regardless, our genetic evidence would suggest this is not a *Trichuris* species.

The majority of people in our study (87.0%) were infected with at least one gastrointestinal parasite. The most common parasite found among the sampled population was *Blastocystis* sp. (81.5%). Several other studies in South America have found lower levels of *Blastocystis* sp., ranging from 22.4-45.0% [23], [45], and [46]. We surmise that higher prevalence of *Blastocystis* sp. in our study is likely due to our use of both genetic and morphological methods for identification. However, because people rarely treated their water, this might also explain the high percentage of *Blastocystis* infections.

Those who boiled their water were less likely to be infected with *Capillaria* sp. and *Blastocystis* sp. In these sampled communities and much of Ecuador, the majority of water is obtained from rivers [47], and is rarely boiled or treated with chlorine [9]. Source waters have been shown to harbor gastrointestinal parasites due to poor sanitation efforts from surrounding communities, and domestic and wildlife fecal contamination [47]. Water from uncontaminated sources does not necessarily mean improved drinking quality though, as treating water at the source leaves multiple opportunities for contamination while collecting, transporting and storing the water [48]. Improvements in source water quality may be undermined by contamination at the point of use, making treatment within the house necessary either through heat, UV radiation, chemical treatment, sedimentation, or filtration [47], [48], and [49].

The presence of specific parasite species was also significantly associated with age, family size, proximity of monkeys to people and farms, and previous administration of anti-

parasitic drugs (Table 2). Everyone positive for *Capillaria* sp. was 28 years or younger, with the highest proportion found in 10-19 year cohort (41.7%) and 0-9 year cohort (40%). A similar relationship was found in several other studies where teenagers had the highest prevalence, potentially due to poor hygiene, or increased densities and contact rates in school settings [50]. Our study found no difference in overall parasite species richness across age groups, though there was a negative trend. Others have found age to be significantly correlated, negatively, with intestinal parasitism [51] and [52].

Larger family size was also associated with a significantly higher prevalence of *Capillaria* sp. when compared to smaller families. Similarly, those with Ascarididae gen. sp. were more likely to be found in families with seven or more individuals ($p=0.000$). Other studies have also reported positive associations between family size and parasitism [53,61].

In our study, those that had previously been treated with anti-parasitic medication were as likely to harbor each parasite species as those who had not taken treatment in the prior year, with the exception of *Capillaria* sp. Even in cases where periodic anti-parasitic treatment is administered, there is an increased likelihood of re-infection as time progresses [29], which might explain why the questionnaire did not reveal significant differences. The other possibility is that de-worming efforts may not be as effective if other public health measures aren't taken as well; for instance, addressing poverty, environmental destruction, hygiene education, and improved sanitation [54]. As a specific example, *A. lumbricoides* was found to persist at high levels despite anti-parasitic treatment [55].

The majority of people in our study (83.3%) described having mantled howler monkeys within 1 km of their house, or 1 km of their farm (68.5%). Contrary to our expectations, detection of Ascarididae gen. sp. and *Capillaria* sp. was more likely in people who described

having no monkeys living near their home or farm, respectively. If there was parasite transmission between wildlife and people, we would have expected higher prevalence in those living near forests. Higher prevalence in those further from forests could conceivably be due to higher densities of people and increased contact rates, lack of fresh water, and reduced sanitation [56]. We are not aware of any studies that have quantified distance between sylvatic populations and people when assessing likelihood of transmission, though evidence of identical bacterial strains has been found in people and chimpanzees living in close proximity [57].

Several risk factors were not significantly associated with parasite prevalence, including gender, hunting activity, or distance to the forest (Table 2). Based on previous research, we did not expect a difference between the sexes [53]. We did expect people who were in contact with wildlife, such as hunters, to carry more parasites [58]. All reported hunters were infected with *Blastocystis* sp. (N=6) while 82.9% of those who didn't hunt were infected, but these differences were not significant (p=0.40). The number of hunters was rather small which likely inhibited our ability to detect any difference.

Admittedly, our sample size is relatively small (N=54) simply because these particular rural, tropical communities numbered only 122 people – many of which were too young to give assent. This means that risk factors which were found to have no effect on parasite communities might indeed have an impact that was not detectable statistically due to small sample size (Type 2 error). Nonetheless, our results did align with previous findings with two exceptions (Table 1). On average, people with *Entamoeba* sp. were slightly older (thought not significantly), which is in contrast to two other studies which found younger children had higher infection rates [23] and [62]. Secondly, the presence of *Capillaria* sp. was associated with younger participants (<28 years) in our study, which has not been reported before. Nominal *Capillaria* species number over

300 throughout the world, and have not been reported in South America communities or wild primates. Making a comparison with other studies from other parts of the world would generally be irrelevant because *Capillaria* life cycles are so diverse and risk of infection would vary accordingly.

Gastrointestinal parasitic infections tend to be chronic, untreated, and largely a problem of developing countries, though areas at greatest risk are those with limited access to sanitation and clean water [59]. Most people in our study (87%) were infected with either Ascarididae gen. sp., *Capillaria* sp., *Strongyloides* sp, or *Entamoeba* spp. which means that many will likely experience some form of associated clinical disease. The result of chronic gastrointestinal parasitic disease goes well beyond immediate health impacts though, as there is ample evidence to suggest that children infected with gastrointestinal parasites can see reductions in growth [17], [19], malnutrition [22] and reduced cognitive function [16], [20], and [21]. The developmental impact can influence everything from physical fitness, school performance, absenteeism at school (or work), decreased work capacity and productivity [24] – all factors that equate to an economic impact as well as social impediment to an already disadvantaged populace [8] and [54]. Avoiding new gastrointestinal parasite infections through education and well-targeted preventative healthcare programs by focusing on those at greatest risk has the potential to reduce parasitic disease and drastically improve education and quality of life.

This study provides baseline data on gastrointestinal parasitism and analyzes factors that might predispose people to specific parasite species in tropical communities. We find our results to be useful in two ways. First, information on intrinsic factors (e.g. age and family size) can help us better understand which groups are at greatest risk of acquiring certain parasites, allowing limited resources to be focused onto those most susceptible in communities. Secondly,

information on extrinsic factors that can be adjusted through education, policy, or infrastructure development (e.g. human behavior, water treatment and sanitation efforts, ecological disturbances) can also be used to help focus efforts in areas with limited funding.

5. Conclusions

Parasite species richness per individual (p=0.004) and presence of *Blastocystis* sp. (p=0.002) were significantly associated with boiling water before consumption. Presence of *Capillaria* sp. was negatively associated with age of participants (p=0.004), boiling water (p=0.05), and treatment with anti-parasitic medication within the previous year (p=0.05), while larger family size (p=0.04) was positively associated with infection. The results of this study demonstrate that there is an increased risk of acquiring certain gastrointestinal parasites for people who don't boil their water, haven't been treated with some form of chemotherapy or anti-helminthic drug, live in larger families, hunt wildlife, live further from monkeys, and are relatively young.

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Fig. 1. Field research took place in Northwestern Ecuador (A) at the Bilsa Biological Station - highlighted with diagonal lines (B). Several small communities surround the ecological reserve, containing numerous families (black dots) (C). A single main road runs loops from La Ye de la Laguna to the local communities and back.

Table 1. Gastrointestinal parasites reported from previous studies in Ecuadorian communities. Route of infection, general clinical characteristics, reported prevalences, and risk factors are provided for each parasite.

		Risk Factors				
Parasite	Route of Infection	Age	Family Size	Water	Anti-parasitic regime	Other reported associations
Entamoeba spp. (specifically *E. histolytica*)	Ingestion of mature cysts.	Higher in 5-15 years,[62] and 9-10 years.[23]	No effect.[66]	Chlorine Treatment[29]; Source (river) has no effect.[9]	Increased prevalence within 1 month after treatment.[29] Chlorine.[29]	Dirt floors.[43] Sanitation[29]; Wash Hands Before Eating[29]; Wash Vegetables[29]; Number domestic animals[9]; Adequate garbage disposal.[9]
Balantidium sp.	Passed in feces. Infection by ingestion of mature cysts.	No affect.[41]	ND	ND	ND	ND
Capillaria spp.	Dependent on species; no reports in S. America.	ND	ND	ND	ND	ND
Ascaris sp.	Passed in feces. Infection by ingestion of eggs.	Highest in teenagers.[13] Increased with child's age.[61] No effect.[16]	No effect.[16,]	Chlorine Treatment.[29]	Rapidly increased prevalence within 6 months after treatment.[29]	Race[65]; Sanitation[55]; Health education[16,31]; Wash Hands Before Eating[29]; Wash Vegetables[29]; Mother's Level of Education[67,69]; Socioeconomic status.[67]
Blastocystis sp.	Passed in feces. Infection by ingestion of mature cysts.[63,64]	Highest in 9-10 years.[23] No effect.[68]	No effect.[68]	Highest in untreated water compared to boiling or filtered.[66]	75% still negative after 6 months.	ND
Strongyloides sp.	Roundworm enters host through exposed skin.	Highest in 3-24 months.[61] In adults, highest in 21-30 years of age.	Higher in smaller families.[61]	ND	ND	Socioeconomic status.[65]

ND: no data available

Table 2. Univariate analysis of risk factors associated with gastrointestinal parasites in two Ecuadorian communities.

Risk Factor	N	*Entamoeba* spp. N (%)	*Balantidium* sp. N (%)	*Capillaria* sp. N (%)	Ascarididae *gen. sp.* N (%)	*Blastocystis* sp. N (%)	*Strongyloides* sp. N (%)
Age group (years)							
0-9	10	1 (10)	0 (0)	4 (40.0)	1 (10.0)	7 (70.0)	0 (0)
10-19	12	1 (8.3)	1 (8.3)	5 (41.7)	2 (16.7)	12 (100.0)	0 (0)
20-29	8	1 (12.5)	0 (0)	2 (25.0)	1 (12.5)	8 (100.0)	1 (25.0)
30-39	5	1 (20.0)	0 (0)	0 (0)	1 (20.0)	3 (60.0)	0 (0)
40-49	11	3 (27.3)	0 (0)	0 (0)	1 (9.1)	8 (72.7)	0 (0)
50-59	4	1 (25.0)	0 (0)	0 (0)	1 (25.0)	3 (75.0)	0 (0)
60-69	0	ND	ND	ND	ND	ND	ND
70-79	1	0 (0)	0 (0)	0 (0)	0 (0)	1 (100.0)	0 (0)
Unreported	3	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)	0 (0)
Total	54	8 (14.8)	1 (1.9)	11 (20.4)	7 (13.0)	44 (81.5)	1 (1.9)
		p=0.36	NA	p=0.004*	p=0.89	p=0.68	NA
Gender							
Male	25	4 (16.0)	1 (4.0)	3 (12.0)	2 (8.0)	18 (76.0)	0 (0)
Female	29	4 (13.8)	0 (0)	8 (27.6)	5 (17.2)	26 (89.7)	1 (3.4)
		p=0.56	NA	p=0.14	p=0.28	p=0.09	NA
Family Size							
1-3	13	2 (15.4)	0 (0)	2 (15.4)	0 (0)	11 (84.6)	1 (16.7)
4-6	9	2 (22.2)	1 (11.1)	0 (0)	0 (0)	6 (66.7)	0 (0)
7-9	9	0 (0)	0 (0)	1 (11.1)	2 (22.2)	7 (77.7)	0 (0)
10-12	10	4 (40.0)	0 (0)	1 (10.0)	2 (20.0)	7 (70.0)	0 (0)
13-15	13	0 (0)	0 (0)	7 (53.8)	3 (23.1)	13 (100.0)	0 (0)
		p=0.09**	NA	p=0.04*	p=0.00*	p=0.35	NA
Treated in Last Year							
Yes	19	3 (15.8)	1 (5.3)	2 (10.5)	1 (5.3)	14 (73.7)	0 (0)
No	27	4 (14.8)	0 (0)	9 (33.3)	4 (14.8)	24 (88.8)	1 (6.3)
Unreported	8	1 (12.5)	0 (0)	0 (0)	2 (25.0)	6 (75.0)	0 (0)
		p=0.98	NA	p=0.05*	p=0.36	p=0.39	NA
Boil Water							
Yes	9	1 (11.1)	0 (0)	0 (0)	0 (0)	4 (44.4)	0 (0)
No	32	3 (9.4)	0 (0)	10 (31.3)	4 (12.5)	30 (93.8)	1 (3.1)
Unreported	13	4 (30.8)	1 (7.7)	1 (7.7)	3 (23.1)	10 (76.9)	0 (0)
		p=0.18	NA	p=0.05*	p=0.30	p=0.002*	NA
Monkeys Near House (<1km)							
Yes	45	7 (15.6)	1 (2.2)	9 (20.0)	2 (4.4)	38 (84.4)	1 (2.2)
No	4	1 (25.0)	0 (0)	0 (0)	3 (75.0)	3 (75.0)	0 (0)
No Response	5	0 (0)	0 (0)	2 (40.0)	1 (20.0)	3 (60.0)	0 (0)
		p=0.56	NA	p=0.34	p=0.00*	p=0.51	NA
Monkeys Near Farm (<1km)							
Yes1	37	4 (10.8)	0 (0)	9 (24.3)	5 (13.5)	31 (83.8)	0 (0)
No0	8	4 (50.0)	1 (12.5)	0 (0)	1 (12.5)	7 (87.5)	1 (12.5)
No Farm	2	0 (0)	0 (0)	2 (100.0)	1 (50.0)	1 (50.0)	0 (0)
No Response	7	0 (0)	0 (0)	0 (0)	0 (0)	5 (71.4)	0 (0)
		p=0.02*	NA	p=0.38	p=0.96	p=0.21	NA

Hunt Wildlife							
Yes	6	1 (16.7)	0 (0)	1 (16.7)	0 (0)	6 (100.0)	0 (0)
No	41	7 (17.1)	1 (2.4)	8 (19.5)	6 (14.6)	33 (80.5)	1 (2.4)
No Response	7	0 (0)	0 (0)	2 (28.6)	1 (14.3)	5 (71.4)	0 (0)
		p=0.51	NA	p=0.84	p=0.62	p=0.73	NA
How Often Hunt							
Everyday	0	ND	ND	ND	ND	ND	ND
2-3 Per Week	3	0 (0)	0 (0)	1 (33.3)	0 (0)	3 (100.0)	0 (0)
Once Per Week	0	ND	ND	ND	ND	ND	ND
Once per Month	1	0 (0)	0 (0)	0 (0)	0 (0)	1 (100.0)	0 (0)
Never	41	7 (17.1)	1 (2.4)	8 (19.5)	6 (14.6)	33 (80.5)	1 (2.4)
No Response	9	1 (11.1)	0 (0)	2 (22.2)	1 (11.1)	7 (77.8)	0 (0)
		p=0.98	NA	p=0.99	p=0.99	p=0.97	NA
Distance from Forest (m)							
≤100m	27	2 (7.4)	0 (0)	8 (29.6)	2 (7.4)	23 (85.2)	0 (0)
>100m	19	3 (15.8)	0 (0)	2 (10.5)	3 (15.8)	16 (84.2)	1 (5.3)
Unreported	8	2 (25.0)	1 (12.5)	1 (12.5)	1 (12.5)	6 (75.0)	0 (0)
		p=0.11	NA	p=0.25	p=0.90	p=0.38	NA

* p-value <0.05; ** p-value <0.10. Not applicable due to small sample size (NA)

Table 3. Logistic regression analysis of *parasite* infections and potential risk factors with odds ratios (OR) and confidence intervals (CI).

Parasite	Characteristics	*Positive*	*Negative*	OR	95% CI	p
Entamoeba spp. (N=8)						
	Age in Years (Mean)	32.6	26.4	0.6	0.1-3.5	0.56
	Gender - Female N (%)	4 (50)	25 (54)	0.8	0.2-3.8	0.82
	Family Size (Mean)	8.0	6.9	1.6	0.3-7.0	0.57
	Treated Last Year N (%)	3 (38)	16 (48)	0.9	0.2-4.7	0.93
	Boil Water N (%)	1 (13)	8 (17)	0.8	0.0-9.1	0.88
	Distance from Forest (m)	1123.0	408.1	0.4	0.1-2.8	0.38
Capillaria sp. (N=11)						
	Age in Years (Mean)	13.7	31.2	9.2	2.0-40.0	0.004*
	Gender - Female N (%)	3 (27)	21 (46)	2.8	0.7-12.0	0.17
	Family Size (Mean)	10.6	7.6	0.3	0.0-1.3	0.10
	Treated Last Year N (%)	2 (18)	17 (40)	4.3	0.8-22.6	0.09
	Boil Water N (%)	NA	0 (0)	NA	NA	NA
	Distance from Forest (m)	598	454.6	3.6	0.7-19.2	0.14
Ascarididae gen. sp. (N=7)						
	Age in Years (Mean)	27.6	27.3	1.6	0.3-8.2	0.57
	Gender - Female N (%)	2 (29)	24 (51)	2.4	0.4-13.6	0.32
	Family Size (Mean)	NA	0 (0)	NA	NA	NA
	Treated Last Year N (%)	1 (14)	18 (38)	3.1	0.3-30.5	0.36
	Boil Water N (%)	NA	0 (0)	NA	NA	NA
	Distance from Forest (m)	508.6	333.3	0.7	0.1-3.7	0.64
Blastocystis sp. (N=44)						
	Age in Years (Mean)	27.0	29.4	1.2	0.3-5.6	0.79
	Gender - Female N (%)	26 (58)	3 (33)	3.4	0.8-14.8	0.11
	Family Size (Mean)	8.2	6.3	0.5	0.1-2.1	0.33
	Treated Last Year N (%)	15 (33)	4 (44)	2.1	0.4-10.9	0.36
	Boil Water N (%)	5 (11)	4 (44)	12	1.7-83.8	0.01*
	Distance from Forest (m)	409.4	911.4	1.1	0.2-5.5	0.93

Not applicable (NA). * p-value <0.05. *Balantidium* sp. and *Strongyloides* sp. removed from analyses due to insufficient sample size.