

EFFECTS OF ECOLOGICAL DISTURBANCE ON PARASITE COMMUNITIES IN BOTH
PEOPLE AND MANTLED HOWLER MONKEYS (*ALOUATTA PALLIATA*
AEQUATORIALIS) LIVING IN ECUADOR

by

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A dissertation
submitted in partial fulfillment
of the requirements for the
Doctor of Philosophy Degree
State University of New York
College of Environmental Science and Forestry
Syracuse, New York
October 2014

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Acknowledgements

I owe many thanks to those who supported me throughout this chapter of my life. Without my family this would not have happened. Thank you especially to: Linda and Ed Garwol, Bill and Sandy Helenbrook, Jen and Aaron Woloszyn, Anna and Ashley Helenbrook, Jeff and Jennifer Drozdowski, Joe and Rose Gala, Debbie and Paul Smolak, Richard Drozdowski, The Verhaagh family, Dave and Debby Helenbrook, Lou and Charlie Hamilton, Chris and Betsy Cain, my extended Helenbrook family, and Dennis, Sandy, and Derek Suarez. And I absolutely couldn't have done this without the lovely and talented Jessica Suarez. Thank you all.

I would like to thank my friends and colleagues – new and old – who I've met along the way, along with all those who contributed to my crowd funding campaign: Brooke and John Zale, Emily Ogburn, Scott Sveiven, Jim Arrigoni, Whitney Lash, Kat DeVilbiss and Kuma, Kim McEathron, Eric Bauer, Becky Fuda, Andrea Thomen, Alex Poisson, Greg Russo, Eric Ungberg, Jesse Robinson, Lewis Grove, Kapil Mandrekar, Carrie Levine, Rebecca May, Jen Yantachka, Carolyn Chang, Emily Gavard, Brette Quale, Andy Kraus, Clint and Lisa Battista, Bill Stelmack, Dave Botticelli, Angie Vitale Tivona Rinoni, Tim McCoy, Stephen Easley, Greg Haines, Jessica Rowland, Fatoumata Jobe, Sandi Dineen, Selena Jattan, Allison Devlin, Lin Perez, Kelly Weinersmith, Lindsay Denhoff, Kelly Fitzsimmons, Katie Volpe Baker, Jimmy MacCarthy, Jennifer Swanson, Lisette Arellano, Joy Falzarano, Amanda Kozlowski, Carie Battista, Stephanie Higgins, Selena Jattan, and Steve Langdon.

Thank you to everyone in the Whipps Lab and all the lab and field research assistants who made this possible: Jenna Sanford, Brook Clemons, Amanda Diebel, Emma Steigerwald, Yelena Prusakova, Sara Glen, and Torin Heavyside.

Thank you to Professors Steve Stehman, James Gibbs, Melissa Fierke, and Tom Horton for their support and input on field and statistical methodologies. Many thanks to all the other researchers and professors who I've had the pleasure of working with over the years, including Profs. Susan Wade, Larry Wolf, and Amy McMillan.

I'm indebted to the co-directors at Bilsa Biological Station, Juliet Bermingham and Carlos Aulestia, who were invaluable in helping develop field methodologies, in obtaining permits and conveying their overall knowledge about the Bilsa rainforests.

Last but not least, thank you to my incredibly patient and encouraging academic fathers: Dr. Chris Whipps and Bill Shields. Each of them was instrumental in helping mold me into the researcher and scientist that I am today. I can only hope to thank them enough by doing my best to follow their example as exemplary researchers and teachers.

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Abstract

W. D. Helenbrook. Effects of ecological disturbance on parasite communities in both people and mantled howler monkeys (*Alouatta palliata aequatorialis*) living in Ecuador, 227 pages, 14 tables, 16 figures, 2014.

Understanding the relationship between anthropogenic disturbances and wildlife gastrointestinal parasite communities is important to both human health and conservation efforts. Forest logging and fragmentation, burgeoning human population growth, wildlife extraction, and expansion of livestock into formerly undisturbed landscapes can affect and compound the transmission of various pathogens between wildlife and people. This study therefore aims to further understand the relationship between two types of anthropogenic disturbance (forest degradation and human encroachment), and gastrointestinal parasite communities in both humans and mantled howler monkeys, *Alouatta palliata aequatorialis* by addressing the following: 1) chronicle primate parasitism, 2) investigate association of environmental degradation and parasitism, and 3) assess human attributes and actions associated with parasitism and potential transmission between human and howler monkey populations. Human and monkey endoparasite communities were characterized using morphological and genetic analyses, and people from surrounding communities were administered demographic surveys to evaluate risk factors associated with parasitism. Of 96 howler monkey fecal samples collected, 2 species of apicomplexan, 6 other protozoa, 4 nematodes, and 1 platyhelminth were detected. Four congeners were found in howlers and people: *Entamoeba* sp., *Balantidium* sp., *Blastocystis* sp., and *Strongyloides* spp. Several key parasites were non-randomly distributed throughout the sampled population. Proximity of agricultural plots and a local biological research station were both associated with the presence of *Strongyloides* spp. Individuals were more than four times likely to harbor *Strongyloides* spp. if they lived in areas considered disturbed forest. Individuals infected with *Controrchis* sp. were found further from human settlements than uninfected individuals and nearly ten times more likely to be found in primary forest. No evidence of shared *Blastocystis* subtypes were found between howlers and people, though *Capillaria* sequence types were similar, suggesting either zoonotic transmission or a common source. Several significant human factors were associated with parasite communities. The results from this study support the hypothesis that anthropogenic disturbances can place both primate populations and humans at risk of select gastrointestinal parasites. Aside from the various direct impacts of anthropogenic disturbances, additional focus should be placed on the indirect effects changing ecological systems have on parasite communities in threatened hosts.

Key Words: Mantled howler monkeys, *Alouatta palliata aequatorialias*, zoonotic, gastrointestinal parasitism, Ecuador, human epidemiology, *Cyclospora*, *Isospora*, *Balantidium*, *Blastocystis*, *Chilomastix*, *Dientamoeba*, *Entamoeba*, *Iodamoeba*, *Enterobius*, *Capillaria*, *Strongyloides*, *Trypanoxyuris*, *Controrchis*, structural equation modeling, species richness, group size, nematodes, apicomplexan, protozoa, platyhelminth, zoonoses.

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CHAPTER 1: INTRODUCTION

OVERVIEW

Anthropogenic disturbances throughout the world have had a profound impact on the abiotic and biotic environment (Rosa et al., 2004; Rosenzweig et al., 2008; Wilcox and Murphy, 1985; Gibson et al., 2011), affecting both human and wildlife populations (Chapman and Peres, 2001; Patz et al., 2005). Climate change, large scale rain-forest destruction, intensified farming, invasive species introduction, land-use change, as well as the bushmeat trade are some of the most serious human impacts which have been inextricably tied to globalization, exponential population growth, and increased resource use (Harvell et al., 2002; McMichael, 2004; Mora et al., 2011). Consequences of rampant worldwide environmental degradation affect biological systems at the ecosystem, community, population, and organismal level (Daszak et al., 2000; Gibbons et al., 2000; Acevedo-Whitehouse and Duffus, 2009; Gillespie et al., 2005). These ecological disturbances have placed wildlife in peril worldwide, affecting health and fitness (Acevedo-Whitehouse and Duffus, 2009; Junge et al., 2011), behavior (Cristobal-Azkarate and Arroyo-Rodriguez, 2007), influencing disease risk (Daszak et al., 2000; Harvell et al., 2002), and driving species to extinction (Cowlshaw, 1999; Ceballos and Ehrlich, 2002; Michalski and Peres, 2005). In unprecedented fashion, humans are transforming the world as we know it, bringing increased uncertainty of future adaptability to environmental change (McMichael et al., 2006; Scholze et al., 2006).

Anthropogenic disturbance and parasite communities

The effect anthropogenic disturbances have on parasite communities and associated emerging infectious diseases is particularly troubling, as ecological and environmental changes have the potential to accelerate the spread of pathogenic communities within already existing

populations, or to increase zoonotic transmission between people, wildlife and domestic animals (Schrag and Weiner, 1995; Daszak et al., 2000; Patz et al., 2000; Daszak et al., 2001; Pavelka et al., 2003; Wells et al., 2007; Puttker et al., 2008; Cristobal-Azkarate et al., 2010; Schwitzer et al., 2010; Trejo-Macias and Estrada, 2012). The majority of these newly emerging human infectious diseases are caused by pathogens that are considered zoonotic (Cleaveland et al., 2001; Taylor et al., 2001; Bengis et al., 2004), often spilling-over from wildlife populations into surrounding communities (Daszak et al., 2001; Anita et al., 2003; Eisenberg et al., 2007). Specific to the tropics, 40% of infectious parasitic diseases in humans originate in wild primates (Wolfe et al., 2007) whose habitat is under intense environmental degradation (Achard et al., 2002). In the case of primate populations, humans, livestock, or other domestic and wild animals can also be a source for pathogen transmission (Daszak et al., 2000; Goldberg et al., 2008). The result is that we are likely to see increased zoonotic transmission as humans expand into previously uninhabited areas and as forest habitats are modified (Chapman et al., 2005; Wells et al., 2007; Gillespie et al., 2005; Daszak and Cunningham, 2003; Puttker et al., 2008), the result of which could lead to mortality and morbidity in both people and wildlife (McMichael, 2004; McMichael et al., 2006).

Anthropogenic disturbances such as increasing human encroachment of tropical forests, logging (and subsequent fragmentation, habitat degradation, and habitat loss), ecotourism, livestock introduction, and agriculture are all likely sources for changing parasite communities in both people and primates throughout the world, as illustrated in Figure 1.1 (Daszak et al., 2000; Daszak et al., 2001; McCallum 2002; Patz et al., 2004; Chapman et al., 2005; Goldberg et al., 2007; Wells et al., 2007; Smith et al., 2009; Valdespino et al., 2010).

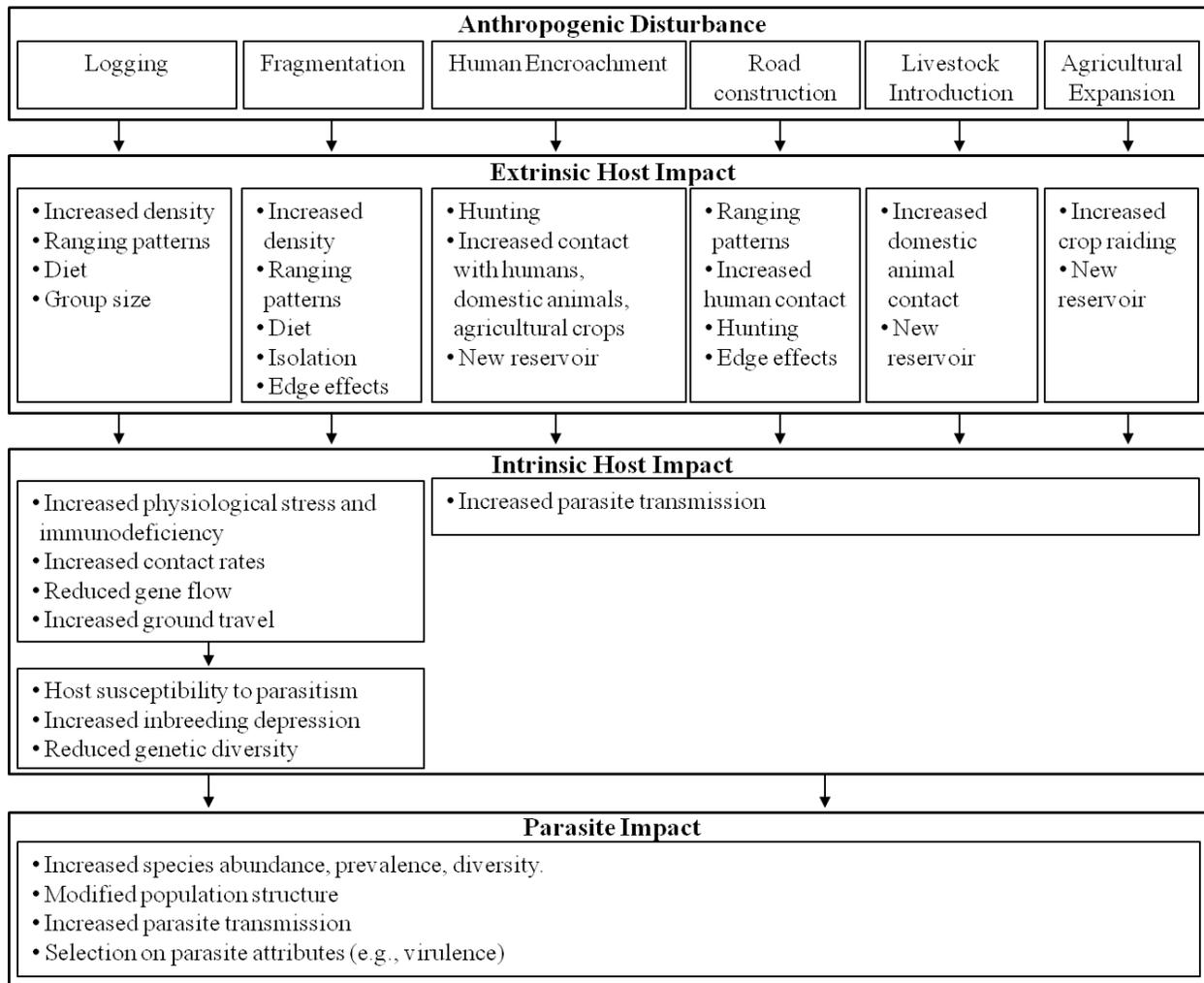


Figure 1.1. Hypothesized anthropogenic disturbances and associated impact on host and parasite communities. For any one ecological disturbance there are numerous potential pathways and impacts (both extrinsic and intrinsic). The end result is a modification of parasite communities. Relevant to this study, primates are largely thought to be directly impacted by forest degradation and loss, and hunting. However, these same disturbances can have an indirect impact on primate parasitism.

Human encroachment

Humans and primates interacting in the wild have been shown to share genetically similar parasites, suggesting that activities that increase interaction or overlap of humans and wildlife can lead to parasite transmission (Muriuki et al., 1998; Wolfe et al., 1998; Gillespie et al., 2005; Goldberg et al., 2008; Rwego et al., 2008). Likewise, introduction of livestock can provide a new zoonotic reservoir that places wildlife at risk or vice versa, and agricultural expansion into

rainforests can increase the likelihood of parasite transmission (Daszak et al., 2000; Goldberg et al., 2008). The majority of studies assessing the degree of parasite transmission between primates, humans, and domestic livestock have focused primarily on microparasite communities (i.e., viral and bacterial) (Wolfe et al., 1998; Wallis and Lee, 1999; Bastone et al., 2003; Chapman et al., 2005; Rwego et al., 2008), though some studies have compared macroparasite transmission (i.e., protozoa, nematodes and platyhelminths) between wildlife and people or domestic livestock (Ashford et al., 1990; Muriuki et al., 1998; Wallis and Lee, 1999; Lilly et al., 2002; Teichroeb et al., 2009; Kowalewski et al., 2010; Pederson and Davies, 2010; Wenz et al., 2010; Messenger et al., 2014). To our knowledge, none of these studies have genetically compared macroparasite communities in both wild neotropical monkeys and people, and none have paired this information with the risk associated with increased anthropogenic disturbance.

Forest degradation and habitat loss

Primate habitat modifications can lead to changes in parasite communities, a potential cause of increased transmission into human populations and subsequent associative infectious disease (Chapman et al., 2005; Wells et al., 2007; Gillespie et al., 2005; Daszak and Cunningham, 2003; Puttker et al., 2008). Selective logging and forest fragmentation can contribute to changes in parasite abundance, prevalence, and richness, either through increased or modified ranging patterns, increased edge effects, or altered habitat characteristics which increase exposure to various gastrointestinal parasite species (McCallum and Dobson, 2002; Fahrig, 2003; Gillespie et al., 2005; Gillespie et al., 2008; Trejo-Macias et al., 2007). Reduction of habitat can force animals into smaller areas, increasing the likelihood of direct transmission as host densities increase. Fragmented habitats may also drive selection by favoring smaller primate groups due to limited carrying capacity (Onderdonk and Chapman, 2000; Irwin, 2008). In turn, these small, isolated populations can lead to inbreeding depression (Chang et al., 2012) and

potentially increase their susceptibility to parasitism (O'Brien and Evermann, 1998; Charpentier et al., 2008). Monkeys along forest edges also show a noticeable increase in intensity of infection compared to those primates in undisturbed forest (Chapman et al., 2006a). These same monkeys are more likely to raid agricultural land, potentially increasing transmission of parasites between themselves and humans. Dietary stress may also contribute to increased parasitism in logged forests, as howler monkeys (*Alouatta* spp.) living in fragmented habitats have higher cortisol levels which is associated with suppression of the immune system – potentially increasing their susceptibility to new infections (Koski and Scott, 2001; Martinez-Mota et al., 2007).

POPULATION DYNAMICS

Primate behavior

If we look at causal pathways between anthropogenic disturbance and parasitism, a complicated, dynamic set of interactions involving modified primate behaviors is found. Forest fragmentation and degradation are associated with increased primate contact rates and host densities, and decreased home ranges (Pinto et al., 1993; Gillespie and Chapman, 2008; Mbora et al., 2009). Other behavioral changes of primate species living in logged forests include increased resting and decreased travel and feeding – all of which can be attributed to reductions in preferred food types (Johns, 1986). Bigger groups are also found in undisturbed forest, areas with increased tree and food density, and high quality food patches (Dunbar, 1987; Wrangham et al., 1993; Chapman et al., 1995; Peres, 1997; Chapman and Chapman, 2000; Struhsaker et al., 2004; Gillespie et al., 2005; Snaith and Chapman, 2007; Irwin, 2008); however, in some primate species, differences in group size between logged and unlogged forests were not always supported (Gillespie et al. 2005; Gillespie and Chapman, 2008). Reduced high quality food sources, often associated with degraded habitats, were found to negatively impact daily travel,

foraging area, time spent feeding, and dietary diversity – all factors that likely have an impact on parasite communities (John, 1986; Nunn and Dokey, 2006; Harris et al., 2010).

Group size

Increased parasite species richness has been documented in larger groups, those with reduced home ranges, and in higher density populations (Freeland, 1979; Nunn et al., 2003). Unfortunately, the effect may or may not be real, as some of these studies fail to account for sampling effort - larger groups are expected to have more parasites simply because more individuals are sampled. Individuals do have a higher contact rate with other conspecifics and increased likelihood of being infected with directly transmitted gastrointestinal parasites in larger groups (Altizer et al., 2003; Chapman, 2008). Presumably, maintaining groups could be advantageous against the spread of parasitism if clustering of individuals reduced the likelihood of an infected individual entering a new group (Black, 1966; Freeland, 1976; Freeland, 1979; Wilson et al., 2003). Other benefits of large groups include predator detection, ability to outcompete other groups for prized resources, and available mates (Schaik, 1983; Loehle, 1995; Koenig, 2002; Altizer et al., 2003; Majolo et al., 2008). Conversely, larger group limitations include increased intra-group parasite transmission along with higher localized host density, agonistic interactions, competition for food resources, and stress levels, larger travel requirements in search of food, greater detection by predators from increased travel, reduced infant development, longer interbirth interval in females, and higher levels of infanticide (Hamilton, 1971; Wrangham, 1980; Janson and van Schaik, 1988; Cote and Poulin, 1995; Sterck et al., 1997; Nunn et al., 2006; Borries et al., 2008; Majolo et al., 2008). Group size is positively associated with overall parasite species richness across primate species, and correlated with helminth diversity and protozoan species richness (Nunn et al., 2003); though, modeling by Wilson et al. (2003) suggests that per capita risk of infection from directly transmitted parasites

is actually decreased in larger groups. Mbora et al. (2009) found smaller groups had higher parasite prevalence and species richness, though the results were not significant. Intensity of infections has also been linked to increases in group size (Stoner and Di Pierro, 2006). If forest degradation influences group size (Onderdonk and Chapman, 2000), then changes in group size could in turn affect parasite abundance, prevalence and species richness. Alternatively, forest degradation and fragmentation could influence parasite communities, which in turn could regulate group size.

Associations between modified ranging and feeding behaviors with parasitism are not as clear. Monkeys living in disturbed forest have been reported to travel further, have larger home ranges, and spend more time feeding (Harris et al., 2010; Mbora et al., 2009). Presumably reduced food abundance in disturbed forests led smaller groups to travel further, which led individuals to more diverse environments, subsequently increasing their contact with additional parasites. However, overall parasite species richness and specifically helminth species richness all increase with reduced home range size, suggesting that infection is correlated with use of contaminated areas and not distance travelled (Nunn and Tae-Won Dokey, 2006; Bordes et al., 2009). In areas with habitat fragmentation, home ranges are reduced, population density increases, and groups are more likely to revisit areas where they've previously defecated or traverse on the ground (and increase exposure to soil-transmitted gastrointestinal parasites), resulting in increased parasite species richness and greater helminth and protozoan diversity.

Higher host population densities are expected to increase contact rates of directly transmitted gastrointestinal parasite species between infected and uninfected individuals and increase the number of available hosts in a particular area (Stuart et al., 1990; Arneberg et al., 1998; Morand and Poulin, 1998; Arneberg, 2001; Nunn et al., 2003; Gillespie et al., 2008). Higher howler monkey densities were also associated with larger group sizes, which helps

explain the higher contact rates (Fedigan et al., 1998). Prevalence and abundance of specific gastrointestinal parasite species are also correlated with host density in mammals (Arneberg et al., 1998; Arneberg, 2001). Across primate species, a positive relationship was found between population density and overall parasite species richness, helminth diversity, and protozoan richness (Nunn et al., 2003; Mbona et al., 2009). This trend was seen across several studies; though in some cases, no relationship was found (Gillespie et al., 2005). If forest degradation influences host densities, then modified parasite communities are expected.

Diet may also play a role in host-parasite communities. Folivores consume a larger volume of resources compared to frugivores, which places them at increased risk of acquiring parasites (Nunn et al., 2006). If forest degradation influences quality and desirable food sources, then this could influence group size, host density, and subsequently impact the risk of acquiring certain parasites (Wrangham et al., 1993). Folivorous primates living in areas with reduced food sources exhibited increased daily path length, spent more time feeding, and increased physiological costs which were associated with elevated parasite loads (Harris et al., 2010). If nutritional resources are suboptimal or greater risks are taken to acquire these resources, then the animals may become stressed or have less energy to invest in reproduction. Changes to diet associated with forest degradation were also found to suppress the immune system leading to increased parasitic burden (Munck et al., 1984; Chapman et al., 2005; Chapman et al., 2006b).

PARASITIC DISEASE

Humans

Parasitism and associated infectious diseases play a critical role in both non-human primate conservation and human health throughout evolutionary history (McCallum and Dobson, 1995; Daszak et al., 2000; Monath, 2001; McMichael, 2004; Chapman et al., 2005; Altizer et al.,

2007), causing massive mortality and morbidity from viral infections (HIV, hepatitis C, yellow fever), bacterial infections (e.g., *Mycobacterium tuberculosis* and *Vibrio cholerae*), protozoans (e.g. *Plasmodium* spp., and *Trypanosoma* spp., *Giardia* sp., *Entamoeba histolytica*), and nematodes (e.g., *Trichuris* sp., *Strongyloides* spp., *Ascaris* sp.) (Pickering, 2003). Today, the impact may be less pronounced in some more developed areas, yet billions are still affected worldwide - helminths alone infecting one-quarter of all humans worldwide (Miguel and Kremer, 2004). The impact of parasitic disease is particularly problematic in tropical climates of the developing world where some of the most prolific – and most neglected - gastrointestinal pathogens are found, including *Taenia* spp. (50 million), *Ascaris lumbricoides* (1.2 billion), *Trichuris trichiura* (795 million), *Ancylostoma duodenale* (1.2 billion), *Strongyloides* spp. (30-100 million), *Necator americanus* (~740 million), *Giardia intestinalis* (2.8 million), *Entamoeba histolytica* (50 million), and *Cryptosporidium* spp. (Asaolu and Ofoezie, 2003; Haque, 2007; Hotez et al., 2007; Olsen et al., 2009).

Reports on gastrointestinal parasitism in tropical communities are widespread and the effect such infections have on people has been documented quite extensively, both from a physical and developmental standpoint (e.g., Dickson et al., 2000; Boeke et al., 2010). Helminth infections may impair appetite, leading to varying degrees of malnourishment (Stephensen et al., 2000; Capello, 2004). Gastrointestinal malabsorption may arise from *Ascaris* infections and hookworms. Impaired cognitive function has been shown to be associated with hookworm infection in some cases (Jardin-Botelho et al., 2008), while others showed no impairment (Dickson et al., 2000). A meta-analysis of thirty different studies did find anthelmintic treatment to be positively associated with weight gain, height and other anthropometric measurements (Dickson et al., 2000).

Non-human primates

In the case of non-human primates, parasitic disease can exacerbate an already precarious situation for endangered species or even well established species (Milton, 1996; Chapman et al., 2005; Mborá et al., 2009). The vast majority of primate studies have focused on the effects of microparasitic disease (i.e., viral and bacterial) and ectoparasitism (e.g., ticks and fleas) on primate health (Crockett, 1998; Koontz et al., 1994; Wallis and Lee, 1999; Leendertz et al., 2006), while far fewer have studied the effect of macroparasites (i.e., protozoa, nematodes and platyhelminths) (Kuntz, 1982; Nunn and Altizer, 2005; Pedersen et al., 2005; Cormier, 2010). The focus on viruses is likely due to the immediate and observable effect they have on primate populations, including massive die-offs that have occurred due to outbreaks such as yellow fever which caused 50% mortality in Panamanian howler monkey population over a 20 year period, and Ebola outbreaks in chimpanzee and gorillas that likely resulted in tens of thousands of deaths (Collias and Southwick, 1952; Huijbregts and Wachter, 2003; Chapman et al., 2005). In large part, the impact of macroparasites on primate populations has largely focused on behavioral effects such as sexual selection strategies, avoidance behaviors, and self-medication (Hart, 1990; Huffman, 1997).

MANTLED HOWLER MONKEYS, *ALOUATTA PALLIATA*

In order to test the relationship of anthropogenic disturbance and parasitism in both a non-human primate species and people, I chose a primate species that is found throughout the Neotropics, often in areas with extensive ecological disturbance, and frequently living sympatrically with people. The genus *Alouatta* consists of 6 species and 22 subspecies spanning most of Central and South America, making it the most widely distributed neotropical primate (Crockett and Eisenberg, 1987; Peres, 1997; Cortes-Ortiz et al., 2003). Five subspecies of

mantled howler monkeys, *Alouatta palliata*, are recognized, *Alouatta palliata palliata* found in Honduras, Nicaragua, Costa Rica, Panama, and El Salvador; *Alouatta palliata mexicana* found in southern Mexico and Guatemala; *Alouatta palliata aequatorialis* found in Panama, W. Colombia, W. Ecuador and N.W. Peru; *Alouatta palliata trabeata* and *Alouatta palliata coibensis* found solely in Panama (Crockett, 1998; IUCN, 2012).

Howlers are one of the largest (4-9kg) arboreal, neotropical monkeys, where females weigh roughly 84% of adult males (Nagy and Milton, 1979; Thorington et al., 1979). Group size in *Alouatta palliata* can exceed 40 individuals, though average size is 14 individuals (Cuaron et al., 2008). Fluctuations in group size are primarily dependent on the number of females in a group, as the number of adult males tends to stay constant (Fedigan et al., 1998). Both sexes disperse as juveniles (Glander, 1992); however, in a single population of Costa Rican howlers, males tend to disperse earlier than females and spend four times as long solitarily (Fedigan et al., 1998). Home ranges for howlers are generally less than 25 ha and can be as small as 10 ha (Crockett and Eisenberg, 1987). The upper density limits of howlers are mainly restricted by food abundance within their habitat (Butynski, 1990). Specifically, *Alouatta palliata* density estimates range widely from 1.2 individuals per km² to 91.7 individuals per km² across several studies summarized in Crockett (1998).

Like the vast majority of primate species worldwide, the most pressing problems facing neotropical monkey species are loss of forested habitat, fragmentation (Struhsaker, 1972; Fedigan et al., 1998; Sih et al., 2000; Chapman and Peres, 2001; Mittermeier et al., 2006; Cristobal-Azkarate and Arroyo-Rodriguez, 2007; Hansen et al., 2008; Peres et al., 2010), and over-harvesting (Peres, 1990). Other non-anthropogenic effects on howler populations include natural disasters, predation, intra-specific aggression, and disease (Peres, 1997). Howlers have a strong ecological tolerance (e.g., occupy multiple vegetation types, broad diet, able to survive

and reproduce in disturbed landscapes), which likely explains their wide range of occupied habitats including seasonal to non-seasonal forest types (Baumgarten and Williamson, 2007), tropical to dry rainforest, and pre-montane and lower montane forests to high terra firma forest (Crockett, 1998). This adaptability also allows them to flourish in degraded habitat and regenerating forests, though they may be limited by colonizing trees that are largely wind-dispersed and thus harbor few fruits (Janzen, 1988; Crockett, 1998; Fedigan et al., 1998). Consumption of a diverse set of food sources is another reason howlers are likely to adapt to changing forests (Chapman, 1987; Crockett and Eisenberg, 1987; Boyle, 2008; Bicca-Marques and Calegario-Marques, 1994; Cristobal-Azkarate and Arroyo-Rodriguez, 2007). Young leaves are the favored food source (25-79%), and they also eat fruit (21.5%) and flowers (12.6%) when available (Julliot and Sabatier, 1993; Crockett, 1998). A largely folivorous diet also allows howlers to live in smaller fragmented patches, unlike frugivores which need much larger home ranges to gather sufficient resources (Gilbert and Setz, 2001). In the absence of hunting, howlers are capable of surviving in fragmented and disturbed forests and in close proximity to people (Crockett, 1998 and Fedigan et al., 1998). Several life history traits also tend to buffer howler populations from some ecological disturbance, including a short birth interval (Mean, $M = 19.9$ months), year round breeding, an early age of sexual maturity ($M = 36$ months in females and 42 months in males), and long life span ($M = 20-25$ years) (Glander, 1980; Fedigan and Rose, 1995; Crockett, 1998).

Parasite community

An array of gastrointestinal macroparasites have been found in several howler monkey species throughout their home ranges, many of which are pathogenic and zoonotic (Table 1.1, Appendix 1). Gastrointestinal parasite species of particular interest are those that are zoonotic,

have expanded in response to ecological and environmental disturbance in other studies, and those that have the potential to affect humans and wildlife health.

The majority of the intestinal gastroparasite species previously reported in mantled howler monkeys have also been reported in humans, with the exception of *Chilomastix* sp., *Retortamonas* sp., *Trypanoxyuris* sp., *Parabronema* sp., *Physaloptera* sp., *Controrchis* sp., and *Prosthenorchis elegans* (Table 1.1). Pathogenicity of these parasites in primates is largely unknown simply due to fewer autopsies of wildlife and lack of direct evidence outside the laboratory (Evans, 1976; Hanson, 1988). There is morphological evidence that similar parasite species have been found in sympatric human and other non-human primate populations (Muriuki et al., 1998; Wallis and Lee, 1999; Lilly et al., 2002; Teichroeb et al., 2009; Wenz et al., 2010; Messenger et al., 2014). Evidence of gastrointestinal parasite transmission to and from howler monkeys and people is limited to two studies which showed a higher prevalence of *Giardia* sp. at sites associated with livestock and/or humans, suggesting cross-species transmission (Vitazkova and Wade, 2006; Kowalewski et al., 2011). However, the only direct evidence of gastrointestinal parasite transmission involving primates is limited to bacterial exchange between people and chimpanzees in Uganda (Goldberg et al., 2007; Rwego et al., 2008; Johnston et al., 2010).

Only four of the gastrointestinal parasite species found in previous mantled howler monkey studies - *Giardia* sp., *Strongyloides* spp., *Trypanoxyuris* sp., and *Controrchis* sp. – varied in response to anthropogenic disturbances (Table 1.1). The relationship between human and livestock and *Giardia* sp. has previously been mentioned. *Controrchis* sp. are more likely to be found in disturbed habitat, while *Trypanoxyuris* sp. are more likely to be found in undisturbed habitat (Vitazkova and Wade, 2007).

Table 1.1. Previously described gastrointestinal parasites and potential pathogenicity in howler monkey species. Zoonotic potential represents those parasites found in both howler monkeys and people.

Taxon	Genus	<i>Alouatta palliata</i>	Zoonotic Potential	Associated with anthropogenic changes in <i>Alouatta</i> spp.	Citation
Apicomplexa					
	<i>Cyclospora</i> sp.		x		1-3
	<i>Isospora</i> sp.	x	x		4-7,35
	<i>Toxoplasma</i> sp.	x	x		8-10
	<i>Cryptosporidium</i> spp.		x		3
Other Protozoa					
	<i>Blastocystis</i> sp.		x		6,11,12
	<i>Giardia</i> sp.	x	x	x	5,13-16,18
	<i>Entamoeba</i> spp.	x	x		6,7,13,14,18
	<i>Balantidium</i> sp.		x		8
	<i>Iodamoeba butschlii</i>		x		11,19
	<i>Chilomastix</i> sp.				11,13
	<i>Retortamonas</i> sp.	x			13
Nematode					
	<i>Strongyloides</i> spp.	x	x		3,7,11,18,36
	<i>Trichuris</i> spp.		x		8,11,16
	<i>Enterobius</i> sp.		x		18
	<i>Dipetalonema</i> sp.		x		20
	<i>Ascaris</i> spp.	x	x		7,19,22
	<i>Trypanoxyuris</i> sp.	x		x	7,8,14,15,18,22-27,36
	<i>Parabronema</i> sp.	x			7,18,28,29,30
	<i>Trichostrongyloides</i> sp.		x		6
	<i>Physaloptera dilatata</i>	x			23
Platyhelminth					
	<i>Controrchis biliophilus</i>	x		x	7,14,15,18,26,31-32,36
	<i>Raillietina</i> spp.	x	x		18,29,33,34
Acanthocephala					
	<i>Prosthenorchis elegans</i>	x			29
Bacteria					
	<i>Escherichia coli</i>		x		6

1. Bern et al. (2002); 2. Karanja et al. (2007); 3. Roncancio-Duque and Benavides Montano (2013); 4. Hendricks (1977); 5. Stuart et al. (1990); 6. Stoner and Gonzalez (2006); 7. Cristobal-Azkarate et al. (2010); 8. Stiles (1929); 9. Frenkel and Sousa (1983); 10. Molina et al. (2014); 11. Phillips et al. (2004); 12. Parkar et al. (2007); 13. Hegner (1935); 14. Vitazkova and Wade (2006); 15. Vitazkova and Wade (2007); 16. Kowalewski et al. (2010); 17. Sa et al. (2013); 18. Trejo-Macias et al. (2007); 19. Eckert et al. (2006); 20. Thoisy et al. (2001); 21. Kowalewski and Gillespie (2009); 22. Inglis (1961); 23. Huggins (1963); 24. Stuart (1998); 25. Prieto et al. (2002); 26. Valdespino et al. (2010); 27. Gonzalez-Hernandez et al. (2014); 28. Van Thiel (1925); 29. Thatcher and Porter (1968); 30. Stoner (1996); 31. Gomes and Pinto (1978); 32. Gonzalez (1983); 33. Baylis (1947); 34. Dunn (1963); 35. Teichroeb et al. (2009); 36. Maldonado-Lopez et al. (2014).

Numerous *Alouatta palliata* gastrointestinal parasites across multiple primate species were more prevalent during the wet season, including *E. histolytica/dispar*, *Giardia* sp., *Isospora* sp., *Ascaris* sp., *Enterobius* sp., *Strongyloides* sp., and *Trichuris* sp. (Teichroeb et al., 2009; Valdespino et al., 2010; Cristobal-Azkarate et al., 2010; Trejo-Macias and Estrada, 2012).

Blastocystis sp. prevalence was higher during the dry season (Stoner and Gonzalez, 2006), and conflicting results were found for *Trypanoxyuris minutes* and *E. coli* (Vitazkova and Wade, 2006; Cristobal-Azkarate et al., 2010).

FIELD AND LABORATORY DIAGNOSTIC EVALUATION

Non-invasive fecal sampling is a particularly useful method for assessing gastrointestinal parasitic infections through both morphological analyses of cysts, eggs and adult worms, and by means of PCR-based amplification of parasite DNA (Stoner, 1996; Verweij et al., 2004). Zinc poly-vinyl alcohol (PVA) is considered the gold-standard for long-term storage of parasites for morphological analysis (Garcia et al., 1993). For host and parasite DNA preservation, RNAlater® has been proven effective, providing significant advantages over alternative methods because it is transportable via commercial airlines and provides high quality DNA yields (Helenbrook, 2006).

Several morphological methods can be used to maximize retrieval of gastrointestinal parasites, including fecal smears, flotations, and sedimentations (Hendricks, 2006). Combined results from each methodology can then be pooled to assess presence or absence of particular parasites species. Once parasite specimens have been isolated, identification of parasite species based solely on morphological methods can be difficult for several reasons: species may appear morphologically similar but are genetically distinct (i.e., cryptic species), certain life stages are not available in samples, making identification more difficult, or they are simply scarce and can't be consistently retrieved. In these cases, traditional microscopic techniques can be supplemented by DNA based methods to improve the accuracy of species identification (e.g., McManus and Bowles, 1996; Blaxter, 2004; Bartosch et al., 2004; Monaghan et al., 2005; Blouin, 2002; Hebert

et al., 2003; Chilton, 2004; Powers, 2004; Stensvold et al., 2011). Several reviews outline the different genomic regions that can be targeted for molecular diagnoses (Power 2004).

Molecular genetic analysis of pathogen species – in particular, gastrointestinal species - generally involves two regions of the genome: the ribosomal DNA array (rDNA) and portions of mitochondrial DNA (Powers, 2004). Ribosomal genes have been extensively used as a molecular diagnostic tool, namely because some of these genes are highly conserved (i.e., small subunit, 5.8S, and large subunit), allowing for varying degrees of diagnostic value across evolutionary time scales. Other ribosomal regions are highly variable (internal transcribed spacer, ITS) and can be used for more shallow phylogenies (McManus and Bowles, 1996). Similarly, the cytochrome oxidase gene – a portion of the mitochondrial genome is also highly variable and useful for species-specific diagnostics (Besansky et al., 2003; Powers, 2004).

Difficulty arises when trying to obtain sequence data specific to a particular parasite species or individual specimen within a fecal sample that contains DNA from the host, the gastrointestinal flora of the host, consumed food, and any other environmental contamination. Primers can be specifically designed to limit amplification of other DNA present in the sample; however, in many cases there are multiple strains of the same parasite species with few differences between individuals and little information available from previous studies to help focus primer design. The Single Strand Conformation Polymorphism (SSCP) is particularly useful for finding different strains from parasites that appear morphologically similar (Gasser et al., 2006). PCR-based amplification paired with SSCP allows researchers to obtain DNA sequence which can then be compared with other parasite sequences from the same individual, among individuals, or across studies.

RESEARCH HYPOTHESIS AND OBJECTIVES

I propose to identify the gastrointestinal parasites of mantled howler monkeys, *Alouatta palliata aequatorialis*, living in a Northwestern Ecuadorian tropical pre-montane rainforest. I aim to assess the spatial distribution of gastrointestinal parasites in a local population of howler monkeys, determine if howler group size was associated with the presence of specific parasite species and species richness, and establish whether co-infection patterns or inhibitory effects occur between parasite species. To achieve these objectives I will test the following hypotheses:

Hypothesis 1 – If certain individual monkeys are more prone to parasitic infection, then there will be a non-random distribution of parasites through the sampled population.

Hypothesis 2 – If gastrointestinal parasites infecting howler monkeys are transmitted directly or via fecal-oral route, then larger groups will have more parasite species per individual.

Hypothesis 3 – If the presence of a parasite species inhibits the ability of other parasites to infect a host, or if they increase host susceptibility to secondary infections, then the presence of parasites of any two species will be more or less than expected by random distribution.

I propose to determine the role that anthropogenic disturbance plays in parasite communities of mantled howler monkeys, *Alouatta palliata aequatorialis*. I aim to assess the role that both human encroachment and forest degradation have on the presence of parasite communities. To achieve these objectives I will test the following hypotheses:

Hypothesis 4 – If human encroachment influences parasite communities in howler monkeys, then monkeys living closer to people should harbor more parasites and the prevalence of specific parasite species should be highest in those living closest to people.

Hypothesis 5 – If forest degradation influences parasite communities in howler monkeys, then monkeys living in degraded habitat should harbor more parasites species and the prevalence of specific parasite species should be highest in degraded habitats.

Hypothesis 6 – If howler monkey group size is affected by forest degradation, then I expect that groups in degraded secondary forest will tend to be smaller on average compared to those in undisturbed primary forest.

I propose to determine the risk factors associated with increased gastrointestinal parasite infection in humans. To achieve these objectives I will test the following hypotheses:

Hypothesis 7 – If individual attributes are important risk factors, then individuals who are younger, male and living in larger families will have higher parasite species richness and the prevalence of specific parasite species should be highest in these groups as well.

Hypothesis 8 – If certain behaviors are important risk factors, then individuals who have not been treated for gastrointestinal parasites within the last year, do not treat their water before consumption, and live near monkeys, forests, and other wildlife, or hunt wildlife, will have higher parasite species richness and the prevalence of specific parasite species should be highest in these groups as well.

I propose to determine whether parasite transmission occurs between humans and mantled howler monkeys living in close proximity. I aim to use molecular sequencing to determine if parasite species that were found in both people and howler monkeys morphologically, were similar in nature to one another. To achieve these objectives I will test the following hypotheses:

Hypothesis 9 - If there is parasite transmission between human and primate populations, then similar *Blastocystis* subtypes should be found in individuals of both hosts living closest to each other.

Hypothesis 10 - If there is parasite transmission between human and primate populations, then similar *Capillaria* strains should be found in individuals of both hosts living closest to each other.

This is a manuscript-style dissertation and all of the chapters included in the body of the dissertation have been prepared for publication. The second chapter, accepted in the Journal of Parasitology, reports on the gastrointestinal parasite community of a howler monkey population. The association of group size with parasite species richness and prevalence are reported, along with information on parasite co-occurrence. The third chapter addresses the association between human encroachment and forest degradation on howler monkey parasite communities. The fourth chapter, accepted in the journal Parasitology Research, reports on the presence of *Blastocystis* parasite strains in both humans and mantled howler monkeys – addressing the question of whether zoonotic parasite transmission is occurring. Chapter five reports on risk factors predisposing individuals from an Ecuadorian community to gastrointestinal parasites. Chapter six applies Structural Equation Modeling to our understanding of the dynamic relationship between environment, host, and parasite. Evidence of *Capillaria* sp. transmission between howler monkeys and people is also reported.

LITERATURE CITED

Acevedo-Whitehouse K., and A. L. Duffus. 2009. Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364: 3429-3438.

Achard, F., H. D. Eva, H. J. Stibig, P. Mayaux, J. Gallego, T. Richards, and J. P. Malingreau. 2002. Determination of deforestation rates of the world's humid tropical forests. *Science* 297: 999-1002.

Altizer, S., C. L. Nunn, P. H. Thrall, J. L. Gittleman, and J. Antonovics, A. A. Cunningham, A. P. Dobson, V. Ezenwa, K. E. Jones, A. B. Pedersen, V. Ezenwa, K. E. Jones, A. B. Pedersen, M. Poss, and J. R. C. Pulliam. 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics* 34: 517-547.

Altizer, S., C. L. Nunn, and P. Lindenfors. 2007. Do threatened hosts have fewer parasites? A comparative study in primates. *Journal of Animal Ecology* 76: 304-314.

- Anita, R., R. Regoes, J. Koella, and C. Bergstrom. 2003. The role of evolution in the emergence of infectious diseases. *Nature* 426: 658-661.
- Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 265: 1283-1289.
- Arneberg, P. 2001. An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. *Ecography*, 24: 352-358.
- Asaolu, S. O., and I. E. Ofoezie. 2003. The role of health education and sanitation in the control of helminth infections. *Acta Tropica* 86: 283-294.
- Ashford, R. W., G. D. Reid, and T. M. Butynski. 1990. The intestinal faunas of man and mountain gorillas in a shared habitat. *Annals of Tropical Medicine and Parasitology* 84: 337-340.
- Bartosch, S., A. Fite, G. MacFarlane, and M. McMurdo. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Applied and Environmental Microbiology* 70: 3575-3581.
- Bastone, P., U. Truyen, and M. Löchelt. 2003. Potential of zoonotic transmission of non-primate foamy viruses to humans. *Journal of Veterinary Medicine, Series B* 50: 417-423.
- Baumgarten, A., and G. B. Williamson. 2007. The distributions of howling monkeys (*Alouatta pigra* and *A. palliata*) in southeastern Mexico and Central America. *Primates* 48: 310-315.
- Baylis, H. A. 1947. Roundworms and flatworms from the West Indies and Surinam. *Zoological Journal of the Linnean Society* 41: 406-414.
- Bengis, R. G., F. A. Leighton, J. R. Fischer, M. Artois, T. Morner, and C. M. Tate. 2004. The role of wildlife in emerging and re-emerging zoonoses. *Revue Scientifique et Technique-Office International des Epizooties* 23: 497-512.
- Bern, C., Y. Ortega, W. Checkley, J. M. Roberts, A. G. Lescano, L. Cabrera, and M. Verastegui, R. E. Black, C. Sterling, and R. H. Gilman. 2002. Epidemiologic differences between cyclosporiasis and cryptosporidiosis in Peruvian children. *Emerging Infectious Diseases* 8: 581-585.
- Besansky, N. J., D. W. Severson, and M. T. Ferdig. 2003. DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you. *TRENDS in Parasitology* 19: 545-546.
- Bicca-Marques, J. C., and C. Calegario-Marques. 1994. Exotic plant species can serve as staple food sources for wild howler populations. *Folia Primatologica* 63: 209-211.

- Black, F. L. 1966. Measles endemicity in insular populations: critical community size and its evolutionary implication. *Journal of Theoretical Biology* 11: 207-211.
- Blaxter, M. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London Series B* 359: 669-679.
- Blouin, M. 2002. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *International Journal of Parasitology* 32: 527-531.
- Boeke, C. E., M. Mora-Plazas, Y. Forero, and E. Villamor. 2010. Intestinal protozoan infections in relation to nutritional status and gastrointestinal morbidity in Colombian school children. *Journal of Tropical Pediatrics* 56: 299-306.
- Bordes, F., S. Morand, D. A. Kelt, and D. H. Van Vuren. 2009. Home range and parasite diversity in mammals. *The American Naturalist* 173: 467-474.
- Borries, C., E. Larney, A. Lu, K. Ossi, and A. Koenig. 2008. Costs of group size: lower developmental and reproductive rates in larger groups of leaf monkeys. *Behavioral Ecology* 19: 1186-1191.
- Boyle, S. A. 2008. The effects of forest fragmentation on primates in the Brazilian Amazon. Ph.D. dissertation. Tempe, Arizona State University.
- Butynski, T. M., 1990. Comparative ecology of blue monkeys (*Cercopithecus mitis*) in high- and low-density subpopulations. *Ecological Monographs* 60: 1-26.
- Cappello, M. 2004. Global health impact of soil-transmitted nematodes. *The Pediatric Infectious Disease Journal* 23: 663-664.
- Ceballos, G., and P. R. Ehrlich. 2002. Mammal population losses and the extinction crisis. *Science* 296: 904-907.
- Chang, Z. F., M. F. Luo, Z. J. Liu, J. Y. Yang, Z. F. Xiang, M. Li, and L. Vigilant. 2012. Human influence on the population decline and loss of genetic diversity in a small and isolated population of Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*). *Genetica* 140: 105-114.
- Chapman, C. A. 1987. Flexibility in diets of three species of Costa Rican primates. *Folia Primatologica* 49: 90-105.
- Chapman, C. A., L. J. Chapman, and R. W. Wrangham. 1995. Ecological constraints on group size: an analysis of spider monkey and chimpanzee subgroups. *Behavioral Ecology and Sociobiology* 36: 59-70.
- Chapman, C. A., and S. R. Balcomb. 1998. Population characteristics of howlers: ecological conditions or group history. *International Journal of Primatology* 19: 385-403.

- Chapman, C. A., and L. J. Chapman. 2000. Constraints on group size in red colobus and red-tailed guenons: examining the generality of the ecological constraints model. *International Journal of Primatology* 21: 565-585.
- Chapman, C. A., and C. A. Peres. 2001. Primate conservation in the new millennium: the role of scientists. *Evolutionary Anthropology* 10: 16-33.
- Chapman, C., T. Gillespie, and T. Goldberg. 2005. Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology* 14: 134-144.
- Chapman, C., M. Speirs, T. Gillespie, T. Holland, and K. Austad. 2006a. Life on the edge: gastrointestinal parasites from the forest edge and interior primate groups. *American Journal of Primatology* 68: 397-409.
- Chapman, C., M. Wasserman, T. Gillespie, M. Speirs, M. Lawes, T. Saj, and T. Ziegler. 2006b. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology* 131: 525-534.
- Chapman, C., T. Saj, and T. Snaith. 2007. Temporal dynamics of nutrition, parasitism, and stress in colobus monkeys: Implications for population regulation and conservation. *American Journal of Physical Anthropology* 134: 240-250.
- Charpentier, M. J., C. V. Williams, and C. M. Drea. 2008. Inbreeding depression in ring-tailed lemurs (*Lemur catta*): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics* 9: 1605-1615.
- Chilton, N. 2004. The use of nuclear ribosomal DNA markers for the identification of bursate nematodes (order Strongylida) and for the diagnosis of infections. *Animal Health Research Reviews* 5: 173-187.
- Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 356: 991-999.
- Collias, N., and C. Southwick. 1952. A field study of population density and social organization in howling monkeys. *Proceedings of the American Philosophical Society* 96: 143-156.
- Cormier, L. A. 2010. The historical ecology of human and wild primate malarias in the New World. *Diversity* 2: 256-280.
- Cortes-Ortiz, E. Bermingham, C. Rico, E. Rodriguez-Luna, I. Sampaio, and M. Ruiz-Garcia. 2003. Molecular systematic and biogeography of the Neotropical monkey genus, *Alouatta*. *Molecular Phylogenetics and Evolution* 26: 64-81.
- Côté, I. M., and R. Poulin. 1995. Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* 6: 159-165.

Cowlishaw, G. 1999. Predicting the pattern of decline of African primate diversity: an extinction debt from historical deforestation. *Conservation Biology* 13: 1183-1193.

Cristóbal-Azkarate, J., and V. Arroyo-Rodríguez. 2007. Diet and activity pattern of howler monkeys (*Alouatta palliata*) in Los Tuxtlas, Mexico: effects of habitat fragmentation and implications for conservation. *American Journal of Primatology* 69: 1013-1029.

Cristóbal-Azkarate, J., B. Hervier, S. Vegas-Carrillo, D. Osorio-Sarabia, E. Rodríguez-Luna, and J. J. Veà. 2010. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. *Primates* 51: 231-239.

Crockett, C. M. and J. F. Eisenberg. 1987. Howlers: variations in group size and demography. *In* *Primate Societies*. B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham, and T. T. Struhsaker (eds.). University of Chicago Press, Chicago, pp. 54-68.

Crockett, C. M. 1998. Conservation biology of the genus *Alouatta*. *International Journal of Primatology* 19: 549-578.

Cuarón, A.D., A. Shedden, E. Rodríguez-Luna, P. C. de Grammont, A. Link, E. Palacios, A. Morales, and L. Cortés-Ortiz. 2008. *Alouatta palliata*. The IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Accessed 16 July 2014.

Daszak, P., A. Cunningham, and A. Hyatt. 2000. Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* 287: 443-449.

Daszak, P., A. Cunningham, and A. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* 78: 103-116.

Daszak, P., and A. Cunningham. 2003. Anthropogenic change, biodiversity loss, and a new agenda for emerging diseases. *Journal of Parasitology* 89: S37-41.

Dickson, R., S. Awasthi, P. Williamson, C. Demellweek, P. Garner. 2000. Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. *BMJ* 320: 1697-1701.

Dunbar, R. I. 1987. Habitat quality, population dynamics, and group composition in colobus monkeys (*Colobus guereza*). *International Journal of Primatology* 8: 299-329.

Dunn, F. L. 1963. Acanthocephalans and cestodes of South American monkeys and marmosets. *Journal of Parasitology* 49: 717-722.

Eckert, K., N. Hahn, A. Genz, D. Kitchen, M. Stuart, G. Averbek, B. Stromberg, and H. Markowitz. 2006. *International Journal of Primatology* 27: 227-238.

Eisenberg, J., M. Desai, K. Levy, S. Bates, S. Liang, K. Naumoff, and J. Scott. 2007. Environmental determinants of infectious disease: A framework for tracking causal links and guiding public health research. *Environmental Health Perspectives* 115: 1216-1223.

- Evans, A. S. 1976. Causation and disease: the Henle-Koch postulates revisited. *The Yale Journal of Biology and Medicine* 49: 175.
- Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 487-515.
- Fedigan, L. M., and L. M. Rose. 1995. Interbirth interval variation in three sympatric species of neotropical monkey. *American Journal of Primatology* 37: 9-24.
- Fedigan, L. M., L. M. Rose, and R. M. Avila. 1998. Growth of mantled howler groups in a regenerating Costa Rican dry forest. *International Journal of Primatology* 19: 405-432.
- Freeland, W. J. 1976. Pathogens and the evolution of primate sociality. *Biotropica* 12-24.
- Freeland, W. J. 1979. Primate social groups as biological islands. *Ecology* 60: 719-728.
- Frenkel, J. K., and O. E. Sousa. 1983. Antibodies to *Toxoplasma* in panamanian mammals. *The Journal of Parasitology* 69: 244.
- Garcia, L. S., R. Y. Shimizu, A. Shum, and D. A. Bruckner. 1993. Evaluation of intestinal protozoan morphology in polyvinyl alcohol preservative: comparison of zinc sulfate-and mercuric chloride-based compounds for use in Schaudinn's fixative. *Journal of Clinical Microbiology* 31: 307-310.
- Gasser, R. B. 2006. Molecular tools—advances, opportunities and prospects. *Veterinary parasitology* 136: 69-89.
- Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. T. Winne. 2000. The Global Decline of Reptiles, Déjà Vu Amphibians. *BioScience* 50: 653-666.
- Gibson, L., T. M. Lee, L. P. Koh, B. W. Brook, T. A. Gardner, J. Barlow, C. A. Peres, C. J. Bradshaw, W. F. Laurance, T. E. Lovejoy, and N. S. Sodhi. 2011. Primary forests are irreplaceable for sustaining tropical biodiversity. *Nature* 478: 378-381.
- Gilbert, K. A. 1994. Endoparasitic infection in red howling monkeys (*Alouatta seniculus*) in the Central Amazonian Basin: a cost of sociality? Doctoral dissertation, Rutgers University.
- Gilbert, K. A., and E. Z. F. Setz. 2001. Primates in a fragmented landscape: six species in central Amazonia. *Lessons from Amazonia: The Ecology and Conservation of a Fragmented Forest* 262-270.
- Gillespie, T., C. Chapman, and E. Greiner. 2005. Effects of logging on gastrointestinal parasite infections and infection risk in African primates. *Journal of Applied Ecology* 42: 699-707.

Gillespie, T. R., and C. A. Chapman. 2008. Forest fragmentation, the decline of an endangered primate, and changes in host–parasite interactions relative to an unfragmented forest. *American Journal of Primatology* 70: 222-230.

Glander, K. 1980. Reproduction and population growth in free-ranging mantled howler monkeys. *American Journal of Physical Anthropology* 53: 25-36.

Glander, K. E. 1992. Dispersal patterns in Costa Rican mantled howling monkeys. *International Journal of Primatology* 13: 415-436.

Goldberg, T., T. Gillespie, I. Rwego, E. Wheeler, E. Estoff, and C. Chapman. 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation* 135: 511-517.

Goldberg, T., T. Gillespie, I. Rwego, E. Estoff, and C. Chapman. 2008. Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. *Emerging Infectious Diseases* 14: 1375-1382.

Gomes, D. C., and R. M. Pinto. A contribution to the knowledge of the helminth fauna of the Amazonian Region-trematodes. *Atas da Sociedade de Biologia do Rio de Janeiro* 19: 43-46.

Gonzales, B., L. H. Paasch, and P. A. Paasch. 1983. Identificación de parasitos metazoarios en cortes histológicos. *Veterinaria México* 14: 159-174.

González-Hernández, M., A. Rangel-Negrín, V. A. Schoof, C. A. Chapman, D. Canales-Espinosa, and P. A. D. Dias. 2014. Transmission patterns of pinworms in two sympatric congeneric primate species. *International Journal of Primatology* 35: 445-462.

Hamilton, W. D. 1971. Geometry for the selfish herd. *Journal of theoretical Biology* 31: 295-311.

Hanson, R. P. 1988. Koch is dead. *Journal of Wildlife Diseases* 24: 193-200.

Hansen, M. C., S. V. Stehman, P. V. Potapov, T. R. Loveland, J. R. Townshend, R. S. DeFries, K. W. Pittman, B. Arunarwati, F. Stolle, M. K. Steininger, M. Carroll, and C. DiMiceli. 2008. Humid tropical forest clearing from 2000 to 2005 quantified by using multitemporal and multiresolution remotely sensed data. *Proceedings of the National Academy of Sciences* 105: 9439-9444.

Haque, R. 2007. Human intestinal parasites. *Journal of Health, Population, and Nutrition* 25: 387.

Harris, T. R., C. A. Chapman, and S. L. Monfort. 2010. Small folivorous primate groups exhibit behavioral and physiological effects of food scarcity. *Behavioral Ecology* 21: 46-56.

Hart, B. L. 1990. Behavioral adaptations to pathogens and parasites: five strategies. *Neuroscience and Biobehavioral Reviews* 14: 273-294.

- Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158-2162.
- Hebert, P., A. Cywinska, S. Ball, J. deWaard. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313-321.
- Hegner, R. 1935. Intestinal protozoa from Panama monkeys. *The Journal of Parasitology* 21: 60-61.
- Helenbrook, W. D. 2006. Non-invasive DNA sampling of mandrills: An evaluation of preservation and extraction techniques [thesis]. Buffalo (NY): State University College at Buffalo.
- Hendrix, C. M., and E. D. Robinson. 2006. *Diagnostic parasitology for veterinary technicians*. St. Louis: Mosby Elsevier.
- Hotez, P. J., D. H. Molyneux, A. Fenwick, J. Kumaresan, S. E. Sachs, J. D. Sachs, and L. Savioli. 2007. Control of neglected tropical diseases. *New England Journal of Medicine* 357: 1018-1027.
- Huffman, M. A. 1997. Current evidence for self-medication in primates: a multidisciplinary perspective. *American Journal of Physical Anthropology* 104: 171-200.
- Hughhins, E. J. 1969. Spirurid and oxyurid nematodes from a red howler monkey in Columbia. *Journal of Parasitology* 55: 680.
- Huijbregts, B., P. De Wachter, L. Sosthene, N. Obiang, and M. E. Akou. 2003. Ebola and the decline of gorilla, *Gorilla gorilla*, and chimpanzee, *Pan troglodytes*, populations in Minkebe Forest, north-eastern Gabon. *Oryx* 37: 437-443.
- Inglis, W. G. 1961. The oxyurid parasites (Nematoda) of primates. *In Proceedings of the Zoological Society of London*, Blackwell Publishing Ltd. 136, p. 103-122.
- Irwin, M. T. 2008. Diademed sifaka (*Propithecus diadema*) ranging and habitat use in continuous and fragmented forest: higher density but lower viability in fragments?. *Biotropica* 40: 231-240.
- IUCN 2012. The IUCN Red List of Threatened Species. Version 2012.2. <<http://www.iucnredlist.org>>. Accessed 17 October 2012.
- Janson, C. H., and C. P. Van Schaik. 1988. Recognizing the many faces of primate food competition: methods. *Behaviour* 165-186.
- Janzen, D. H. 1988. Management of habitat fragments in a tropical dry forest: growth. *Annals of the Missouri Botanical Garden* 75: 105-116.

Jardin-Botelho A., S. Raff, R. A. Rodrigues, H. J. Hoffman, J. H. Diemert, R. Correa-Oliviera, J. M. Bethony, M. F. Gazzinelli. 2008. Hookworm, *Ascaris lumbricoides* infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. *Tropical Medicine and International Health* 13: 994–1004.

Johns, A. D. 1986. Effects of selective logging on the behavioral ecology of West Malaysian primates. *Ecology* 684-694.

Johnston, A. R., T. R. Gillespie, I. B. Rwego, T. L. T. McLachlan, A. D. Kent, and T. L. Goldberg. 2010. Molecular epidemiology of cross-species *Giardia duodenalis* transmission in western Uganda. *PLoS Neglected Tropical Diseases* 4: e683.

Julliot, C., and D. Sabatier. 1993. Diet of the red howler monkey (*Alouatta seniculus*) in French Guiana. *International Journal of Primatology* 14: 527-550.

Junge, R. E., M. A. Barrett, and A. D. Yoder. 2011. Effects of anthropogenic disturbance on indri (*Indri indri*) health in Madagascar. *American Journal of Primatology* 73: 632-642.

Karanja, R. M., W. Gatei, and N. Wamae. 2007. Cyclosporiasis: an emerging public health concern around the world and in Africa. *African Health Sciences* 7: 62-67.

Koenig, A. 2002. Competition for resources and its behavioral consequences among female primates. *International Journal of Primatology* 23: 759-783.

Koontz, F., R. Horwich, R. Saqui, H. Saqui, K. Glander C. Koontz, and W. Westrom. 1994. Reintroduction of black howler monkeys (*Alouatta pigra*) into the Cockscomb basin sanctuary, Belize. *AZA Annual Conference Proceedings*, p. 104-111.

Koski, K. G., and M. E. Scott. 2001. Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annual Review of Nutrition* 21: 297-321.

Kowalewski, M. M., and T. R. Gillespie. 2009. Ecological and anthropogenic influences on patterns of parasitism in free-ranging primates: A meta-analysis of the genus *Alouatta*. In *South American Primates*, P.A. Garber et al. (eds.). Springer, New York. p. 433-461.

Kowalewski, M. M., J. S. Salzer, , J. C. Deutsch, M. Raño, M. S. Kuhlenschmidt, T. R. Gillespie. 2010. Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: patterns of zoonotic protozoa infection relative to degree of human–primate contact. *American Journal of Primatology* 71: 1-9.

Kuntz, R. E. 1982. Significant infections in primate parasitology. *Journal of Human Evolution* 11: 185-194.

Leendertz, F. H., G. Pauli, K. Maetz-Rensing, W. Boardman, C. Nunn, H. Ellerbrok, S. A. Jensen, S. Junglen, and C. Boesch. 2006. Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. *Biological Conservation* 131: 325-337.

- Lilly, A. A., P. T. Mehlman, and D. Doran. 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika research site, Dzanga-Ndoki National Park, Central African Republic. *International Journal of Primatology* 23: 555-573.
- Loehle, C. 1995. Social barriers to pathogen transmission in wild animal populations. *Ecology* 326-335.
- Majolo, B., A. de Bortoli Vizioli, and G. Schino. 2008. Costs and benefits of group living in primates: group size effects on behaviour and demography. *Animal Behaviour* 76: 1235-1247.
- Maldonado-López, S., Y. Maldonado-López, A. G. T. Ch, P. Cuevas-Reyes, K. E. Stoner. 2014. Patterns of infection by intestinal parasites in sympatric howler monkey (*Alouatta palliata*) and spider monkey (*Ateles geoffroyi*) populations in a tropical dry forest in Costa Rica. *Primates* 1-10.
- Martinez-Mota, R., C. Valdespino, M. Sanchez-Ramos, and J. Serio-Silva. 2007. Effects of forest fragmentation on the physiological stress response of black howler monkeys. *Animal Conservation* 10: 374-379.
- Mbora, D. N., J. Wieczkowski, and E. Munene. 2009. Links between habitat degradation, and social group size, ranging, fecundity, and parasite prevalence in the Tana River mangabey (*Cercocebus galeritus*). *American Journal of Physical Anthropology* 140: 562-571.
- McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology and Evolution* 10: 190-194.
- McCallum, H., and A. Dobson. 2002. Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269: 2041-2049.
- McManus, D., and J. Bowles. 1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematic. *International Journal of Parasitology* 26: 687-704.
- McMichael, A. J. 2004. Environmental and social influences on emerging infectious diseases: past, present and future. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 1049-1058.
- McMichael, A. J., Woodruff, R. E., and S. Hales. 2006. Climate change and human health: present and future risks. *The Lancet* 367: 859-869.
- Messenger, A. M., A. N. Barnes, and G. C. Gray. 2014. Reverse zoonotic disease transmission (zooanthroponosis): A systematic review of seldom-documented human biological threats to animals. *PLoS ONE* 9: e89055.
- Michalski, F., and C. A. Peres. 2005. Anthropogenic determinants of primate and carnivore local extinctions in a fragmented forest landscape of southern Amazonia. *Biological Conservation* 124: 383-396.

- Miguel, E., and M. Kremer. 2004. Worms: identifying impacts on education and health in the presence of treatment externalities. *Econometrica* 72: 159-217.
- Milton, K. 1996. Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. *Journal of Zoology* 239: 39-63.
- Mittermeier, R. A., C. Valladares-Pádua, A. B. Rylands, A. A. Eudey, T. M. Butynski, J. U. Ganzhorn, and R. Kormos, J. M. Aguiar, and S. Walker. 2006. Primates in peril: The world's 25 most endangered primates, 2004-2006. *Primate Conservation* 1-28.
- Molina, C. V., J. L. Catão-Dias, J. S. Ferreira Neto, S. A. Vasconcellos, S. M. Gennari, R. D. R. Valle, and M. G. Bueno. 2014. Sero-epidemiological survey for brucellosis, leptospirosis, and toxoplasmosis in free-ranging *Alouatta caraya* and *Callithrix penicillata* from São Paulo State, Brazil. *Journal of Medical Primatology* 43: 197-201.
- Monaghan, M., M. Balke, T. Gregory, and A. Vogler. 2005. DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions of the Royal Society B* 360: 1925-1933.
- Monath, T. P. 2001. Yellow fever: an update. *The Lancet Infectious Diseases* 1: 11-20.
- Mora, C., O. Aburto-Oropeza, A. A. Bocos, et al. 2011. Global human footprint on the linkage between biodiversity and ecosystem functioning in reef fishes. *PLoS Biology* 9: e1000606.
- Morand, S., and R. Poulin. 1998. Density, body mass and parasite species richness of terrestrial mammals. *Evolutionary Ecology* 12: 717-727.
- Munck, A., P. Guyre, and N. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews* 5: 25-44.
- Muriuki, S. M. K., R. K. Murugu, E. Munene, G. M. Karere, and D. C. Chai. 1998. Some gastrointestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica* 71: 73-82.
- Nagy, K. A., and K. Milton. 1979. Energy metabolism and food consumption by wild howler monkeys (*Alouatta palliata*). *Ecology* 475-480.
- Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative tests of parasite species richness in primates. *The American Naturalist* 162: 597-614.
- Nunn, C. L., and S. M. Altizer. 2005. The global mammal parasite database: an online resource for infectious disease records in wild primates. *Evolutionary Anthropology: Issues, News, and Reviews* 14: 1-2.
- Nunn, C. L., and A. T. W. Dokey. 2006. Ranging patterns and parasitism in primates. *Biology Letters* 2: 351-354.

- O'Brien, S. J., and J. F. Evermann. 1988. Interactive influence of infectious disease and genetic diversity in natural populations. *Trends in Ecology and Evolution* 3: 254-259.
- Olsen, A., L. van Lieshout, H. Marti, T. Polderman, K. Polman, P. Steinmann, R. Stothard, S. Thybo, J. J. Verweij, and P. Magnussen. 2009. Strongyloidiasis—the most neglected of the neglected tropical diseases?. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103: 967-972.
- Onderdonk, D. A., and C. A. Chapman. 2000. Coping with forest fragmentation: the primates of Kibale National Park, Uganda. *International Journal of Primatology* 21: 587-611.
- Parkar, U., R. J. Traub, S. Kumar, M. Mungthin, S. Vitali, S. Leelayoova, K. Morris, and R. C. A. Thompson. 2007. Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. *Parasitology* 134: 359-367.
- Patz, J. A., T. K. Graczyk, N. Geller, and A. Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30: 1395-1405.
- Patz, J., P. Daszak, G. Tabor, A. Aguirre, M. Pearl, J. Epstein, N. Wolfe, A. Kilpatrick, J. Fofopoulos, D. Molyneux, D. Bradley, and Members of the Working Group on Land Use Change and Disease Emergence. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. *Environmental Health Perspectives* 112: 1092-1098.
- Patz, J. A., D. Campbell-Lendrum, T. Holloway, and J. A. Foley. 2005. Impact of regional climate change on human health. *Nature* 438: 310-317.
- Pedersen, A. B., S. Altizer, M. Poss, A. A. Cunningham, and C. L. Nunn. 2005. Patterns of host specificity and transmission among parasites of wild primates. *International Journal for Parasitology* 35: 647-657.
- Pedersen, A. B., and T. J. Davies. 2009. Cross-species pathogen transmission and disease emergence in primates. *EcoHealth* 6: 496-508.
- Peres, C. A. 1990. Effects of hunting on western Amazonian primate communities. *Biological Conservation* 54: 47-59.
- Peres, C. 1997. Effects of habitat quality and hunting pressure on arboreal folivore densities in neotropical forests: A case study of howler monkeys (*Alouatta* spp.). *Folia Primatologica* 68: 199-222.
- Peres, C. A., T. A. Gardner, J. Barlow, J. Zuanon, F. Michalski, A. C. Lees, I. C. G. Vieira, F. Moreira, and K. J. Feeley. 2010. Biodiversity conservation in human-modified Amazonian forest landscapes. *Biological Conservation* 143: 2314-2327.
- Phillips, K. A., M. E. Haas, B. W. Grafton, and M. Yrivarren. 2004. Survey of the gastrointestinal parasites of the primate community at Tambopata National Reserve, Peru. *Journal of Zoology* 264: 149-151.

- Pickering, L. K. 2003. Red book®: 2003 report of the committee on infectious diseases (26th Edition). American Academy of Pediatrics. Pennsylvania State University, U.S.
- Pinto, L. P. S., C. M. R. Costa, K. B. Strier, and G. A. B. Da Fonseca. 1993. Habitat, density and group size of primates in a Brazilian tropical forest. *Folia Primatologica* 61: 135-143.
- Powers, T. 2004. Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* 42: 367-383.
- Prieto, O. H., A. M. S. Cruz, N. Scheibler, J. T. Borda, and L. G. Gomez. 2002. Incidence and external morphology of the nematode *Trypanoxyuris* (*Hapaloxuyuris*) *callithricis*, isolated from black-and-gold howler monkeys (*Alouatta caraya*) in Corrientes, Argentina. *Laboratory Primate Newsletter* 41: 12-14.
- Puttker, T., Y. Meyer-Lucht, and S. Sommer. 2008. Effects of fragmentation on parasite burden (nematodes) of generalist and specialist small mammal species in secondary forest fragments of the coastal Atlantic Forest, Brazil. *Ecological Research* 23: 207-215.
- Roncancio-Duque, N., and J. A. Benavides Montaña. 2013. Intestinal parasites in small and isolated population of Red-howler monkey (*Alouatta seniculus*) and Brown-spider monkey (*Ateles hybridus*), Atelidae-Primates in the Colombian Magdalena Medio. *Veterinaria y Zootecnia* 7: 71-89.
- Rosa, E. A., R. York, and T. Dietz. 2004. Tracking the anthropogenic drivers of ecological impacts. *AMBIO: A Journal of the Human Environment* 33: 509-512.
- Rosenzweig, C., D. Karoly, M. Vicarelli, P. Neofotis, Q. Wu, G. Casassa, A. Menzel, T. L. Root, N. Estrella, B. Seguin, P. Tryjanowski, C. Liu, S. Rawlins, and A. Imeson. 2008. Attributing physical and biological impacts to anthropogenic climate change. *Nature* 453: 353-357.
- Rwego, I. B., G. Isabirye-Basuta, T. R. Gillespie, and T. L. Goldberg. 2008. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. *Conservation Biology* 22: 1600-1607.
- Sá, R. M., J. Petrášová, K. Pomajbíková, I. Profousová, K. J. Petrželková, C. Sousa, J. Cable, M. W. Bruford, and D. Modrý. 2013. Gastrointestinal symbionts of chimpanzees in Cantanhez National Park, Guinea-Bissau with respect to habitat fragmentation. *American Journal of Primatology* 75: 1032-1041.
- Scholze, M., W. Knorr, N. W. Arnell, and I. C. Prentice. 2006. A climate-change risk analysis for world ecosystems. *Proceedings of the National Academy of Sciences* 103: 13116-13120.
- Schrag, S. J., and P. Wiener. 1995. Emerging infectious disease: what are the relative roles of ecology and evolution?. *Trends in Ecology and Evolution* 10: 319-324.

- Schwitzer, N., D. Clough, H. Zahner, W. Kaumanns, P. Kappeler, and C. Schwitzer. 2010. Parasite prevalence in blue-eyed black lemurs *Eulemur flavifrons* in differently degraded forest fragments. *Endangered Species Research* 12: 215-225.
- Sih, A., B. G. Jonsson, and G. Luikart. 2000. Habitat loss: ecological, evolutionary and genetic consequences. *Trends in Ecology and Evolution* 15: 132-134.
- Smith, K. F., K. Acevedo-Whitehouse, and A. B. Pedersen. 2009. The role of infectious diseases in biological conservation. *Animal Conservation* 12: 1-12.
- Snaith, T. V., and C. A. Chapman. 2007. Primate group size and interpreting socioecological models: do folivores really play by different rules?. *Evolutionary Anthropology: Issues, News, and Reviews* 16: 94-106.
- Snaith, T. V., C. A. Chapman, J. M. Rothman, and M. D. Wasserman. 2008. Bigger groups have fewer parasites and similar cortisol levels: a multi-group analysis in red colobus monkeys. *American Journal of Primatology* 70: 1072-1080.
- Stensvold, C. R., M. Lebbad, and J. J. Verweij. 2011. The impact of genetic diversity in protozoa on molecular diagnostics. *Trends in Parasitology* 27: 53-58.
- Stephenson, L. S., M. C. Latham, and E. A. Ottesen. 2000. Malnutrition and parasitic helminth infections. *Parasitology* 121: S23-S38.
- Sterck, E. H., D. P. Watts, and C. P. van Schaik. 1997. The evolution of female social relationships in nonhuman primates. *Behavioral Ecology and Sociobiology* 41: 291-309.
- Stiles, C. W., A. Hassall, and O. Nolan. 1929. Key-catalogue of parasites reported for primates (monkeys and lemurs) with their possible public health importance, and key catalogue of primates for which parasites are reported. U.S. Treasury Department, Public Health Service, Hygienic Laboratory Bulletin No. 152, p. 409-601.
- Stoner, K. E. 1996. Prevalence and intensity of intestinal parasites in mantled howling monkeys (*Alouatta palliata*) in northeastern Costa Rica: Implications for conservation biology. *Conservation Biology* 10: 539-546.
- Stoner, K. E., and A. M. G. Di Piero. 2006. Intestinal parasitic infections in *Alouatta pigra* in tropical rainforest in Lacandona, Chiapas, Mexico: implications for behavioral ecology and conservation. *In New Perspectives in the Study of Mesoamerican Primates*, E. Alejandro, P. A. Garber, M. S. M. Pavelka, L. Luecke (eds.). Spring, U.S. p. 215-240.
- Struhsaker, T. T. 1972. Rain-forest conservation in Africa. *Primates* 13: 103-109.
- Struhsaker, T. T., A. R. Marshall, K. Detwiler, K. Siex, C. Ehardt, D. D. Lisbjerg, and T. M. Butynski. 2004. Demographic variation among Udzungwa red colobus in relation to gross ecological and sociological parameters. *International Journal of Primatology* 25: 615-658.

- Stuart, M., L. Greenspan, K. Glander, and M. Clarke. 1990. A coprological survey of parasites of wild mantled howler monkeys, *Alouatta palliata palliata*. *Journal of Wildlife Disease* 26: 547-549.
- Stuart, M., V. Pendergast, S. Rumfelt, S. Pierberg, L. Greenspan, K. Glander, and M. Clarke. 1998. Parasite of wild howlers (*Alouatta* spp.) *International Journal of Primatology* 19: 493-512.
- Taylor, L., S. Latham, and M. Woolhouse. 2001. Risk factors for human disease emergence. *Philosophical Transactions Royal Society of London Series B* 356: 983-989.
- Teichroeb, J. A., S. J. Kutz, U. Parkar, R. C. Thompson, and P. Sicotte. 2009. Ecology of the gastrointestinal parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: Possible anthrozoootic transmission. *American Journal of Physical Anthropology* 140: 498-507.
- Thatcher, V. E., and J. A. Porter Jr. 1968. Some helminth parasites of Panamanian primates. *Transactions of the American Microscopical Society* 186-196.
- Thoisy, B., I. Vogel, J. M. Reynes, J. F. Pouliquen, B. Carme, M. Kazanji, and J. C. Vié. 2001. Health evaluation of translocated free-ranging primates in French Guiana. *American Journal of Primatology* 54: 1-16.
- Thorington Jr, R. W., R. Rudran, and D. Mack. 1979. Sexual dimorphism of *Alouatta seniculus* and observations on capture techniques. *Vertebrate Ecology in the Northern Neotropics*, Smithsonian Institution Press, Washington, DC, 97-106.
- Trejo-Macías, G., A. Estrada, and M. Á. M. Cabrera. 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in southern Mexico. *International Journal of Primatology* 28: 931-945.
- Trejo-Macias, G., and A. Estrada. 2012. Risk factors connected to gastrointestinal parasites in mantled *Alouatta palliata mexicana* and black howler monkeys *Alouatta pigra* living in continuous and in fragmented rainforests in Mexico. *Current Zoology* 58: 375-383.
- Valdespino, C., G. Rico-Hernández, and S. Mandujano. 2010. Gastrointestinal parasites of howler monkeys (*Alouatta palliata*) inhabiting the fragmented landscape of the Santa Marta mountain range, Veracruz, Mexico. *American Journal of Primatology* 72: 539-548.
- Van Schaik, C. P. 1983. Why are diurnal primates living in groups? *Behaviour* 120-144.
- Van Thiel, P. H. 1925. Deux nematodes nouveaux d'un singe hurleur de Surinam. *Annals Parasitology* 3: 171-182.
- Verweij, J. J., R. A. Blangé, K. Templeton, J. Schinkel, E. A. Brienen, M. A. van Rooyen, L. van Lieshout, and A. M. Polderman. 2004. Simultaneous detection of *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* in fecal samples by using multiplex real-time PCR. *Journal of Clinical Microbiology* 42: 1220-1223.

- Vitazkova, S. and S. Wade. 2006. Parasite of the free-ranging black howler monkeys (*Alouatta pigra*) from Belize and Mexico. *American Journal of Primatology* 68: 1089-1097.
- Vitazkova, S. K., and S. E. Wade. 2007. Effects of ecology on the gastrointestinal parasites of *Alouatta pigra*. *International Journal of Primatology* 28: 1327-1343.
- Vitazkova, S. K. 2009. Overview of parasites infecting howler monkeys, *Alouatta* sp., and human-howler interactions. *In Primate parasite ecology: the dynamics and study of host-parasite relationships*, M. A. Huffman and C. A. Chapman (eds.). Cambridge University Press, Cambridge, p. 371-385.
- Wallis, J., and D. R. Lee. 1999. Primate conservation: the prevention of disease transmission. *International Journal of Primatology* 20: 803-826.
- Wells, K., L. Smales, E. Kalko, and M. Pfeiffer. 2007. Impact of rain-forest logging on helminth assemblages in small mammals (Muridae, Tupaiidae) from Borneo. *Journal of Tropical Ecology* 23: 35-43.
- Wenz, A., E. W. Heymann, T. N. Petney, and H. F. Taraschewski. 2010. The influence of human settlements on the parasite community in two species of Peruvian tamarin. *Parasitology* 137: 675-684.
- Wilcox, B. A., and D. D. Murphy. 1985. Conservation strategy: the effects of fragmentation on extinction. *The American Naturalist* 125: 879-887.
- Wilson, K., R. Knell, M. Boots, and J. Koch-Osborne. 2003. Group living and investment in immune defence: an interspecific analysis. *Journal of Animal Ecology* 72: 133-143.
- Wolfe, N. D., A. A. Escalante, W. B. Karesh, A. Kilbourn, A. Spielman, and A. A. Lal. 1998. Wild primate populations in emerging infectious disease research: the missing link?. *Emerging Infectious Diseases* 4: 149-158.
- Wolfe, N., C. Dunavan, and J. Diamond. 2007. Origins of major human infectious disease. *Nature* 447: 279-283.
- Wrangham, R. W. 1980. An ecological model of female-bonded primate groups. *Behaviour* 262-300.
- Wrangham, R. W., J. L. Gittleman, and C. A. Chapman. 1993. Constraints on group size in primates and carnivores: population density and day-range as assays of exploitation competition. *Behavioral Ecology and Sociobiology* 32: 199-209.

Chapter 2

GASTROINTESTINAL PARASITES OF ECUADORIAN MANTLED HOWLER MONKEYS (*ALOUATTA PALLIATA AEQUATORIALIS*) BASED ON FECAL ANALYSIS

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ABSTRACT

An analysis of gastrointestinal parasites of Ecuadorian mantled howler monkeys, *Alouatta palliata aequatorialis*, was conducted based on parasites from fecal samples examined using fecal smears, flotations and sedimentations. At least one type of parasite was detected in 97% of the 96 fecal samples screened across 19 howler monkey groups using a combination of smear, flotation, and sedimentation techniques. Samples averaged 3.6 parasite species per individual (\pm 1.4 SD). Parasites included species representing genera of: 2 apicomplexans - *Cyclospora* sp. (18% of individual samples) and *Isospora* sp. (3%); 6 other protozoa - *Balantidium* sp. (9%), *Blastocystis* sp. (60%), *Chilomastix* sp. (4%), *Dientamoeba* sp. (3%), *Entamoeba* spp. (56%), *Iodamoeba* sp. (5%); 4 nematodes - *Enterobius* sp. (3%), *Capillaria* sp. (78%), *Strongyloides* sp. (88%) which included 2 morphotypes, *Trypanoxyuris* sp. (12%); and the platyhelminth *Controrchis* sp. (15%). A statistically significant positive correlation was found between group size and each of three different estimators of parasite species richness adjusted for sampling effort (ICE: $r^2=0.24$, $p=0.05$; Chao2: $r^2=0.25$, $p=0.05$, and Jackknife: $r^2=0.31$, $p=0.03$). Two significant associations between co-infecting parasites were identified. Based on the prevalence data, individuals infected with *Balantidium* sp. were more likely to also be infected with *Isospora* sp. ($\chi^2=6.02$, $p=0.01$), while individuals harboring *Chilomastix* sp. were less likely to have *Capillaria* sp. present ($\chi^2=4.03$, $p=0.04$).

Key words: gastrointestinal parasite, Ecuador, monkey, *Alouatta palliata*

INTRODUCTION

In the last decade an increasing number of studies have focused on parasitism in wild primate populations emanating mainly from an interest in zoonotic pathogen transmission (Muriuki et al., 1998; Pedersen et al., 2005; Howells et al., 2011), conserving host species

(Wallis and Lee, 1999; Gillespie et al., 2005; Smith et al., 2008), and ecosystem health (Marcogliese, 2005). Many of the parasitic diseases in primates are zoonotic (Daszak, 2003; Jones et al., 2008) and concern is growing that anthropogenic disturbances are changing the dynamics of these ecological systems (Morse, 1995; Patz et al., 2000; Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008). The first step in understanding this relationship is examining the parasite community of a primate that is intricately associated with humans and is often detrimentally affected by their actions, such as howler monkeys (*Alouatta* spp.).

The genus *Alouatta* consists of 6 species and 22 subspecies spanning most of Central and South America (Crockett, 1998). Five subspecies of *Alouatta palliata* are recognized, *Alouatta palliata palliata* found in Honduras, Nicaragua, Costa Rica, Panama, and El Salvador; *Alouatta palliata mexicana* found in southern Mexico and Guatemala; *Alouatta palliata aequatorialis* found in Panama, W. Colombia, W. Ecuador and N.W. Peru; *Alouatta palliata trabeata* and *Alouatta palliata coibensis* found solely in Panama (Crockett, 1998; IUCN, 2012). *Alouatta* spp. are among the largest New World primates, living in groups averaging 14 individuals and ranging from 4-21 individuals per group (Rowe, 1996; IUCN, 2012). Howlers are arboreal, spending the vast majority of their time in the forest canopy, seasonal and non-seasonal forests, in mangroves, and swamps providing key ecological services such as seed dispersal (Anzures-Dadda et al., 2011). They are largely folivorous, though it has also been shown that upwards of 40% of their diet is fruit during favorable seasons (Estrada et al., 1999; Pinto et al., 2003; Vitazkova, 2009). An indiscriminate diet is likely one of the main reasons that they are so adaptable to changing ecological landscapes, whether natural or anthropogenic.

The gastrointestinal parasite communities of several howler monkey species have been studied throughout Central and South America, including *A. palliata* (Stuart et al., 1998; Trejo-Macias, 2007), *Alouatta pigra* (Stoner and Gonzalez Di Pierro, 2006; Eckert, 2006; Vitazkova

and Wade, 2006; Trejo-Macias, 2007; Cristobal-Azkarate et al., 2010), *Alouatta caraya* (Stiles et al., 1929; Cruz et al., 2000), and *Alouatta seniculus* (Gilbert, 1994; Phillips et al., 2004). These howler monkey studies have found an array of gastrointestinal parasites including protozoans, nematodes and platyhelminths (Pope, 1966; Stuart et al., 1990; Amato et al., 2002; Godoy et al., 2004; Phillips et al., 2004; Eckert et al., 2006; Vitazkova and Wade, 2007; Cristobal-Azkarate et al., 2010; Valdespino et al., 2010). A complete literature review, including non-gastrointestinal parasites, can be found in Stuart et al. (1998) and Vitazkova (2009).

Gastrointestinal parasite communities of mantled howler monkeys (*A. p. aequatorialis*) from Ecuador have yet to be described, so one objective of this study was to establish baseline data on this threatened howler monkey subspecies. These data will subsequently be used in our long-term research project designed to study the effects of habitat disturbance (i.e., human proximity and basal area) on parasite communities in the mantled howler monkey, and to assess zoonotic transmission of gastrointestinal parasites. I anticipate that a broad array of protozoans, nematodes, and platyhelminths will be recovered based on findings from other related howler monkey studies throughout Central and South America.

We also examined the relationship between group size and gastrointestinal parasite species richness, because several studies have documented increased parasite species richness in larger groups (Freeland, 1979; Nunn et al., 2003). Individual parasitism is expected to increase with larger social groups due to higher densities and increased contact rate (Cote and Poulin, 1995; Arneberg et al., 1998; Nunn et al., 2003). Unfortunately, the effect may or may not be real, as some of these studies fail to account for sampling effort - larger groups are expected to have more parasites simply because more individuals are sampled. Modeling by Wilson et al. (2003) suggests that per capita risk of infection from directly transmitted parasites is actually decreased in larger groups, and Mborá et al. (2009) found smaller groups had higher parasite prevalence

and species richness, though the results were not significant. Presumably, maintaining groups could be advantageous against the spread of parasitism if clustering of individuals reduced the likelihood of an infected individual entering a new group (Black, 1966; Freeland, 1976; Freeland, 1979; Wilson et al., 2003). Conversely, larger group limitations include increased intra-group parasite transmission along with higher localized host density, increased stress levels, and larger travel requirements in search of food (Hamilton, 1971; Wrangham, 1980; Janson and van Schaik, 1988; Cote and Poulin, 1995; Sterck et al., 1997; Nunn et al., 2006; Borries et al., 2008; Majolo et al., 2008). If gastrointestinal parasites infecting howler monkeys are transmitted directly or via fecal-oral route, then larger groups are expected to have more parasite species per individual.

Lastly, gastrointestinal parasite species have previously been shown to interact with each other both experimentally and in the wild (Poulin, 2001; Nacher, 2011). Two main relationships have been previously reported: competitive interactions where one parasite species outcompetes another, causing an inhibitory effect (Poulin, 2001), and infection of a host by 1 parasite species increases host susceptibility to secondary infection (Murphy et al., 2013). We will investigate this phenomenon using fecal analysis to evaluate whether certain parasites co-occur or some occur in the absence of others.

METHODS

The Bilsa Biological Station, a private reserve of 3,300 ha in northwestern Ecuador (35 km west of Quininde, 0°21'N, 79°44'W), is located in a pre-montane tropical forest along the Pacific coast at an altitude of 350-576 m (Figure 2.1A and 2.1B). This area is part of the Tumbes-Choco-Magdalena bioregion and is currently under threat due to increased logging pressure, but still maintains a mixture of disturbed and undisturbed forest (Ortega-Andrade et al.,

2010). At Bilsa, 20% of the reserve is comprised of secondary forest, while only 4% of primary rainforest remains throughout the rest of Northwest Ecuador (Ortega-Andrade et al., 2010). Annual precipitation ranges from 1,500-2,000 cm, making it one of the wettest areas in the world.

Field teams consisting of two to five people collected 96 fecal samples from 19 primate groups, plus 4 samples from solitary individuals, starting June until August 2010. Primate groups were sampled along two 5-km transects at the Bilsa Biological Reserve (Figure 2.1C). Howler monkey groups were found based on vocalization or visualizations while walking transects, or by scent with the use of a dog. All primate groups found along each transect were sampled, and every effort was made to ensure that the same group wasn't re-sampled. In order to avoid re-sampling, I systematically moved down transects, noting the location of the last sampled group at night, then moving further down the transect to the next group the following morning. Knowing the location of the last sampled group ensured that individuals from that group would not be re-sampled with nearby groups the following day. It is possible that groups were missed, but our main purpose was to avoid repeat sampling. Although these transects were initially designed for use as part of a larger study to evaluate impacts of human proximity to primate groups, they also served to provide an objective protocol for detecting howler groups to include in the study. All individuals detected in a foraging group were sampled when feasible, though some individuals did not defecate or field teams could not locate a fecal sample. Three additional samples were collected away from transects near the village of Dogola (Figure 2.1C). Group size ranged from solitary to 10 individuals. Four individuals were solitary, 5 groups had 2-3 individuals, 7 groups had 4-5 individuals, 6 groups had 6-7 individuals, and one group had 10 individuals.

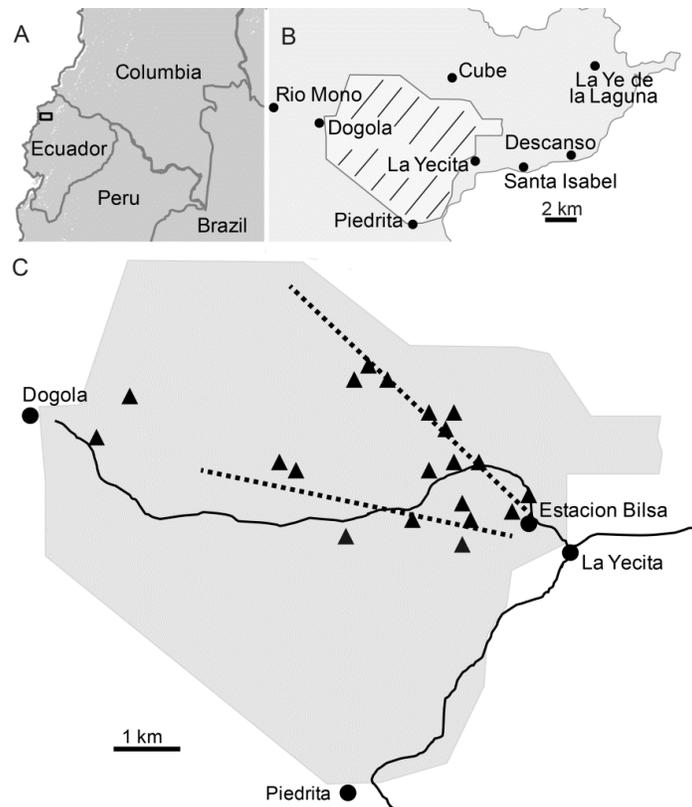


Figure 2.1. The Mache Chindul Reserve in North-western Ecuador (A) is part of the Tumbes-Choco-Magdalena bioregion and surrounds the Bilsa Ecological Station highlighted with diagonal lines (B). The field station is within a 1 km of La Yecita on the Eastern edge of the reserve. Two transects are indicated, running through both secondary and primary forest, along which mantled howler monkey groups were sampled (triangles). Communities surrounding the reserve include Dogola, Cube, La Yecita and Piedrita with additional homes found along much of the southeastern border (C).

Fecal samples were immediately collected following defecation and environmental contamination was minimized by only collecting the upper portion of the feces that had not contacted the ground and leaf litter. Location of each sample was recorded using GPS (global positioning system) and howler monkey group demographics noted. Contamination was minimized by wearing disposable gloves and collecting each sample with a new set of wooden tongue depressors to evenly distribute samples into the three 50 ml containers - zinc polyvinyl alcohol (Zn-PVA) was used to preserve the feces for parasite recovery, RNAlater® (Qiagen Inc., Valencia, California) was used to preserve parasite DNA for *Blastocystis* PCR analysis, and a third preservation solution of 50% ethanol was used for a separate study. All individuals within a

group were sampled as logistically feasible. A description of each sample was recorded and included consistency of feces, presence of blood, mucus, and macroscopic parasites.

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 Garcia (1999) and Hendrix and Robinson (2006) with the following modifications. A NaNO₃ solution (SG 1.2) was used for optimal retrieval of parasite eggs in flotations (Hendrix and Robinson, 2006). 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 (Hendrix and Robinson, 2006). I also used smears which are useful for obtaining protozoan parasites (Garcia et al., 1993). 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. An index of individual parasite abundance was made by counting the number of parasite eggs or cysts on a single cover slide at 20x magnification (22x22mm) (Hendrix and Robinson, 2006; Gillespie, 2006; Vitaskova and Wade, 2012).

A PCR-based method of detection was used to confirm the presence of *Blastocystis* sp. because it is a cryptic but common gastrointestinal parasite found in primates (Stensvold et al.,

2009). In addition, generated molecular data are being used to study *Blastocystis* phylogenetics (Chapter 4), so the data was included for completeness. For *Blastocystis* species PCR detection, DNA was extracted from approximately 200 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. *Blastocystis* spp. were confirmed using protocols and PCR primers BH1F/ BHRDr, and BLF / BLR and protocols described in Whipps et al. (2010) and Menounos et al. (2008), respectively.

Every attempt was made to ensure that duplicate samples were not collected from the same individual by attempting to identify specific monkeys prior to defecation and collecting samples immediately. However, because it is possible that more than 1 sample may have been collected for some individuals, I consider my calculations to be an index of prevalence, where an equal sampling bias exists across all individuals and groups (Chapman et al., 2011). Parasite species richness and prevalence were calculated across all samples and howler groups. In order to assess prevalence, I considered an individual positive for a parasite species if it was found for any one fecal extraction method. Prevalence values are given with 95% confidence intervals which were calculated with the modified Wald method.

The relationship of parasite species richness to howler monkey group size was evaluated as follows. Larger howler groups may be expected to harbor more parasites simply because there are more samples collected (Gregory, 1990; Walther et al., 1995; Poulin, 1998; Walther and Martin, 2001; Colwell et al., 2004; Yurkov et al., 2011). Several methods have been proposed to control for the uneven sampling effort attributable to number of individual monkeys sampled per group. I controlled for larger sample sizes in bigger groups by using three non-parametric species richness estimators, available in the software program EstimateS (Colwell, 2009), that adjust for different sampling effort, including Chao 2 (Chao, 1987), Jackknife (Chao and Lee, 1992), and Incidence - based Coverage Estimator (ICE) (Walther and Martin, 2001; Gotelli and

Colwell, 2001; Gotelli and Colwell, 2011). I investigated three adjusted species richness estimators to ensure the observed results were robust to choice of adjustment. Groups with two or fewer individuals were not included in analyses because estimates of adjusted species richness would be unreliable based on so few individuals. Estimated species richness adjusted for sampling effort was then modeled as a function of group size to assess the relationship between species richness and group size. Estimated species richness adjusted for sampling effort is a continuous variable, so ordinary least squares regression was used to quantify the association between estimated species richness and group size.

Attribute agreement analysis coupled with Cohen's kappa was used to evaluate the ability of the three methods employed to detect parasites. No gold-standard for presence of each parasite species is available, so each method was compared to the final assessment which was a summation of fecal smears, flotations, and sedimentation results. In the case of *Blastocystis* sp., I used PCR-based results as a gold standard. Co-infection was assessed using the Chi-square test of independence (with the Yates adjustment) for pairs of parasite species. Unless otherwise specified, all statistical analyses were conducted using STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, OK).

RESULTS

Almost all samples (97%) contained at least one type of gastrointestinal parasite. Parasite species richness per fecal sample ranged from 0 to 7 species. Of the 96 fecal samples, 23 had 5-7 parasite species, 68 had 2-4 parasite species, 3 had a single parasite, and 2 had no parasites detected. Sample mean was 3.6 parasite species per individual (± 1.4 SD). Thirteen parasites encompassing 13 genera were identified, representing 2 apicomplexans, 6 other protozoa, 4 nematodes, and 1 platyhelminth (Figure 2.2; Table 2.1). At least one apicomplexan was found in

20% of all samples, other protozoans were present in 88% of samples, and nematodes detected in 95% of the samples.

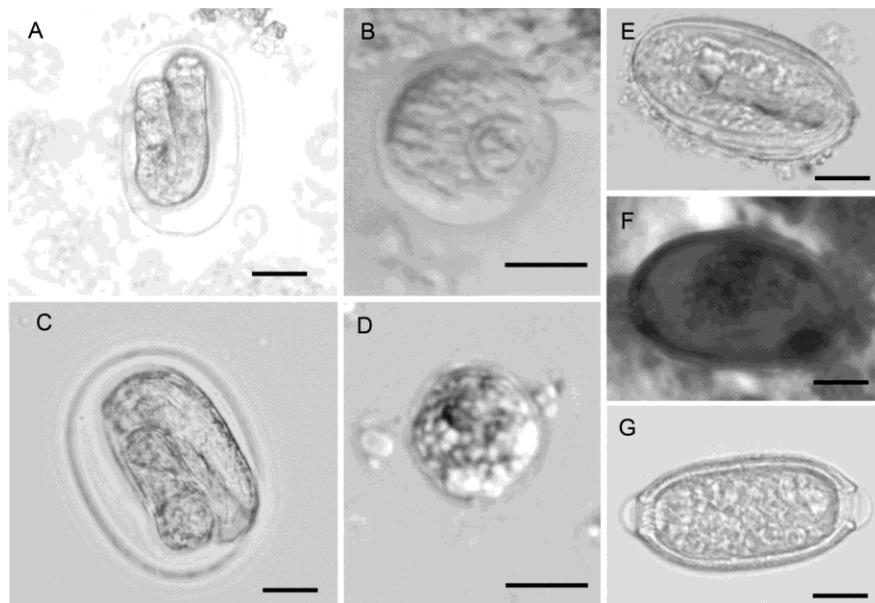


Figure 2.2. Examples of parasites and parasite eggs recovered from howler monkeys. Bar = 10 μm . (A) *Strongyloides* sp. 1 (B) *Entamoeba* sp. 1 (C) *Strongyloides* sp. 2 (D) *Entamoeba* sp. 2 (E) *Trypanoxyuris* sp. (F) *Enterobius* sp. (G) *Capillaria* sp.

Average length and range of parasites are listed in Table 2.1. Three parasite groups require additional explanation. First, *Strongyloides* spp. egg length ranged from 14.4 μm to 71.3 μm . When frequency distribution of the lengths was plotted, a bimodal curve was produced with peaks at 36 μm and at 52.8 μm (figure not shown). Over half (52%) of identified eggs fell below 48 μm in length, a reported minimum for *Strongyloides* species in howlers. The two apparent morphotypes observed were considered a single entity for the purposes of analysis here. Secondly, 58% of samples contained *Entamoeba* spp. Several morphotypes existed with cysts that averaged 12.8 μm in length and ranged from 9.2-16.3 μm . The number of nuclei within each cyst could not reliably be determined, thus all findings were categorized as *Entamoeba* spp. Lastly, PCR-based confirmation was conducted for *Blastocystis* sp. Seven percent (7%) of fecal

smears were positive, 1% of flotations, and 4% of sedimentations, while 60% were positive using PCR detection.

Recovery efficiency was dependent on fecal extraction method (Table 2.1). For example, *Strongyloides* spp. were found in 88% of samples based on all methods combined, but only 58% of smears, 3% of flotations, and 73% of fecal sedimentations were positive. Similarly, a *Capillaria* species was present in 78% of samples, yet only 64% of smears, 56% of flotations, and 38% of sedimentations were positive. Thirteen parasite species were recovered from fecal smears compared to nine species from flotations, and 10 from sedimentations. The highest parasite prevalence for six species was found using smears, while only one species had the highest prevalence from flotations, and three species from sedimentations. There is clearly a benefit to using multiple extraction methods as each is suited to recovering different parasite species (Table 2.2).

Considering the 19 howler groups with at least 2 individuals (N=19), parasite species richness varied from 3 to 11 parasite species, an averaged 3.5 species per group (± 0.6 SD; Figure 2.3). Sixteen groups (84%) harbored five or more distinct parasite species, and three groups (16%) had 2-4 species of parasite. Eleven groups (58%) exhibited at least one apicomplexan. Nematode and protozoan species were found in all other groups. Parasite species richness increased with group size, regardless of the method used to adjust for sampling effort (Figure 2.4): Jackknife ($F_{1,14} = 6.2$, $r^2 = 0.31$, $p=0.03$), Chao2 ($F_{1,14} = 4.7$, $r^2 = 0.25$, $p=0.05$), and ICE ($F_{1,14} = 4.5$, $r^2 = 0.24$, $p=0.05$).

Table 2.1. Percent parasite prevalence (P) with 95% confidence intervals (CI), mean intensity (MI) estimated as number of eggs per gram of feces using three fecal extraction techniques, and mean and range of length of eggs or cysts.

	Parasite species	Smear			Flotation			Sedimentation			Total Sampled (n=96)		Mean Length	
		P %	CI	MI	P %	CI	MI	P %	CI	MI	P %	CI	(μ m)	Range (μ m)
Apicomplexa	<i>Cyclospora</i> sp.	8.3	4-16	7.0	3.1	1-9	134.0	9.4	5-17	13.4	17.7	11-27	14.7	10.2-23.4
	<i>Isoospora</i> sp.	1.0	0-6	1.0	1.0	0-6	1.0	1.0	0-6	1.0	3.1	1-9	35.2	11.2-68.6
Other Protozoa	<i>Balantidium</i> sp.	8.3	4-16	2.4	1.0	0-6	1.0	0.0	0-5	0.0	9.4	5-17	44.5	30.1-107.3
	<i>Blastocystis</i> sp.	7.3	3-15	1.1	1.0	0-6	1.0	4.2	1-11	8.5	60.4*	60-78	18.6	14.0-33.4
	<i>Chilomastix</i> sp.	3.1	1-9	3.0	0.0	0-5	0.0	1.0	0-6	1.0	4.2	1-11	23.6	16.0-27.3
	<i>Dientamoeba</i> sp.	3.1	1-9	1.0	0.0	0-5	0.0	0.0	0-5	0.0	3.1	1-9	11.8	5.4-15.9
	<i>Entamoeba</i> sp.	55.0	45-65	6.8	2.0	0-5	1.0	7.3	2-13	1.3	56.3	46-66	12.8	9.2-16.3
	<i>Iodamoeba</i> sp.	5.2	2-12	1.0	0.0	0-5	0.0	0.0	0-5	0.0	5.2	2-12	19.2	11.2-30.1
Nematoda	<i>Enterobius</i> sp.	1.0	0-6	1.0	0.0	0-5	0.0	2.1	0-8	1.0	3.1	1-9	41.5	36.7-46.3
	<i>Capillaria</i> sp.	63.5	53-72	7.3	56.3	46-66	17.2	37.5	28-48	3.2	78.1	69-85	48.8	24.5-54.0
	<i>Strongyloides</i> sp.	58.3	48-68	3.8	3.1	1-9	1.0	72.9	63-81	4.4	87.5	79-93	44.6	14.4-71.3
	<i>Trypanoxyuris</i> sp.	6.3	3-13	1.8	1.0	0-6	7.0	6.3	3-13	6.0	11.5	6-20	42.5	41.7-43.3
Platyhelminth	<i>Controrchis</i> sp.	3.1	1-9	1.0	9.4	5-17	3.0	2.1	0-8	1.0	14.6	9-23	35.5	25.9-54.1

* *Blastocystis* confirmation using PCR-based detection.

Table 2.2. Percent agreement and Kappa statistics for parasite detection using 3 extraction methods (fecal flotation, sedimentation and smear). Aggregated data from all methods were used as the gold standard. N=96 fecal samples assessed by all three methods.

	Flotation		Sedimentation		Smear	
	Agreement %	Kappa	Agreement %	Kappa	Agreement %	Kappa
<i>Cyclospora</i>	85	0.26	92	0.65	91	0.59
<i>Isospora</i>	98	0.49	98	0.49	98	0.49
<i>Balantidium</i>	92	0.18	91	0.00	99	0.94
<i>Blastocystis</i> [†]	40	-0.02	39	-0.05	38	-0.08
<i>Chilomastix</i>	96	0.00	97	0.39	99	0.85
<i>Dientamoeba</i>	97	0.00	97	0.00	100	1.00
<i>Entamoeba</i>	44	0.03	49	0.11	97*	0.94
<i>Iodamoeba</i>	90	0.15	95	0.68	95	0.68
<i>Enterobius</i>	97	0.00	99	0.79	98	0.49
<i>Capillaria</i>	77	0.51	58	0.27	85*	0.65
<i>Strongyloides</i>	18	0.02	88*	0.63	71*	0.39
<i>Trypanoxyuris</i>	95	0.00	95	0.00	100	1.00
<i>Controrchis</i>	95	0.75	88	0.22	89	0.32

*Significantly higher prevalence than other unmarked methods based on 95% confidence interval.

[†]For *Blastocystis* sp., the gold-standard was PCR data.

Kappa values >0.81 are considered near perfect agreement, 0.41 to 0.60 corresponds to moderate agreement, and <0.20 is considered poor.

Co-infection status was evaluated for all parasite pair combinations. Two significant associations were found. Of those individuals infected with *Balantidium* sp., 22% were also infected with *Isospora* sp. which is significantly greater than expected (3%) under the hypothesis of no association between the two species ($\chi^2=6.02$, $p=0.01$). A negative association was found between *Chilomastix* sp. and *Capillaria* sp. Only 25% of the individuals infected with *Chilomastix* sp. were also infected with *Capillaria* sp., far fewer than the expected *Capillaria* sp. prevalence of 78% under the null hypothesis of no association between the two species ($\chi^2=4.03$, $p=0.04$).

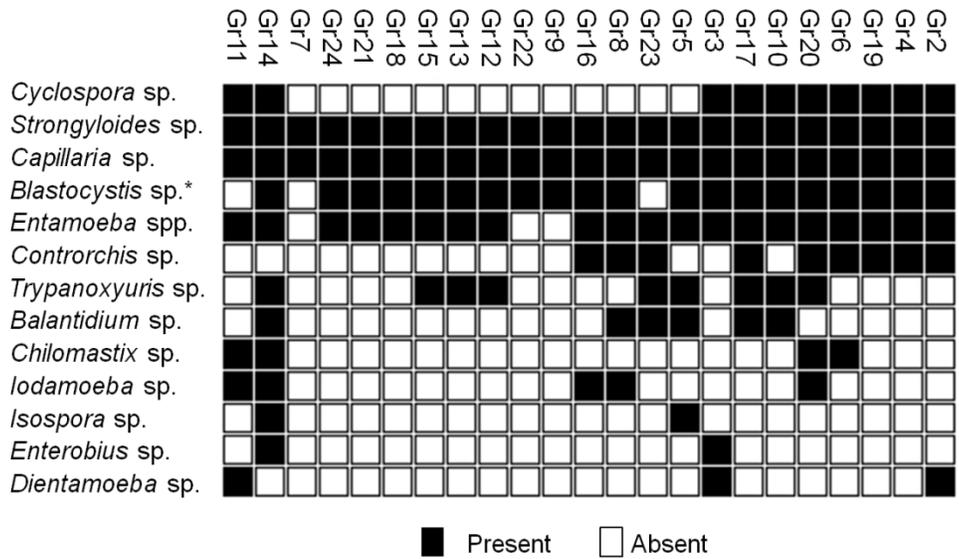


Figure 2.3. Similarity of parasite communities in howler monkey groups based on parasite species presence/absence. A group was considered positive if a parasite was found present in at least one individual using any of the three fecal extraction methods (i.e., smear, flotation, or sedimentation). *The presence of *Blastocystis* species was determined using PCR-based sequencing described in Chapter 4.

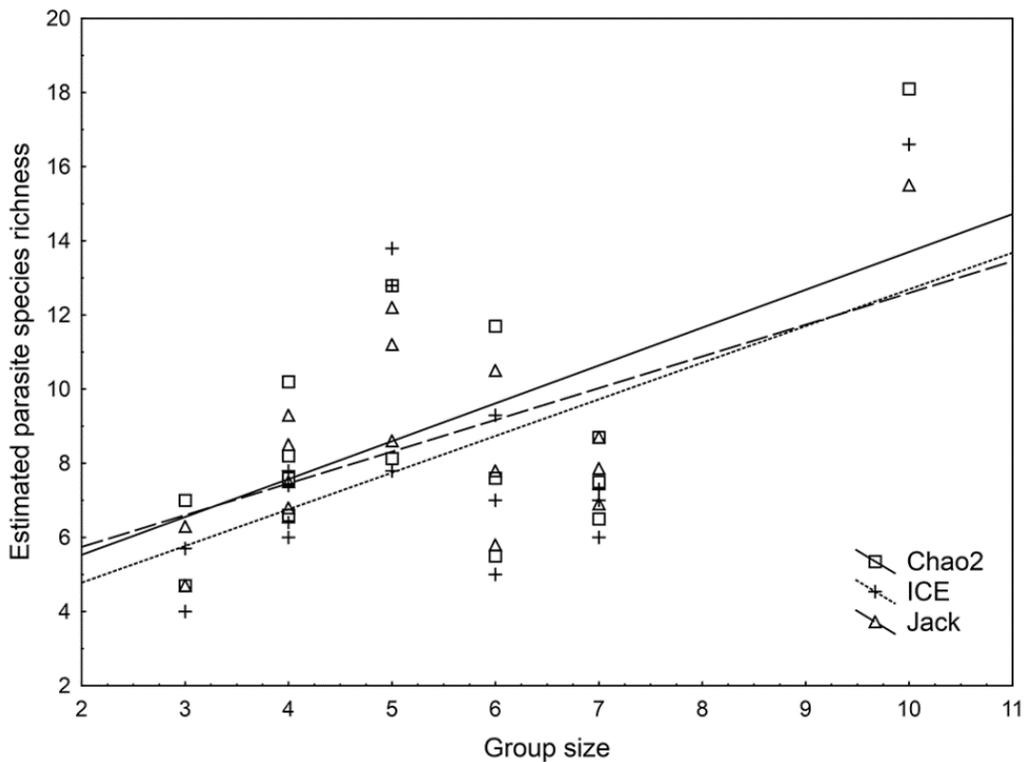


Figure 2.4. Association of Y=estimated parasite species richness (adjusted for sampling effort) with x=howler monkey group size. Larger groups have more gastrointestinal parasite species even after accounting for sampling effort using three non-parametric richness estimators. The ordinary least squares prediction equations are Chao 2 estimated species richness (solid line, square) ($Y = 3.48 + 1.02x$); Jackknife (dashed line, triangle) ($Y = 4.03 + 0.86x$); and ICE estimated species richness (dotted line, plus sign) ($Y = 2.80 + 0.99x$).

DISCUSSION

Parasite species from 13 genera were found in 19 groups of Ecuadorian mantled howler monkeys. These 13 genera represent a higher number than observed in previous howler monkey studies. The higher parasite species richness observed for these howler groups relative to previous studies could be due to several factors, including differences in sampling effort and technique, extraction methodology, environmental conditions (Stoner, 1996; Stuart et al., 1990; 1993; Eckert et al., 2006; Cristobal-Azkarate et al., 2010), anthropogenic disturbance (Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008), host densities, and parasite attributes such as intermittent shedding of parasites by hosts in various seasons, differences in host immunity (Stear et al., 1995), initial infection dose (Christensen et al., 1995), and parasite fecundity (Dineen et al., 1965). This study may simply have recovered more parasites because I used three different methods to retrieve parasites from feces (plus PCR-based detection for *Blastocystis*) in order to maximize the likelihood of recovering various types of parasites, whereas the other studies limited retrieval and detection to one or two extraction methods. I am not suggesting that this is the only explanation for increased species richness. However, looking only at our fecal flotation results, 9 species were recovered as opposed to 13 species recovered when all three extraction methods were combined. Sedimentation methods resulted in the recovery of ten parasite species. Fecal smears did result in the detection of all 13 species and also had the highest number of positive matches (when compared to a combination of all methods) for seven parasite species (Table 2.2). Yet, only two other howler monkey studies have utilized this method (Eckert, 2006; Stoner and Gonzalez Di Pierro, 2006).

Group size

Group size was associated with parasite species richness; larger groups harbored a greater number of parasite species (Figure 2.4). This finding is consistent with the hypothesis proposed

by Freeland (1979) and summarized by Altizer et al. (2003) in which the number of intestinal, protozoan species is a function of group size. In larger groups, individuals are hypothetically more likely to be infected either from increased environmental contamination, from an increased exposure to immigrants (Freeland, 1976), or from expanded traveling needed to secure food resources (Chapman et al., 2008). Conversely, other studies have found no association of parasitism with group size (Chapman et al., 2008), or a negative association (Snaith et al., 2008). The positive relationship found between group size and parasite richness is likely a product of several contributing factors closely associated with the number of individuals within a group (Cote and Poulin, 1995; Altizer et al., 2003), including group density, individual stress levels, food availability, and subsequent altered ranging behaviors that could bring individuals into contact with fecal contaminated areas. This could mean that fluctuations in certain environmental factors, such as food sources, would result in a change in stress levels, group density, and number of individuals (Chapman et al., 2006).

Parasite interactions

There was one case where a host was more likely to harbor a parasite species in the presence of another - individuals positive for *Isospora* sp. were much more likely to be found in individuals with *Balantidium* sp. There was also only one case where 2 parasites showed a negative association. Individuals infected with *Chilomastix* sp. were much less likely to be infected with *Capillaria* sp. There is little evidence in the literature to suggest *Chilomastix* sp. actually inhibits or influences the presence of *Capillaria* sp. In fact, *Chilomastix mesneli* is found in primates of all sorts, and is often associated with other parasitic infections (Cox, 2001; Ekanayake et al., 2006; Chapman et al., 2011). In either case, it is not possible to definitively identify what type of interaction is occurring simply based on patterns of co-infection; however, there is certainly evidence elsewhere to suggest competitive interactions between parasites

occurs (Petney and Andrews, 1998; Graham, 2008; Pedersen and Fenton, 2007), as well as parasite-induced immunosuppression – where the presence of one parasite species benefits another (Cox, 2001). Poulin (2001) summarizes possible causes of gastrointestinal parasite relationships, including the possibility that certain hosts might be more susceptible to parasitism than other individuals.

Parasite species identification

Two distinct *Strongyloides* morphotypes may have been encountered during this study as measured eggs ranged in length from 14 μm to 71.3 μm , and the distribution of egg lengths was bimodal with modes at 36 μm and at 52.8 μm . Because of significant overlap, I could not confidently separate large and small eggs for the purposes of our analysis here. Multiple *Strongyloides* species have been found in the same red colobus monkey population, though it was not reported whether both were in a single individual (Gillespie and Chapman, 2008). The howler parasite literature typically only lists genus because these are difficult to identify to species level from eggs and there are more than 50 described species throughout the world (Speare, 1989). It is possible that some of the recovered eggs longer than 48 μm that we found are attributable to *Strongyloides stercoralis* and this species does have a distribution throughout humans in the Neotropics (Olsen et al., 2009). However, 52% of identified *Strongyloides* eggs were less than 48 μm long, which puts them outside the previous size estimates for any species reported from howlers. Besides egg length, there were no other discernible morphological differences between the morphotypes (Figure 2.2A and 2.2C).

All but three parasite genera (*Cyclospora* sp., *Capillaria* sp., and *Balantidium* sp.) have been previously reported in other howler monkey species (Gilbert, 1994; Stoner, 1996; Eckert et al., 2006; Stoner and Gonzalez Di Pierro, 2006; Vitazkova and Wade, 2006; Trejo-Macias et al., 2007; Cristobal-Azkarate et al., 2010; Kowalewski et al., 2010). In most cases I was not able to

identify to a species level based simply on morphology, yet I can speculate based on other factors. *Entamoeba coli* could have been responsible for some howler infections as cyst morphology was consistent with other reports (Table 2.1). It has also been reported in humans living in the same province as our field study (Gatti et al., 2002). Nonetheless, we cannot rule out the presence of other *Entamoeba* spp.

Trypanoxyuris sp. eggs averaged 42.5 μm in length, similar to previous findings which ranged from 31.2-49.3 μm in *A. palliata* in Mexico (Table 2.1; Cristobal-Azkarate et al., 2010). *Trypanoxyuris minutus* has been reported in nearly every other Central and South American country which leads us to believe that this is *T. minutus* (Thatcher and Porter, 1968; Gilbert, 1994; Stuart, 1998; Cristobal-Azkarate et al., 2010). This parasite is relatively important because it has previously been reported as the cause of death of a howler in Brazil (Amato et al., 2002).

We observed eggs of a *Capillaria* species in 78% of our samples with eggs averaging 48.8 μm long. Over 250 *Capillaria* spp. have been described in vertebrates, though only eggs from *C. hepatica* and *C. brochieri* have been reported from primates (Brack et al., 1994; Graczyk et al., 1999). *Capillaria hepatica* eggs were reported to be from 50 μm (Brack et al., 1994) to $54.3 \pm 0.5 \mu\text{m}$ (Graczyk et al., 1999). *Capillaria brochieri* eggs in chimpanzees were documented from 45-55 μm which would encompass our observations (Justine, 1987). However, *Capillaria* species have only been found in *A. caraya*, but not *A. palliata* (Godoy et al., 2004).

Balantidium coli is the only known ciliated protozoan to infect humans, and has been found in Ecuadorian human populations (Chiriboga Urquiza et al., 1985). However, no cases of *B. coli* have been reported in howler monkeys, though *Balantidium* species have been reported in *A. caraya* (Stiles et al., 1929). Trophozoites averaged 44.5 μm (30.1-107.3 μm), which is within the normal variable size limit of 30-300 μm in length (Figure 2.1). *Cyclospora* species have been previously reported from Ethiopian and Kenyan primates (Eberhard et al., 1999, 2001).

However, *Cyclospora* in South American non-human primates has not been reported. Human infections of *Cyclospora cayetanensis* have been reported in South American countries including Peru (Ortega et al., 1994) and Venezuela (Chacin-Bonilla et al., 2001), which suggests that this parasite might be found in Ecuador. *Chilomastix* sp. was also identified to the genus level. Only one other howler study (*A. caraya*) reported this parasite, in Brazil (Stiles, 1929).

Kowalzik et al. (2010) previously hypothesized that black howler monkeys (*A. pigra*) who eat the leaves, fruit and stems of the cecropia tree, *Cecropia peltata*, are likely to become infected with *Controrchis* sp. through the ingestion of infected ants. Our study found only 15% of samples positive for *Controrchis* sp. compared to 80-89% prevalence in black howler monkeys (Vitazkova and Wade, 2006; Kowalzik et al., 2010). Aside from the difference in host species, there is the possibility that forest structure differences between the studies might play a role. The cecropia tree is a pioneer species and thus we would expect *Controrchis* sp. to be more prevalent in disturbed forest. Only 20% of the Bilsa Biological Reserve is considered secondary forest (Ortega-Andrade et al., 2010) while Kowalzik et al. (2010) describe their site as a “hurricane-damaged” forest and Vitazkova and Wade (2006) sampled monkeys in secondary growth forest, cattle pastures and plantations. If indeed these *Controrchis* species were limited to degraded environments, then this might very well explain the reduced prevalence in our study. Vitazkova and Wade (2006) identified the parasite from black howlers as *C. biliophilus*. Based on descriptions and size estimates of eggs (41 μm to 50 μm) from other studies (Stuart et al. 1990), the eggs we observed are consistent with *C. biliophilus*.

Pathogenicity

Although we took a conservative approach to identify species only to genus, some of the species in this study are from genera where most of the members are considered asymptomatic. Others may be associated with host impacts ranging from mild signs of infection to death

(Abbott and Majeed, 1984; Foreyt, 2001; Chapman et al., 2005; Trejo-Macias et al., 2007). In the case of *Blastocystis* sp., *Iodamoeba* sp., and *Entamoeba coli* there is little evidence to suggest pathogenicity (Toft, 1982; Chapman et al., 2005; Coyle et al., 2011). Alternatively, *Strongyloides* spp. (Abbott and Majeed, 1984; Chapman et al., 2005), *Capillaria* spp. (Abbott and Majeed, 1984), *Balantidium* sp. (Kuntz, 1982; Toft, 1982), *Enterobius* sp. (Toft, 1982; Chapman et al., 2005), and *Chilomastix* sp. (Chapman et al., 2005) have been shown to be pathogenic in other primates. *Entamoeba* spp. (Kuntz, 1982) could be either asymptomatic (*E. coli*) or cause amoebiasis (*E. histolytica*), but is limited in this case by our inability to identify these parasites to a species level (Chapman et al., 2005). Similarly, *Isospora* sp. may or may not lead to coccidiosis in nonhuman primates (Lindsay et al., 1997; Duszynski et al., 1999). Information on the effect of these parasite species in *A. palliata* is limited, especially as it relates to multiple infections; however, based on evidence found in other organisms there is the possibility that host health and fitness could be affected (Murray et al., 1998; Chapman et al., 2007; Cristobal-Azkarate, 2010). Multiple infections in combination or single infections coupled with reduced host immune response could influence pathogenicity, leading to illness or in some circumstances, death (Amato et al., 2002).

In conclusion, our study found several parasite genera which may have relevance to primate and human health: *Balantidium* sp., *Isospora* sp., *Enterobius* sp., and *Strongyloides* sp. We also found that group size was positively correlated with gastrointestinal parasitism – a finding which builds on previous primate studies. Describing gastrointestinal parasites infecting nonhuman primate species and understanding the factors that impact parasite communities is important to both human health and primate conservation, particularly in a time when both natural and man-made disturbances are predicted to increase spill-over events between wildlife and human populations.

LITERATURE CITED

- Abbott, D. P., and S. K. Majeed. 1984. A survey of parasitic lesions in wild-caught, laboratory-maintained primates: (Rhesus, Cynomolgus, and Baboon). *Veterinary Pathology* 21: 198-207.
- Altizer, S., C. L. Nunn, P. H. Thrall, J. L. Gittleman, and J. Antonovics, A. A. Cunningham, A. P. Dobson, V. Ezenwa, K. E. Jones, A. B. Pedersen, V. Ezenwa, K. E. Jones, A. B. Pedersen, M. Poss, and J. R. C. Pulliam. 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics* 34: 517-547.
- Amato, J. F., S. B. Amato, C. Calegario-Marques, and J. C. Bicca-Marques. 2002. *Trypanoxyuris (Trypanoxyuris) minutus* associated with the death of a wild southern brown howler monkey, *Alouatta guariba clamitans*, in Rio Grande do Sul, Brazil. *Arquivos do Instituto Biologico, Sao Paulo* 69: 99-102.
- Anzures-Dadda, A., E. Andresen, M. L. Martínez, and R. H. Manson. 2011. Absence of howlers (*Alouatta palliata*) influences tree seedling densities in tropical rain forest fragments in southern Mexico. *International Journal of Primatology* 32: 634-651.
- Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society B* 265: 1283-1289.
- Brack, M., H. Gass, and F. Stirnberg. 1994. Intestinal capillariasis in New World monkeys. *Journal of Medical Primatology* 23: 37-41.
- Chacin-Bonilla, L., J. Estevez, F. Monsalve, and L. Quijada. 2001. *Cyclospora cayetanensis* infections among diarrheal patients from Venezuela. *American Journal of Tropical Medicine and Hygiene* 65: 351-354.
- Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43: 783-791.
- Chao, A. and S. M. Lee. 1992. Estimating the number of classes via sample coverage. *Journal of American Statistical Association* 87: 210-217.
- Chapman, C. A., T. R. Gillespie, and T. L. Goldberg. 2005. Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology* 14: 134-144.
- Chapman, C. A., M. D. Wasserman, T. R. Gillespie, M. L. Speirs, M. J. Lawes, T. L. Saj, and T. E. Ziegler. 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology* 131: 525-534.
- Chapman, C. A., J. M. Rothman, and S. A. Hodder. 2008. Can parasite infections be a selective force influencing primate group size? A test with red colobus. *In Primate parasite ecology: the*

dynamics and study of host-parasite relationships, M. A. Huffman, C. A. Chapman (eds.). Cambridge University Press, Cambridge, p. 371-385.

Chapman, C. A., D. D. Bowman, R. R. Ghai, J. F. Gogarten, T. L. Goldberg, J. M. Rothman, D. Twinomugisha, and C. Walsh. 2011. Protozoan parasites in group-living primates: testing the biological island hypothesis. *American Journal of Primatology* 73: 1-8.

Chiriboga Urquizo, M., N. Falconi, J. Calderon, C. Paladines, and R. Irigoyen. 1985. Enteroparasitosis in school children of different regions of Ecuador. *Revista de la Facultad de Ciencias Medicas* 20: 99-103.

Christensen, C. M., E. H. Barnes, A. Roepstorff, and H. C. Slotved. 1995. Experimental *Oesophagostomum dentatum* infection in the pig: Worm populations resulting from single infections with three doses of larvae. *International Journal of Parasitology* 25: 1491-1498.

Colwell, R. K., C. X. Mao, and J. Chang. 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85: 2717-2727.

Colwell, R. K. 2009. EstimateS 8.2: Statistical estimation of species richness and shared species from samples. User's Guide and Application. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs. <<http://viceroy.eeb.uconn.edu/estimates/>>. Accessed 23 July 2014.

Cote, I. M., and R. Poulin. 1995. Parasitism and group size in social animals: A meta-analysis. *Behavioral Ecology* 6: 159-165.

Cox, F. E. G. 2001. Concomitant infections, parasites and immune responses. *Parasitology* 122: S23-S38.

Coyle, C. M., J. Varughese, L. M. Weiss, and H. B. Tanowitz. 2011. *Blastocystis*: To treat or not to treat... *Clinical Infectious Disease* 54: 105-110.

Cristobal-Azkarate, J., B. Hervier, S. Vegas-Carrillo, D. Osorio-Sarabia, E. Rodriguez-Luna, and J. J. Veá. 2010. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. *Primates* 51: 231-239.

Crockett, C. M. 1998. Conservation biology of the genus *Alouatta*. *International Journal of Primatology* 19: 549-578.

Cruz, A. C., J. T. Borda, E. M. Patino, L. Gomez, and G. E. Zunino. 2000. Habitat fragmentation and parasitism in howler monkeys (*Alouatta caray*). *Neotropical Primates* 8: 146-148.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science* 287: 443-449.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* 78: 103-116.

- Dineen, J. K., A. D. Donald, B. M. Wagland, and J. H. Turner. 1965. The dynamics of the host-parasite relationship. *Parasitology* 55: 163-171.
- Duszynski, D. W., W. D. Wilson, S. J. Upton, and N. D. Levine. 1999. Coccidia (Apicomplexa: Eimeriidae) in the primates and the Scandentia. *International Journal of Primatology* 20: 761-797.
- Eberhard, M. L., A. J da Silva, B. G. Lilley, and N. J. Pieniazek. 1999. Morphologic and molecular characterization of new *Cyclospora* species from Ethiopian monkeys: *C. cercopithecii* sp.n., *C. colobi* sp.n., and *C. papionis* sp.n. *Emerging Infectious Diseases* 5: 651-658.
- Eberhard, M. L., E. Kovacs-Nace, J. Blotkamp, J. J. Verwij, V. A. Asigri, and A. M. Polderman. 2001. Experimental *Oesophagostomum bifurcum* in monkeys. *Journal of Helminthology* 75: 51-56.
- Eckert, K. A., N. E. Hahn, A. Genz, D. M. Kitchen, M. D. Stuart, G. A. Averbek, B. E. Stromberg, and H. Markowitz. 2006. Coprological surveys of *Alouatta pigra* at two sites in Belize. *International Journal of Primatology* 27: 227-238.
- Ekanayake, D. K., A. Arulkanthan, N. U. Horadagoda, G. K. Sanjeevani, R. Kieft, S. Gunatilake, and W. P. Dittus. 2006. Prevalence of *Cryptosporidium* and other enteric parasites among wild non-human primates in Polonnaruwa, Sri Lanka. *American Journal of Tropical Medicine and Hygiene* 74: 322-329.
- Estrada, A., S. Juan-Solano, T. O. Martinez, and R. Coates-Estrada. 1999. Feeding and general activity patterns of a howler monkey (*Alouatta palliata*) troop living in a forest fragment at Los Tuxtlas, Mexico. *American Journal of Primatology* 48: 167-183.
- Foreyt, W. J. 2001. *Veterinary parasitology reference manual*, 2nd ed. Washington State University, Pullman, Washington, 165 p.
- Freeland, W. J. 1976. Pathogens and the evolution of primate sociality. *Biotropica* 8: 12-24.
- Freeland, W. J. 1979. Primate social groups as biological islands. *Ecology* 60: 719-728.
- Garcia, L. S., R. Y. Shimizu, A. Shum, and D. A. Bruckner. 1993. Evaluation of intestinal protozoan morphology in polyvinyl alcohol preservative: Comparison of zinc sulfate- and mercuric chloride-based compounds for use in Schaudinn's fixative. *Journal of Clinical Microbiology* 31: 307-310.
- Garcia, L. S. 1999. *Practical guide to diagnostic parasitology*. American Society of Microbiology Washington, D.C., 88 p.
- Gatti, S., G. Swierczynski, F. Robinson, M. Anselmi, J. Corrales, J. Moreira, G. Montalvo, A. Bruno, R. Maserati, Z. Bisoffi, and M. Scaglia. 2002. Amebic infections due to the *Entamoeba histolytica-Entamoeba dispar* complex: A study of the incidence in a remote rural area of Ecuador. *American Journal of Tropical Medical Hygiene* 67: 123-127.

- Gilbert, K. A. 1994. Endoparasitic infection in red howling monkeys (*Alouatta seniculus*) in the central Amazonian basin: A cost of sociality? Ph.D. Thesis. Rutgers. State University of New Jersey, New Brunswick, New Jersey, 156 p.
- Gillespie, T. R., C. A. Chapman, and E. C. Greiner. 2005. Effects of logging on gastrointestinal parasite infections and infection risk in African primates. *Journal of Applied Ecology* 42: 699-707.
- Gillespie, T. R. 2006. Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. *International Journal of Primatology* 27: 1129-1143.
- Gillespie, T. R., and C. A. Chapman. 2008. Forest fragmentation, the decline of an endangered primate, and changes in host-parasite interactions relative to an unfragmented forest. *American Journal of Primatology* 70: 222-230.
- Godoy, K. C., A. Odalia-Rimoli, and J. Rimoli. 2004. Infecção por endoparasitas em um grupo de bugios-pretos (*Alouatta caraya*) em um fragmento florestal no estado do Mato Grosso do Sul, Brasil. *Neotropical Primates* 12: 63-68.
- Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4: 379-391.
- Gotelli, N. J., and R. K. Colwell. 2011. Estimating species richness. *In* *Frontiers in Measurement and Assessment*, A. E. Magurran, B. J. McGill (eds.). Oxford University Press, New York, p. 39-54.
- Graczyk, T. K., L. J. Lowenstine, and M. R. Cranfield. 1999. *Capillaria hepatica* (Nematoda) infections in human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Parc National de Volcans, Rwanda. *Journal of Parasitology* 85: 1168-1170.
- Graham, A. L. 2008. Ecological rules governing helminth-microparasite coinfection. *Proceedings of the National Academy of Science* 105: 566-570.
- Gregory, R. D. 1990. Parasites and host geographic range as illustrated by waterfowl. *Functional Ecology* 4: 645-654.
- Hendrix, C. M., and E. Robinson. 2006. *Diagnostic Parasitology for Veterinary Technicians*, 3rd ed. Mosby Inc., St. Louis, Missouri, 416 p.
- Howells, M. E., J. Pruetz, and T. R. Gillespie. 2011. Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: The case of the sympatric western chimpanzee (*Pan troglodytes verus*) and Guinea Baboons (*Papio hamadryas papio*) at Fongoli, Senegal. *American Journal of Primatology* 73: 173-179.
- IUCN, 2012. The IUCN Red List of Threatened Species. Version 2012.2. <<http://www.iucnredlist.org>>. Accessed 17 October 2012.

- Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451: 990-993.
- Justine, J. L. 1987. *Capillaria brochieri* n. sp. (Nematoda: Capillariinae) intestinal parasite of the chimpanzee (*Pan paniscus*) in Zaire. *Annales de Parasitologie Humaine et Comparée* 63: 420-438.
- Kowalzik, B. K., M. S. Pavelka, S. J. Kutz, and A. Behie. 2010. Parasites, primates, and ant-plants: clues to the life cycle of *Controrchis* spp. in black howler monkeys (*Alouatta pigra*) in southern Belize. *Journal of Wildlife Disease* 46: 1330-1334.
- Kowalewski, M. M., J. S. Salzer, J. C. Deutsch, M. Rano, M. S. Kuhlenschmidt, and T. R. Gillespie. 2010. Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: patterns of zoonotic protozoa infection relative to degree of human-primate contact. *American Journal of Primatology* 73: 75-83.
- Kuntz, R. E. 1982. Significant infections in primate parasitology. *Journal of Human Evolution* 11: 185-194.
- Lindsay, D. S., J. P. Dubey, and B. L. Blagburn. 1997. Biology of *Isospora* spp. from humans, nonhuman primates, and domestic animals. *Clinical Microbiology Reviews* 10: 19-34.
- Marcogliese, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health. *International Journal of Parasitology* 35: 705-716.
- Menounos, P. G., G. Spanakos, N. Tegos, C. M. Vassalos, C. Papadopoulou, and N. C. Vakalis. 2008. Direct detection of *Blastocystis* sp. in human faecal samples and subtype assignment using single strand conformational polymorphism and sequencing. *Molecular and Cellular Probes* 22: 24-29.
- Morse, S. S. 1995. Factors in the emergence of infectious diseases. *Emerging Infectious Diseases* 1: 7-15.
- Muriuki, S. M., R. K. Murugu, E. Munene, G. M. Karere, and D. C. Chai. 1998. Some gastrointestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica* 71: 73-82.
- Murphy, L., A. K. Pathak, and I. M. Cattadori. 2013. A co-infection with two gastrointestinal nematodes alters host immune responses and only partially parasite dynamics. *Parasite Immunology* 35: 421-432.
- Murray, D. L., L. B. Keith, and J. R. Cary. 1998. Do parasitism and nutritional status interact to affect production in snowshoe hares? *Ecology* 79: 1209-1222.
- Nacher, M. 2011. Interactions between worms and malaria: Good worms or bad worms? *Malaria Journal* 10: 259-264.

- Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative tests of parasite species richness in primates. *The American Naturalist* 162: 597-614.
- Olsen, A., L. van Lieshout, H. Marti, T. Polderman, K. Polman, P. Steinmann, R. Stothard, S. Thybo, J. J. Verweij, and P. Magnussen. 2009. Strongyloidiasis – the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103: 967-972.
- Ortega, Y. R., R. H. Gilman, and C. R. Sterling. 1994. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. *Journal of Parasitology* 80: 625-629.
- Ortega-Andrade, H., J. Bermingham, C. Aulestia, and C. Paucar. 2010. Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. *Check List* 6: 119-154.
- Patz, J. A., T. K. Graczyk, N. Geller, and A. Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30: 1395-1405.
- Pedersen, A. B., S. Altizer, M. Poss, A. A. Cunningham, and C. L. Nunn. 2005. Patterns of host specificity and transmission among parasites of wild primates. *International Journal of Parasitology* 35: 647-657.
- Pedersen, A. B., and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. *TRENDS in Ecology and Evolution* 22: 133-139.
- Petney, T. N., and R. H. Andrews. 1998. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *International Journal for Parasitology* 28: 377-393.
- Phillips, K. A., M. E. Haas, B. W. Grafton, and M. Yrivarren. 2004. Survey of the gastrointestinal parasites of the primate community at Tambopata National Reserve, Peru. *Journal of Zoology* 264: 149-151.
- Pinto, A. C., C. Azevedo-Ramos, and O. de Carvalho Jr. 2003. Activity patterns and diet of the howler monkey, *Alouatta belzebul*, in areas of logged and unlogged forest in Eastern Amazonia. *Animal Biodiversity and Conservation* 26: 39-49.
- Pope, B. L. 1966. Some parasites of the howler monkey of northern Argentina. *Journal of Parasitology* 52: 166-168.
- Poulin, R. 1998. Comparison of three estimators of species richness in parasite component communities. *Journal of Parasitology* 84: 485-490.
- Poulin, R. 2001. Interactions between species and the structure of helminth communities. *Parasitology* 122: S3-S11.
- Puttker, T., Y. Meyer-Lucht, and S. Sommer. 2008. Effects of fragmentation on parasite burden (nematodes) of generalist and specialist small mammal species in secondary forest fragments of the coastal Atlantic Forest, Brazil. *Ecological Research* 23: 207-215.

- Rowe, N. 1996. The pictorial guide to the living primates. Pogonias Press, Hong Kong, 263 p.
- Smith, K. F., K. Acevedo-Whitehouse, and A. B. Pedersen. 2008. The role of infectious diseases in biological conservation. *Animal Conservation* 12: 1-12.
- Snaith, T. V., C. A. Chapman, J. M. Rothman, and M. D. Wasserman. 2008. Bigger groups have fewer parasites and similar cortisol levels: A multi-group analysis in red colobus monkeys. *American Journal of Primatology* 70: 1072-1080.
- Speare, R. 1989. Identification of species of *Strongyloides*. In *Strongyloidiasis: A major roundworm infection of man*, D. I. Grove (ed.). Taylor & Francis: London, U.K., p. 11-83.
- Stear, M. J., S. C. Bishop, M. Doligalska, J. L. Duncan, P. H. Holmes, J. Irvine, L. McCririe, Q. A. McKellar, E. Sinksi, and M. Murray. 1995. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology* 17: 643-652.
- Stensvold, C. R., M. A. Alfellani, S. Nørskov-Lauritsen, K. Prip, E. L. Victory, C. Maddox, H. V. Nielsen, and C. G. Clark. 2009. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *International Journal for Parasitology* 39: 473-479.
- Stiles, C. W., A. Hassall, and O. Nolan. 1929. Key-catalogue of parasites reported for primates (monkeys and lemurs) with their possible public health importance, and key catalogue of primates for which parasites are reported. U.S. Treasury Department, Public Health Service, Hygienic Laboratory Bulletin 152: 409-601.
- Stoner, K. E., 1996. Prevalence and intensity of intestinal parasites in mantled howler monkeys (*Alouatta palliata*) in Northeastern Costa Rica: Implications for conservation biology. *Conservation Biology* 10: 539-546.
- Stoner, K. E., and A. M. Gonzalez Di Pierro. 2006. Intestinal parasite infections in *Alouatta pigra* in tropical rainforest in Lacandona, Chiapas, Mexico: Implications for behavioral ecology and conservation. In *New perspectives in the study of mesoamerican primates. Distribution, ecology and conservation*, A. Estrada, P. A. Garber, M. Pavelka, and L. Luecke (eds.). Springer, New York, New York, p. 215-240.
- Stuart, M., V., L. L. Greenspan, K. E. Glander, and M. R. Clarke. 1990. A coprological survey of parasites of wild mantled howling monkeys, *Alouatta palliata palliata*. *Journal of Wildlife Disease* 26: 547-549.
- Stuart, M., Pendergast, S. Rumfelt, S. Pierberg, L. Greenspan, K. Glander, and M. Clarke 1998. Parasites of wild howlers (*Alouatta* spp.). *International Journal of Primatology* 19: 493-512.
- Thatcher, V. E., and J. A. Porter. 1968. Some helminth parasites of Panamanian primates. *Transactions of the American Microscopical Society* 87: 186-196.

- Toft, J. D. 1982. The pathoparasitology of the alimentary tract and pancreas of nonhuman primates: A review. *Veterinary Parasitology* 19: 44-92.
- Trejo-Macias, G., A. Estrada, and M. A. Cabrera. 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in Southern Mexico. *International Journal of Primatology* 28: 931-945.
- Valdespino, C., G. Rico-Hernández, and S. Mandujano. 2010. Gastrointestinal parasites of howler monkeys (*Alouatta palliata*) inhabiting the fragmented landscape of the Santa Marta mountain range, Veracruz, Mexico. *American Journal of Primatology* 72: 539-548.
- Vitazkova, S. K., and S. E. Wade. 2006. Parasite of free-ranging black howler monkeys (*Alouatta pigra*) from Belize and Mexico. *American Journal of Primatology* 68: 1089-1097.
- Vitazkova, S. K., and S. E. Wade. 2007. Effects of ecology on the gastrointestinal parasites of *Alouatta pigra*. *International Journal of Primatology* 28: 1327-1343.
- Vitazkova, S. K. 2009. Overview of parasites infecting howler monkeys, *Alouatta* sp., and human-howler interactions. In *Primate parasite ecology: The dynamics and study of host-parasite relationships*, M. A. Huffman, and C. A. Chapman (eds.). Cambridge University Press, Cambridge, UK, p. 371-385.
- Wallis, J., and D. R. Lee. 1999. Primate conservation: the prevention of disease transmission. *International Journal of Primatology* 20: 803-826.
- Walther, B. A., P. Cotgreave, R. D. Price, R. D. Gregory, and D. H. Clayton. 1995. Sampling effort and parasite species richness. *Parasitology* 11: 306-310.
- Walther, B. A., and J. L. Martin. 2001. Species richness estimation of bird communities: how to control for sampling effort. *Ibis* 143: 413-419.
- Whipps, C. M., K. Boorum, L. E. Bermudez, and M. L. Kent. 2010. Molecular characterization of *Blastocystis* species in Oregon identifies multiple subtypes. *Parasitology Research* 106: 827-832.
- Yurkov, A. M., M. Kemler, and D. Begerow. 2011. Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. *PLOS ONE* 6: 1-9.

Chapter 3

EFFECT OF FOREST DISTURBANCE AND HUMAN ENCROACHMENT ON PARASITISM IN ECUADORIAN MANTLED HOWLER MONKEYS

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ABSTRACT

Anthropogenic environmental disturbance is dramatically affecting the health of forests and wildlife throughout the tropics, contributing to a reduction in available habitat, biodiversity loss, reductions in connected areas, and unsustainable harvesting of animals and timber. What is less well-known is the indirect impact of these disturbances on changing parasite disease dynamics. Habitat degradation, fragmentation, and human encroachment have the potential to influence parasite communities by modifying wildlife nutritional health, physiological stress, densities, contact rates, and ranging patterns. The result of habitat modifications is a changing landscape of parasitic disease ecology with the potential to threaten both wildlife and people. My research was designed to measure the effect anthropogenic disturbances have on parasite communities in mantled howler monkeys, *Alouatta palliata aequatorialis*. I assessed the relationship between parasite species, howler monkey group size, and indicators of human disturbance and forest degradation. Presence of several parasite species was associated with forest disturbance and human encroachment. Proximity of agricultural plots and a local biological research station were both associated with the presence of *Strongyloides* spp. Individuals were more than four times as likely to harbor *Strongyloides* spp. if they lived in areas considered disturbed forest. Areas with disturbed forest were also more likely to harbor monkeys infected with *Cyclospora* sp. Individuals infected with *Controrchis* sp. were found further from human settlements than uninfected individuals ($p=0.05$) and monkeys infected with *Controrchis* sp. were nearly ten times more likely to be found in primary forest ($p=0.04$). Indicators of forest structure were not associated with overall parasite species richness at either a group or individual level. Howler monkeys with *Cyclospora* sp., *Isospora* sp., *Balantidium* sp., and *Entamoeba* spp. were found in larger groups than those without each of these parasites. These results support the hypothesis that human encroachment and forest degradation are associated with differences in primate

gastrointestinal parasites. Aside from the various direct impacts of anthropogenic disturbances on wildlife populations, such as reduced habitat and increased hunting in areas adjacent to human settlements, additional focus should be placed on understanding the indirect role of changing ecological systems on parasite communities and their hosts. For instance, modified gastrointestinal parasite communities presumably impact primate health and long-term survival through changes in birth interval, host survival, fecundity, evolutionary fitness, and social behavior.

Keywords: *Alouatta palliata*, primates, parasitism, human disturbance, logging, anthropogenic disturbance

INTRODUCTION

Seventy-five percent of newly emerging parasitic diseases in humans are caused by pathogens considered zoonotic (Taylor et al., 2001), often spilling-over from wildlife populations into surrounding communities (Daszak et al., 2001). Specific to the tropics, nearly 40% of infectious diseases originate in wild primate populations, though most of the research investigating pathogen spill-over has focused on great apes and Old World monkeys (Wolfe et al., 2007; Pedersen and Davies, 2010). The impact of parasitic disease is far reaching, associated with 25% of human deaths worldwide. The significance of wildlife mortality due to parasitic disease is largely unknown, though studies have proposed that birth interval, host survival, fecundity, overall evolutionary fitness, and social behavior are strongly influenced (Dobson, 1988; Mooring and Hart, 1992; Vandegrift et al., 2008; Coop and Holmes, 1996).

Both zoonotic transmission of pathogens and composition of parasite communities in wildlife have been linked to human disturbance (Table 3.1) (Morse, 1995; Patz et al., 2000; Daszak et al., 2001; Chapman et al., 2005; Puttker et al., 2008; Wilcox and Colwell, 2005).

Table 3.1. Mode of infection, morbidity, mortality, and evidence of anthropogenic disturbance (AD) type associated with gastrointestinal parasite species in primate populations. Limited to those parasites previously confirmed in Ecuadorian mantled howler monkeys, *Alouatta palliata aequatorialis*, at the Bilsa Biological Station. *Represent significant ($p < 0.05$) findings.

Parasite Species	Mode of Infection	Morbidity/Mortality	Association with AD*
Apicomplexa			
<i>Cyclospora</i> sp.	Sporulated oocysts ingested	Diarrhea, inflammation and damage to the intestinal lining	No evidence reported.
Other Protozoa			
<i>Cystoisospora belli</i> sp. (Isospora)	Sporulated oocysts ingested	Diarrhea	Higher prevalence nearest to people. ¹⁰ Parasite intensity reduced with increasing anthropogenic disturbance. ¹³
<i>Balantidium</i> sp.	Cyst ingested	Generally asymptomatic but evidence of lethal ulcerative colitis in great apes. ¹⁴	No evidence reported.
<i>Blastocystis</i> sp.	Cyst ingested	Typically asymptomatic	Parasite intensity reduced with increasing anthropogenic disturbance. ¹³
<i>Chilomastix</i> sp.	Cyst or trophozoite ingested	Typically asymptomatic ¹	Increased prevalence in logged areas. ⁷
<i>Dientamoeba</i> sp. <i>Entamoeba</i> spp.	Trophozoite ingested		No evidence reported. Parasite intensity reduced with increasing anthropogenic disturbance. ¹³
<i>E. histolytica</i>	Cyst or trophozoite ingested	Hepatic and gastric, amoebiasis, death ²	Increased prevalence in logged areas. ⁷
<i>E. coli</i>	Cyst or trophozoite ingested	Typically asymptomatic ¹	Increased prevalence in logged areas. ⁷ Increased prevalence along forest edges. ⁸
Nematoda			
<i>Iodamoeba</i> sp.	Cyst or trophozoite ingested	Typically asymptomatic ¹	Increased prevalence in logged areas. ⁷
<i>Enterobius</i> sp.	Embryonated eggs ingested		No effect. ⁸
<i>Capillaria</i> sp.	Unembryonated eggs ingested		No evidence reported.
<i>Strongyloides</i> sp.	Larvae ingested, skin penetration	Mucosal inflammation, ulceration, death ^{3,4}	Increased prevalence in logged areas. ⁷ Increased prevalence in fragmented forest. ^{8,11}
Platyhelminth			
<i>Trypanoxyuris</i> sp.	Unknown.	Unknown. Death. ¹⁵	Increased prevalence in continuous forest, ⁹ versus in fragmented forest. ^{11,12}
<i>Controrchis</i> sp.	Metacercaria ingested in ant	Typically asymptomatic. ^{5,6}	Increased prevalence in fragmented forest. ^{9,11,12}

1. Beaver et al. (1984); 2. Loomis (1983); 3. McClure and Guilloud (1971); 4. Pampiglione and Ricciardi (1972); 5. Beaver et al. (1984); 6. Coombs and Crompton (1991); 7. Gillespie et al. (2005); 8. Chapman et al. (2006); 9. Vitazkova and Wade (2007); 10. Teichroeb et al. (2009); 11. Trejo-Macías et al. (2007); 12. Trejo-Macias and Estrada (2012); 13. Lane et al. (2011). 14. Teare and Loomis (1982). 15. Amato et al. (2002).

A study in Borneo found higher nematode species richness among treeshrews in logged and fragmented habitats relative to those living in undisturbed forest. In the same study they found the opposite trend in rats (Wells et al. 2007). Individuals were more heavily infected with several helminths in unlogged forest compared to disturbed areas. In primates, parasite species richness and prevalence of several gastrointestinal parasites was universally higher in logged forests compared to primary habitat (Gillespie et al., 2005; Salzer et al., 2007; Trejo-Macias et al., 2007; Schwitzer et al., 2010).

Interactions between the environment, host and parasites are dynamic and complex (Figure 3.1). For example, logging and subsequent fragmentation have been shown to influence host ranging patterns, modifying exposure to parasites (Gillespie et al., 2005; Wells et al., 2007). Individuals are presumably forced into new territories or into closer contact with other groups because of altered resource distribution – such as food or mates (Nunn et al., 2003; Mborá and McPeck, 2009; Arroyo-Rodríguez and Dias, 2010). Lower density food resources are associated with larger home ranges, forcing individuals to travel further, increasing their encounters with more parasites, and in turn leading to higher parasite abundance and prevalence (Nunn et al., 2003; Gillespie et al., 2005).

Forest fragmentation can also limit travel route diversity, bringing individuals into greater contact with contaminated foliage or forcing them onto the ground where they are more likely to come into contact with soil-transmitted pathogens (Stoner, 1996; Gillespie et al., 2005; Pozo and Serio-Silva, 2007; Trejo-Macias et al., 2007; Mborá and McPeck, 2009). Living on the forest edge of fragmented habitats increases contact with agricultural plots, areas with domestic animals and human settlements – also potential sources of gastrointestinal parasites (Trejo-Macias et al., 2007). Fragmentation could also limit individual dispersal between troops or populations which could conceivably force groups into “genetic islands” - limiting their ability to

recruit new members and ultimately lead to inbreeding depression (Estrada et al., 2002). The effect of inbreeding depression on wild primate parasite communities is unknown, though evidence from captive studies suggests there is an association between genetic diversity loss and higher prevalence and abundance of certain parasites (Charpentier et al., 2008). Population fragmentation has also been shown to affect food availability which is strongly correlated with parasitism (Chapman et al., 2006).

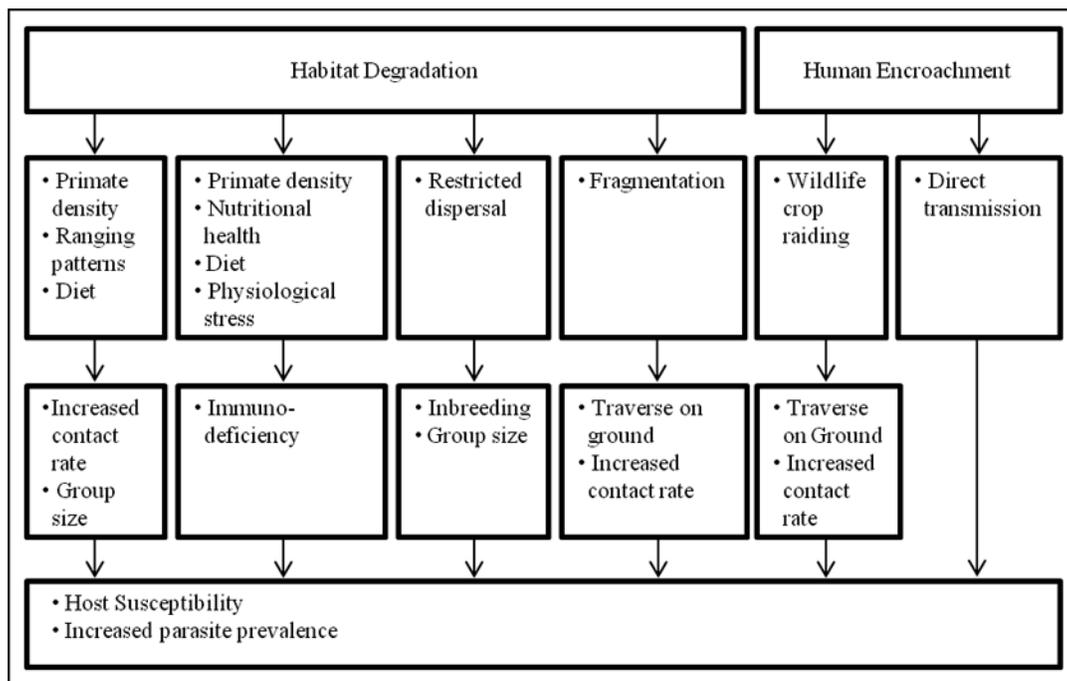


Figure 3.1. Anthropogenic disturbances relevant to this study and the causal chain of events that could hypothetically lead to changes in parasite communities of affected hosts.

In addition to direct impacts on wildlife, increased human encroachment may lead to parasite transmission between species. For example, humans and nonhuman primates interacting in the wild have also been shown to share genetically similar *Escherichia coli* (Goldberg et al., 2007; Goldberg et al., 2008). Another study on mountain gorillas found gastrointestinal parasites of possible human origin, including *Trichuris* sp., *Strongyloides* sp., *Chilomastix* sp., and

Endolimax nana (Sleeman et al., 2000). Colobus and guenons are also possible reservoirs for zoonotic transmission of *Giardia* and *Cryptosporidium* species (Salzer et al., 2007).

Conservation biology

In the last decade an increasing number of studies have focused on parasitism in wild primate populations emanating from an interest in zoonotic pathogen transmission (Muriuki et al., 1998; Pedersen and Davies, 2010; Howells et al., 2011), conserving host species (Wallis and Lee, 1999; Gillespie et al., 2005), and ecosystem health (Marcogliese, 2005; Kowalewski et al., 2010). Modified landscapes due to anthropogenic disturbances have led several researchers to test whether gastrointestinal parasites of host populations vary in response to habitat alteration, primarily focused on Old World primates (e.g., Gillespie et al., 2005; Raharivololona and Ganzhorn, 2009; Valdespino et al., 2010; Lane et al., 2011; Junge et al., 2011; Jones et al., 2013).

Despite being found in close proximity to people and able to resist limited habitat destruction, there is evidence that howler monkeys (*Alouatta palliata*), a New World primate species, are negatively affected by anthropogenic disturbance (Martinez-Mota et al., 2007; Arroyo-Rodriguez and Dias, 2010). I focused on two types of anthropogenic disturbances and their potential affect on parasite communities in mantled howler monkeys, including: 1) forest degradation as quantified from calculated basal area and percent of trees >40cm diameter breast height (percent old growth), and 2) human encroachment as defined by proximity of monkeys to a research station, roads, agricultural plots, and local communities. My aim was to understand how the distribution of parasites in a New World primate was associated with these factors. I hypothesize that monkeys living in areas with greater forest degradation would have greater gastrointestinal parasite species richness and parasite prevalence compared to those living in primary forest. Similarly, based on our encroachment measures, monkeys closer to humans will

have a greater number of parasite species and a greater prevalence of specific parasites relative to monkeys further away. Understanding how anthropogenic disturbances influence gastrointestinal parasite communities in primate populations is important for management and conservation planning purposes; however, there is also a clear benefit to people living near these tropical forests if we can better understand how environmental changes may impact parasitic disease dynamics, and mitigate actions that exacerbate zoonotic transmission.

METHODS

The Bilsa Biological Station ($00^{\circ}21'33''\text{N}$ $79^{\circ}42'02''\text{W}$; 300-750 m; Figure 3.2) is located in northwestern Ecuador, roughly 60 km from the Pacific Ocean. The reserve spans 3300 ha and is surrounded on three sides by the Mache Chindul Ecological Reserve which is adjacent to local communities (Figure 3.2B). This field site is ideal for our study because varying levels of forest degradation are present (Ortega-Andrade et al., 2010) along with local communities where humans and monkeys are sympatric.

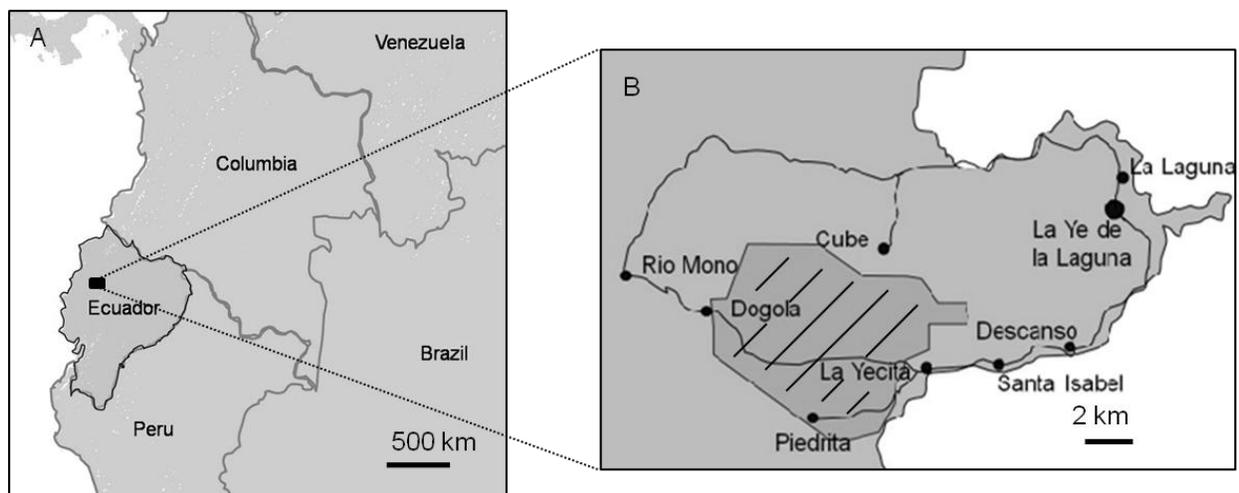


Figure 3.2. Field research took place in Northwestern Ecuador (A) at the Bilsa Ecological Station - highlighted with diagonal lines (B). Two 5km transects were used, running through both secondary and primary forest, along which mantled howler monkey groups were systematically sampled.

To locate primate groups, teams of two to five people systematically traversed two 5-km transects collecting 96 fecal samples from 23 primate groups. All primate groups along each transect were sampled, and every effort was made to ensure that the same group wasn't re-sampled, as previously described in detail (Chapter 2). Location for each individual was recorded using global positioning system (GPS). This information, coupled with satellite imagery was used to ascertain distance from each monkey to roads, agricultural fields, the Bilsa Biological Station, and other human settlements. Fecal samples were collected using disposable gloves and sterile tongue depressors to manipulate a portion of the sample into 50 ml tubes containing 10 mL RNAlater, and some into zinc polyvinyl alcohol (Zn-PVA) fixative. Samples in Zn-PVA were used for morphological identification of parasites using fecal smear, flotation, and sedimentation extraction methods. Samples preserved in RNAlater® (Qiagen Inc., Valencia, California) were used for parasite DNA preservation – specifically, for *Blastocystis* sp. PCR-based identification. 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. Forest complexity was estimated from basal area, calculated using data from point-centered quarter methods in 10 m circle plots and percentage of trees greater than 40 cm diameter at breast height (DBH) (Cottam and Curtis, 1956). Laboratory components of the research took place in the Fish and Wildlife Disease laboratory at the State University of New York College of Environmental Science and Forestry in the Department of Environmental and Forest Biology.

Species richness of parasites within each group of howler monkeys was adjusted for sampling effort by using three non-parametric estimators (Jackknife, ICE, and Chao 2 in EstimateS 9.1.0) (Chapter 2; Colwell, 2013). All three methods were used in the group analyses because of their precision, minimally biased predictions, and relative performance compared to other methods (Walter and Morand, 1998). Measures of human proximity (distance to

settlement, agriculture, and roads) and forest structure (basal area) at both the individual and group level were compared with parasite species richness and group size using Spearman's rank correlation. For each parasite species, I also compared site presence and absence to forest structure and human encroachment characteristics. A Mann-Whitney U Test was used to evaluate whether differences between the medians of the "detect" and "non-detect" sites existed for the various measures of human proximity and forest structure. These comparisons were conducted with presence and absence of the parasite defined at both individual and group levels. Risk factors associated with presence/absence of each parasite species were examined using logistic regression analysis. The impact each risk factor had on the presence or absence of discovered parasite species was expressed as an odds ratio (OR) with 95% confidence intervals (CI). All statistical analyses were done with STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, OK). All methods reported in this manuscript were non-invasive and adhered to guidelines set forth by the Institutional Animal Care and Use Committee at SUNY ESF in New York. Permit to import and transfer biological samples was approved by the Center for Disease Control (Permit #2009-06-089) and research was approved in-country according to guidelines and permit No 033-FAU-DPE-MA approved by the Director Provincial de Esmeraldas, Lic. Guillermo Oleas Zabala from the Ministerio del Ambiente in Quito, Ecuador.

RESULTS

Thirteen parasite species were found among 23 primate groups (Table 3.2). Several key parasites were non-randomly distributed throughout the sampled population (Table 3.3). Monkeys infected with *Strongyloides* sp. were, on average, located significantly closer to agricultural plots than uninfected individuals, and significantly closer to a local research station than uninfected individuals ($p=0.04$). Likewise, monkeys with *Entamoeba* sp. were found

significantly closer to the local research station than uninfected individuals ($p<0.05$). The exact opposite trend was found for individuals with *Controrchis* sp. – infected monkeys were found further from people, on average, than uninfected individuals ($p=0.04$). Roads were not associated with the presence of any parasite species.

Table 3.2. Parasite prevalence among 96 individuals and 23 groups using pooled presence/absence data from fecal smears, flotations, and sedimentations.

	Individuals Positive N (%)	Groups Positive N (%)
Apicomplexa		
<i>Cyclospora</i> sp.	17 (18)	10 (46)
<i>Isospora</i> sp.	3 (3)	2 (9)
Other Protozoa		
<i>Balantidium</i> sp.	9 (9)	6 (26)
<i>Blastocystis</i> sp.	58 (60)*	23 (100)*
<i>Chilomastix</i> sp.	4 (4)	4 (17)
<i>Dientamoeba</i> sp.	3 (3)	3 (13)
<i>Entamoeba</i> spp.	54 (56)	20 (87)
<i>Iodamoeba</i> sp.	5 (5)	5 (22)
Nematoda		
<i>Enterobius</i> sp.	3 (3)	2 (9)
<i>Capillaria</i> sp.	75 (78)	23 (100)
<i>Strongyloides</i> sp.	84 (88)	20 (87)
<i>Trypanoxyuris</i> sp.	11 (12)	3 (13)
Platyhelminth		
<i>Controrchis</i> sp.	14 (15)	9 (39)

*PCR-based detection

Monkeys infected with *Cyclospora* sp. were found in areas with lower basal area compared to those that were uninfected ($p<0.05$) (Table 3.4). The opposite was true for *Controrchis* sp., where infected individuals were more likely to be found in areas with high basal area ($p=0.04$) and a higher percent of trees >40cm DBH ($p=0.03$). Similarly, monkeys with *Blastocystis* sp. were found in areas with a higher percent of trees >40cm DBH ($p=0.04$). No significant correlation was found between species richness and any indicator of human proximity or forest structure at the host group or individual level.

Table 3.3. Impact of human encroachment indicators on presence of gastrointestinal parasites. Median distance for individual monkeys with and without each parasite were compared using a Mann Whitney U Test.

	N	Roads (m)			People (m)			Agriculture (m)			Bilsa (m)		
		Median	IQR	P	Median	IQR	P	Median	IQR	P	Median	IQR	P
<i>Strongyloides</i>													
Present	84	505	238-884	NS	1388	628-2764	NS†	859	379-1356	0.05*	1017	225-2643	0.04*
Absent	12	565	285-265		2099	1506-3012		1525	759-1902		2587	1287-2755	
<i>Cyclospora</i>													
Present	17	453	251-1526	NS	1597	749-2837	NS	1356	658-1851	NS	1017	534-2562	NS
Absent	79	668	238-884		1405	637-2837		859	487-1439		1124	225-2754	
<i>Isospora</i>													
Present	3	677	251-677	NS	1901	132-1901	NS	1693	109-1693	NS	1450	225-1450	NS
Absent	93	453	238-884		1405	682-2837		859	487-1439		1089	225-2724	
<i>Capillaria</i>													
Present	75	556	238-1236	NS	1399	683-2837	NS	931	487-1439	NS	1089	225-2643	NS
Absent	21	453	259-685		1597	128-2692		658	122-1693		1123	225-2724	
<i>Enterobius</i>													
Present	3	263	259-2052	NS	126	126-3186	NS	125	119-1851	NS	225	207-2562	NS
Absent	93	556	238-884		1411	683-2837		910	487-1439		1120	285-2724	
<i>Trypanoxyuris</i>													
Present	11	285	87-685	NS	1081	192-2307	NS	559	112-931	NS	1017	188-2616	NS
Absent	85	556	238-884		1415	733-2837		931	487-1693		1124	285-2724	
<i>Controrchis</i>													
Present	14	369	238-1424	NS	2311	1389-3216	0.04*	1145	859-1953	NS†	2440	1089-2724	NS
Absent	82	612	238-884		1300	620-2692		805	270-1439		1017	225-2616	
<i>Blastocystis</i>													
Present	58	672	254-1234	NS†	1588	728-3184	NS	857	484-1693	NS	1455	423-2737	NS†
Absent	38	254	105-709		1078	298-1902		914	173-1143		1008	213-1455	

<i>Dientamoeba</i>													
Present	3	884	453-2052	NS	1597	733-3186	NS	1356	658-1851	NS	988	540-2562	NS
Absent	93	453	238-884		1405	663-2837		859	487-1439		1120	225-2724	
<i>Chilomastix</i>													
Present	4	272	181-585	NS	429	71-1520	NS	389	60-1306	NS	383	128-1578	NS
Absent	92	612	238-884		1413	682-2837		921	487-1566		1121	255-2724	
<i>Balantidium</i>													
Present	9	677	556-685	NS	1901	793-1901	NS	931	578-1693	NS	1449	434-1449	NS
Absent	87	453	238-884		1405	637-2837		859	487-1439		1089	225-2724	
<i>Entamoeba</i>													
Present	54	446	236-870	NS	1212	638-2306	NS	857	484-1354	NS	966	213-2542	0.05*
Absent	42	678	236-1426		1902	988-3184		929	484-1688		1455	981-2751	
<i>Iodamoeba</i>													
Present	5	884	285-1424	NS	2305	749-2692	NS	859	658-1953	NS	2240	534-2609	NS
Absent	91	453	238-884		1405	663-2837		910	487-1439		1089	225-2724	

Median distance. Interquartile range (IQR=50%). * p<0.05; † p<0.10

Table 3.4. Impact of forest structure and group size on presence of gastrointestinal parasites. Median distance for individual monkeys with and without each parasite were compared using a Mann Whitney U Test. 50% interquartile range (IQR)

	Basal Area (m2/ha)				% Trees >40cm			Group size			
	N	Median	IQR	<i>p</i>	Media n	IQR	<i>p</i>	N	Median	IQR	<i>p</i>
<i>Strongyloides</i>											
Present	80	8.1	5.5-12.9	NS	8.9	6.9-14.0	NS	22	NA	NA	NA
Absent	12	12.9	8.2-16.3		14.4	7.9-22.5		0			
<i>Cyclospora</i>											
Present	17	5.5	5.4-12.0	0.05*	11.1	5.9-14.0	NS	10	5.5	4-7	NS
Absent	75	11.1	6.3-15.4		10.0	6.9-14.0		12	3.0	1.5-5.5	
<i>Isoospora</i>											
Present	3	11.1	6.4-11.1	NS	7.9	7.9-11.1	NS	2	8.5	7-10	NS
Absent	89	8.1	5.5-13.8		10.0	6.9-14.0		20	4.0	2-6.5	
<i>Capillaria</i>											
Present	73	9.8	5.5-13.8	NS	7.9	6.9-14.0	NS	22	NA	NA	NA
Absent	19	8.1	6.4-15.9		11.1	7.9-14.8		0			
<i>Enterobius</i>											
Present	3	6.4	0.9-6.4	NS	11.1	0-11.1	NS	2	7.0	4-10	NS
Absent	89	9.8	5.5-13.8		10.0	6.9-14.0		20	4.0	2-7	
<i>Trypanoxyuris</i>											
Present	11	7.8	5.5-15.4	NS	7.9	7.4-11.1	NS	3	6.0	3-7	NS
Absent	81	9.8	5.5-13.8		11.1	6.9-14.0		19	4.0	2-7	
<i>Controrchis</i>											
Present	13	12.1	11.3-15.9	0.04*	14.0	7.7-25.0	0.03*	9	5.0	4-7	NS
Absent	79	8.1	5.5-13.8		7.9	5.9-14.0		13	3.0	2-6	
<i>Blastocystis</i>											
Present	60	11.1	6.3-15.4	NS	11.1	6.7-14.6	0.04*	22	NA	NA	NA
Absent	31	6.4	5.4-12.1		7.8	6.9-11.1		0			
<i>Dientamoeba</i>											
Present	3	5.4	0.9-8.1	NS	4.9	0.0-25.0	NS	3	5.0	4-5	NS
Absent	89	9.8	5.5-13.8		10.0	6.9-14.0		19	4.0	2-7	
<i>Chilomastix</i>											
Present	4	7.2	4.6-20.5	NS	8.4	5.4-25.1	NS	4	6.0	3.5-8.5	NS
Absent	88	9.8	5.5-13.8		10.0	6.9-14.0		18	4.0	2-7	
<i>Balantidium</i>											
Present	8	11.1	8.7-15.4	NS	7.9	7.6-9.5	NS	6	7.0	6-8	0.01*
Absent	84	8.1	5.4-13.8		11.1	6.4-14.0		16	3.5	2-5	
<i>Entamoeba</i>											
Present	48	8.1	5.4-15.4	NS	10.0	5.4-14.0	NS	16	5.0	4-7	NS
Absent	44	9.8	5.5-13.8		11.1	7.6-14.0		6	2.0	1-3	
<i>Iodamoeba</i>											
Present	4	9.7	7.2-22.1	NS	9.0	5.9-25.1	NS	5	5.0	4-7	NS
Absent	88	9.0	5.5-13.8		10.0	6.9-14.0		17	4.0	2-7	

Median distance. Interquartile range (IQR=50%). * $p < 0.05$; † $p < 0.10$

Continuous data obtained for each risk factor was dichotomously categorized for univariate analysis (Table 3.5). In this analysis, only forest structure indicators were associated with the presence of *Controrchis* sp. Individuals living in forests with higher basal area (>25m²/ha) were ten times more likely to harbor *Controrchis* sp., and these same monkeys were three times more likely to harbor *Controrchis* sp. in forest with >15 percent old growth. Individuals living in areas with a lower percent old growth (<15%) were more than 4 times likely to harbor *Strongyloides* sp.

Group size was statistically relevant with regard to the presence of *Balantidium* sp.; all tended to be larger, on average, than those without ($p=0.01$) (Table 3.4). *Strongyloides* sp., *Capillaria* sp. and *Blastocystis* sp. were found in every group so no comparison could be made. At a group level there were no significant differences between presence or absence of any parasite species with anthropogenic disturbance variables.

Table 3.5. Logistic regression analysis comparing various measured indicators of anthropogenic disturbance with identified parasites found in Ecuadorian mantled howler monkeys, *Alouatta palliata aequatorialis*. The odds ratio (OR) refers to the probability of having each gastrointestinal parasite species in the former category (e.g., 0-500m) versus the second category (e.g., >500m).

	Distance to Road (0-500m vs. >500m)		Distance to Humans (0-500m vs. >500m)		Distance to Agricultural Plots (0-500m vs. >500m)		Distance to Bilsa Biological Station (0-500m vs. >500m)		Basal Area (0-25 m ² /ha vs. >25 m ² /ha)		Percentage Trees >40cm DBH (0-15% vs. >15%)	
	OR (CI)	<i>p</i>	OR (CI)	<i>p</i>	OR (CI)	<i>p</i>	OR (CI)	<i>p</i>	OR (CI)	<i>p</i>	OR (CI)	<i>p</i>
<i>Strongyloides</i>	1 (0.4-1.6)	1.0	3.4 (2.4-4.5)	0.25	1.5 (0.8-2.2)	0.57	5.2 (4.2-6.3)	0.12	3.8 (2.9-4.7)	0.15	4.3 (3.7-5.0)	0.02*
<i>Cyclospora</i>	1.5 (1.0-2.1)	0.42	0.7 (0.0-1.4)	0.64	0.4 (-0.3-1.1)	0.17	0.7 (0.1-1.3)	0.57	1.1 (0.0-2.3)	0.91	1.0 (0.3-1.6)	0.94
<i>Isospora</i>	0.5 (-0.7-1.7)	0.57	1.8 (0.6-3.1)	0.63	1.1 (-0.2-2.3)	0.97	1.2 (0.0-2.4)	0.87	NA	NA	NA	NA
<i>Capillaria</i>	0.9 (0.4-1.4)	0.81	0.5 (-0.1-1.0)	0.16	0.4 (-0.1-0.9)	0.09†	1.0 (0.5-1.6)	0.95	0.8 (-0.3-1.9)	0.8	2.5 (1.9-3.0)	0.11
<i>Enterobius</i>	2.0 (0.8-3.3)	0.57	7.8 (6.6-9.0)	0.1†	4.4 (3.2-5.6)	0.23	5.2 (3.9-6.4)	0.19	NA	NA	NA	NA
<i>Trypanoxyuris</i>	1.2 (0.6-1.9)	0.75	1.4 (0.7-2.1)	0.65	1.9 (1.3-2.5)	0.33	2.2 (1.6-2.9)	0.22	0.7 (-0.5-1.8)	0.71	1.4 (0.6-2.2)	0.7
<i>Controrchis</i>	1.4 (0.8-2.0)	0.56	0.2 (-0.8-1.3)	0.18	0.5 (-0.2-1.2)	0.35	0.2 (-0.9-1.2)	0.08†	0.1 (-0.7-1.0)	0.02*	0.3 (-0.3-0.9)	0.04*
<i>Blastocystis</i>	0.6 (0.2-1.0)	0.24	0.7 (0.3-1.2)	0.55	1.6 (1.2-2.1)	0.29	0.6 (0.2-1.1)	0.84	NA	NA	0.3 (-0.5-1.0)	0.09†
<i>Dientamoeba</i>	0.5 (-0.7-1.7)	0.57	NA	NA	NA	NA	NA	NA	NA	NA	0.6 (-0.6-1.8)	0.67
<i>Chilomastix</i>	3.1 (2.0-4.3)	0.33	3.8 (2.8-4.9)	0.19	2.2 (1.2-3.2)	0.45	2.5 (1.5-3.5)	0.36	0.2 (-1.0-1.4)	0.17	0.9 (-0.3-2.0)	0.92
<i>Balantidium</i>	0.3 (-0.6-1.1)	0.1†	0.4 (-0.7-1.5)	0.42	0.6 (-0.2-1.4)	0.5	1.2 (0.5-2.0)	0.77	NA	NA	2.2 (1.1-3.3)	0.48
<i>Entamoeba</i>	1.5 (1.1-1.9)	0.36	1.1 (0.6-1.6)	0.89	0.7 (0.3-1.1)	0.44	1.7 (1.3-2.2)	0.23	0.6 (-0.3-1.5)	0.59	0.8 (0.2-1.4)	0.67
<i>Iodamoeba</i>	0.7 (-0.3-1.6)	0.65	0.9 (-0.3-2.0)	0.92	0.5 (-0.6-1.6)	0.55	0.6 (-0.5-1.7)	0.65	0.2 (-1.0-1.4)	0.17	0.9 (-0.3-2.0)	0.92

* *p* < 0.05 and † *p* < 0.10

DISCUSSION

This study provides evidence that human encroachment and forest degradation are associated with the presence of specific gastrointestinal parasites in mantled howler monkeys. Three indicators of human proximity (distance to agricultural plots, to a research station, and to closest human settlement) were significantly associated with the presence of both *Strongyloides* sp. and *Controrchis* sp. (Figure 3.3). Two indicators of forest structure (basal area and percentage of trees >40cm DBH) were also significantly associated with the presence of *Cyclospora* sp., *Controrchis* sp., and *Strongyloides* sp. Individuals with *Strongyloides* sp. were more likely to be found near agricultural plots, near a local research station, and in secondary forest types. A previous study found that baboons living closer to agricultural plots are more likely to feed on crops and come in contact with the same soil-transmitted parasites that occur in humans (Weyher et al., 2006). Similarly, I speculate that howlers living in secondary forest are more likely to traverse on the ground during crop raiding, and to travel further in search of food due to reduced resource availability. There is evidence to suggest that the soil-transmitted nematode, *Strongyloides* sp., is quite common in Ecuadorian communities, increasing the chances for interspecies transmission (Olsen et al., 2009). Genetic comparison of parasites from both host species would be useful in determining how likely this transmission might be.

Individuals living in disturbed forest were more likely to harbor *Cyclospora* sp. A coccidian parasite, *Cyclospora* causes prolonged diarrhea and is found in humans and other primates throughout the world (Ortega et al., 1993; Chacin-Bonilla et al., 2010). Other related parasite species have been described, which makes it difficult to tell if this is indeed the same species that infects humans (Eberhard et al., 1999; Lopez et al., 1999). No domestic animals have been characterized as reservoir hosts, though whether other species such as primates are paratenic or reservoir hosts is still unknown (Ghimire and Sherchan, 2008). Because monkeys

living in degraded forest were most likely to be infected, it is possible these monkeys are acquiring *Cyclospora* for the same reasons as those with *Strongyloides* – they descend to the ground more often or travel further to find food thus increasing their risk of coming into contact with contaminated soils. This is purely speculative without further behavioral data from studies assessing gastrointestinal parasitism.

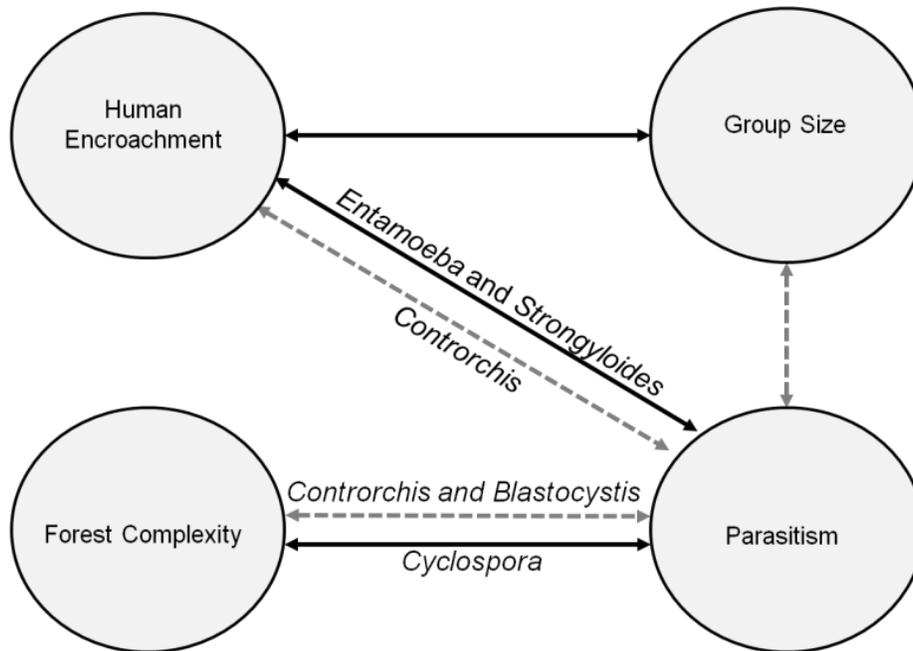


Figure 3.3. Results Measured associations between two indicators of anthropogenic disturbance (human encroachment and forest complexity), group size and presence of observed gastrointestinal parasite species. Arrows represent statistically significant associations. Solid black line (negative association) and dotted gray line (positive association).

Antithetical to our original hypothesis, individual howlers infected with *Controrchis* sp. were more likely to be found in areas of low disturbance. Cecropia trees that harbor ants infected with *Controrchis* sp. are primarily found in disturbed habitat and are often eaten by howler monkeys, which suggests they may be ingesting ants unintentionally (Alvarez-Buylla and Garay, 1994; Kowalzik et al., 2010). As a result, I expected howlers inhabiting secondary forest to show higher prevalence rates, but the opposite was found. Individuals infected with *Controrchis* sp. were also significantly more likely to be found further from people. My only explanation for this

apparent contradiction is that *Cecropia* trees are still found in open gaps in primary forest (Sanford et al., 1986), suggesting that gaps could create a higher prevalence of *Controrchis* sp. in monkeys living in these areas.

Causal Pathway

There are numerous causal pathways that could conceivably explain how anthropogenic disturbance affects parasite communities in primates as previously depicted (Figure 3.1). Host nutrition might be affected by reduced habitat quality which increases their propensity for infection (Junge et al., 2011); physiological stress may be higher in degraded habitats making individuals more prone to infection (Martinez-Mota et al., 2007); individuals may be forced into smaller areas, thereby increasing density, contact rates and subsequent exposure (Stoner, 1996; Vitazkova and Wade, 2007; Mborá and McPeck, 2009); restricted dispersal in fragmented landscapes can increase inbreeding risk and subsequent susceptibility for infection (Estrada et al., 2002; Oklander et al., 2010); or they may simply be more likely to acquire parasites from local human communities due to physical proximity (Graczyk et al., 2002; Goldberg et al. 2007; Goldberg et al., 2008; Kowalewski et al., 2010).

Forest degradation also appears to be linked to primate group size, where larger groups are found in unlogged forests (e.g., Onderdonk and Chapman, 2000). This relationship between group size and forest structure is likely a result of increased food availability in primary forest (Chapman et al., 1995; Clarke et al., 2002; Gillespie et al., 2005; Marshall et al., 2005; Gillespie and Chapman, 2008). In turn, larger groups are associated with increased parasite species richness (Freeland, 1976; Nunn et al., 2003), attributed to higher contact rates and subsequent transmission rates of directly transmitted parasites (Cote and Poulin, 1995; Arneberg et al., 1998; McCallum et al., 2001; Cross et al., 2009). However, based on this logic, larger groups found in primary forest should have the highest parasitism, which has not been reported. Rather,

parasitism is highest in groups found in logged forests (e.g., Gillespie et al., 2005; Gillespie and Chapman, 2008).

Neither forest structure nor human encroachment measurements were significantly associated with group size, though those living nearest to a biological research station, roads, and in secondary forest tended to be larger. The forests in our sampled area have had nearly 15 years to recover, which might actually provide an ideal heterogeneous habitat for howlers (Ortega-Andrade et al., 2010), thus explaining why changes in forest structure may have had a limited impact on overall parasite species richness, and may have mitigated changes in group size. Mantled howler monkeys are also well-adapted to changing food sources as a result of past deforestation (Peres, 1997; Pinto et al., 2003). Perhaps more recent effects of logging would have resulted in a larger impact on ranging patterns, diet, and stress, subsequently providing a more dichotomous comparison with undisturbed primary forest. Pinto et al. (2003) outline other possibilities why howler behavior might not change with modified forest structure, including low logging intensity, untouched forest adjacent to harvested areas, and extended time period between last harvest and sampling. Additional studies looking at more recent and more severe levels of anthropogenic disturbance would prove insightful.

Another possibility is that forest degradation is directly associated with the presence or absence of specific parasites species, and these parasites may influence group size. If primate group size was regulated by frequency dependent transmission of gastrointestinal parasites, then I would expect an association between group size and the presence of certain parasite species (Cote and Poulin, 1995). I did indeed find that groups with *Cyclospora* sp., *Isospora* sp., *Balantidium* sp., and *Entamoeba* spp. all tended to be larger, on average, compared to those without each of these parasites. These four parasites are protozoan and transmitted via the fecal-oral route. Group size could not be compared with the presence of *Strongyloides*, *Capillaria* or

Blastocystis because these parasite species were found in every group. Several of these gastrointestinal parasite species do have the potential to be pathogenic. *Cyclospora* sp., *Isospora* sp., *Balantidium* sp., and *Entamoeba* spp. are all transmitted in humans via consumption of food or water contaminated with feces, causing nausea, vomiting, anorexia, weight loss, and severe diarrhea (Ortega et al., 1993; Soave, 1996; Chacin-Bonilla, 2010; Weiss and Keohane, 1997). Most of these parasites have the potential to reduce fitness, causing morbidity or even death (Table 3.1); however, without empirical testing we cannot determine whether the presence of certain parasites – or combinations of parasites - regulates group size, if group size influences parasite transmission rates, or if group size is simply correlated with other ecological variables (e.g., food patch size, density, or quality) (Chapman et al., 2009).

In conclusion, forest structure and human encroachment had a significant impact on the presence of three parasites. *Strongyloides* sp. was negatively associated with distance to agricultural plots and a local field research station. *Controrchis* sp. was positively associated with distance to people and with two indicators of forest structure. And *Cyclospora* sp. was negatively associated with basal area. Expanding human populations, particularly in tropical countries where many of the world's primate species and other wildlife live, will likely lead to greater interspecies interactions and subsequent expansion of infectious diseases throughout human populations (Wolfe et al., 2007; Vitazkova, 2009; Pedersen and Davies, 2010). At the same time, many primates are in peril, either from logging or hunting, and it would appear that human encroachment could also play an important role in their health. These results are likely to be applicable to disturbed systems throughout the tropics, suggesting a potential threat to other wildlife.

LITERATURE CITED

- Alvarez-Buyllia, E. R., and A. A. Garay. 1994. Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution* 437-453.
- Amato, J. F., S. B. Amato, C. Calegario-Marques, and J. C. Bicca-Marques. 2002. *Trypanoxyuris* (*Trypanoxyuris*) *minutus* associated with the death of a wild southern brown howler monkey, *Alouatta guariba clamitans*. Rio Grande do Sul, Brazil. *Arquivos de Instituto Biológico* 69: 99-102.
- Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society B* 265: 1283-1289.
- Arroyo-Rodriguez, V., and P. A. Dias. 2010. Effects of habitat fragmentation and disturbance on howler monkeys: a review. *American Journal of Primatology* 72: 1-16.
- Beaver, P. C., R. C. Jung, and E. W. Cupp. 1984. *Clinical Parasitology*. Lea & Febiger, Philadelphia, P.A. p. 825.
- Chacín-Bonilla, L. 2010. Epidemiology of *Cyclospora cayetanensis*: A review focusing in endemic areas. *Acta Tropica* 115: 181-193.
- Chapman, C. A., L. J. Chapman, and R. W. Wrangham. 1995. Ecological constraints on group size: an analysis of spider monkey and chimpanzee subgroups. *Behavioral Ecology and Sociobiology* 36: 59-70.
- Chapman, C., T. Gillespie, and T. Goldberg. 2005. Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology* 14: 134-144.
- Chapman, C. A., M. L. Speirs, T. R. Gillespie, T. Holland, and K. M. Austad. 2006. Life on the edge: gastrointestinal parasites from the forest edge and interior primate groups. *American Journal of Primatology* 68: 397-409.
- Chapman, C. A., M. A. Huffman, S. J. Ryan, R. Sengupta, and T. L. Goldberg. 2009. Ways forward in the study of primate parasite ecology. *Primate Parasite Ecology: The Dynamics and Study of Host-Parasite Relationships*: 487-505.
- Charpentier, M. J., C. V. Williams, and C. M. Drea. 2008. Inbreeding depression in ring-tailed lemurs (*Lemur catta*): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics* 9: 1605-1615.
- Clarke M. R., D. A. Collins, and E. L. Zucker. 2002. Responses to deforestation in a group of mantled howlers (*Alouatta palliata*) in Costa Rica. *International Journal of Primatology* 23: 365-381.

- Colwell, R. K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, Connecticut.
- Coombs, I., and D. W. Crompton. 1991. A Guide to Human Helminths. Taylor & Francis, London, UK. p. 192.
- Coop, R. L., and P. H. Holmes. 1996. Nutrition and parasite interaction. *International Journal of Parasitology* 26: 951-962.
- Côté, I. M., and R. Poulin. 1995. Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* 6: 159-165.
- Cottam, G., and J. T. Curtis. 1956. The use of distance measures in phytosociological sampling. *Ecology* 451-460.
- Cross, P. C., J. Drewe, V. Patrek, G. Pearce, M. D. Samuel, and R. J. Delahay. 2009. Wildlife population structure and parasite transmission: implications for disease management. *In: Management of disease in wild mammals*. Delahay, R.J., C.S. Graham, M.R. Hutchings. (eds.). Springer, Japan. p. 9-29.
- Daszak, P., A. Cunningham, and A. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* 78: 103-116.
- Dobson, A. P. 1988. The population biology of parasite-induced changes in host behavior. *Quarterly Review of Biology* 139-165.
- Eberhard, M. L., A. J. da Silva, B. G. Lilley, and N. J. Pieniazek. 1999. Morphologic and molecular characterization of new *Cyclospora* species from Ethiopian monkeys: *C. cercopithecii* sp. n., *C. colobi* sp. n., and *C. papionis* sp. n. *Emerging Infectious Diseases* 5: 651.
- Estrada, A., A. Mendoza, L. Castellanos, R. Pacheco, S. Van Belle, Y. García, and D. Muñoz. 2002. Population of the black howler monkey (*Alouatta pigra*) in a fragmented landscape in Palenque, Chiapas, Mexico. *American Journal of Primatology* 58: 45-55.
- Freeland, W. J. 1976. Pathogens and the evolution of primate sociality. *Biotropica* 8: 12-24.
- Ghimire, T. R., and J. B. Sherchan. 2006. Human Infection of *Cyclospora cayetanensis*: a review on its medico-biological and epidemiological pattern in global scenario. *Journal of Nepal Health Research Council* 4: 31-2.
- Gillespie, T. R., C. A. Chapman, and E. C. Greiner. 2005. Effects of logging on gastrointestinal parasite infections and infection risk in African. *Journal of Applied Ecology* 42: 699-707.
- Gillespie, T. R., and C. A. Chapman. 2008. Forest fragmentation, the decline of an endangered primate, and changes in host-parasite interactions relative to an unfragmented forest. *American Journal of Primatology* 70: 222-230.

- Goldberg, T., T. Gillespie, I. Rwego, E. Wheeler, E. L. Estoff, and C. A. Chapman. 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation* 135: 511-517.
- Goldberg, T. L., T. R. Gillespie, I. B. Rwego, E. L. Estoff, and C. A. Chapman. 2008. Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. *Emerging Infectious Diseases* 14: 1375-1382.
- Graczyk, T. K., J. Bosco-Nizeyi, B. Ssebide, R. C. Thompson, C. Read, and M. R. Cranfield. 2002. Anthropozoonotic *Giardia duodenalis* genotype (assemblage) A infections in habitats of free-ranging human-habituated gorillas, Uganda. *Journal of Parasitology* 88: 905-909.
- Helenbrook, W. 2006. Non-invasive sampling of mandrill and drill monkeys for use in genetic analyses. Thesis, SUNY College at Buffalo, Buffalo, New York.
- Howells, M. E., J. Pruetz, and T. R. Gillespie. 2011. Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: the case of sympatric Western chimpanzees (*Pan troglodytes verus*) and Guinea baboons (*Papio hamadryas papio*) at Fongoli, Senegal. *American Journal of Primatology* 73: 173-179.
- Jones, B. A., D. Grace, R. Kock, S. Alonso, J. Rushton, M. Y. Said, and D. U. Pfeiffer. 2013. Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences* 110: 8399-8404.
- Junge R. E., M. A. Barrett, and A. D. Yoder. 2011. Effects of anthropogenic disturbance on Indri (*Indri indri*) health in Madagascar. *American Journal of Primatology* 73: 632-642.
- Kowalewski, M. M., J. S. Salzer, J. C. Deutsch, M. Raño, M. S. Kuhlenschmidt, and T. R. Gillespie. 2010. Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: patterns of zoonotic protozoa infection relative to degree of human-primate contact. *American Journal of Primatology* 71: 1-9.
- Kowalzik, B. K., M. S. Pavelka, S. J. Kutz, and A. Behie. 2010. Parasites, primates, and ant-plants: clues to the life cycle of *Controrchis* spp. in black howler monkeys (*Alouatta palliata*) in Southern Belize. *Journal of Wildlife Disease* 46: 1330-1334.
- Lane, K. E., C. Holley, H. Hollocher, and A. Fuentes. 2011. The anthropogenic environment lessens the intensity and prevalence of gastrointestinal parasites in Balinese long-tailed macaques (*Macaca fascicularis*). *Primates* 52: 117-128.
- Loomis, M. 1983. Hepatic and gastric amebiasis in black and white colobus monkeys. *Journal of American Veterinary Medicine Association* 183: 1188-1191.
- Lopez, F.A., J. Manglicmot, T. M. Schmidt, C. Yeh, H. V. Smith, and D. A. Relman. 1999. Molecular characterization of *Cyclospora*-like organisms from baboons. *Journal of Infectious Diseases* 179: 670-676.

- Marcogliese, D. J. 2005. Parasites of the superorganism: are they indicators of ecosystem health?. *International Journal for Parasitology* 35: 705-716.
- Marshall, A. R., J. E. Topp-Jørgensen, H. Brink, and E. Fanning. 2005. Monkey abundance and social structure in two high-elevation forest reserves in the Udzungwa Mountains of Tanzania. *International Journal of Primatology* 26: 127-145.
- Martinez-Mota, R., C. Valdespino, M. A. Sanchez-Ramos, and J. C. Serio-Silva. 2007. Effects of forest fragmentation on the physiological stress response of black howler monkeys. *Animal Conservation* 10: 374-379.
- Mbora, D. N., and M. A. PePeeck. 2009. Host density and human activities mediate increased parasite prevalence and richness in primates threatened by habitat loss and fragmentation. *Journal of Animal Ecology* 78: 210-218.
- McCallum, H., N. Barlow, and J. Hone. 2001. How should pathogen transmission be modelled? *Trends in Ecology and Evolution* 16: 295-300.
- McClure, H. M., and N. B. Guilloid. 1971. Comparative pathology of the chimpanzee. *The Chimpanzee* 4: 103-272.
- Mooring, M. S., and B. L. Hart. 1992. Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. *Behaviour* 173-193.
- Morse, S. S. 1995. Factors in the emergence of infectious diseases. *Emerging Infectious Diseases* 1: 7-15.
- Muriuki, S. M. K., R. K. Murugu, E. Munene, G. M. Karere, and D. C. Chai. 1998. Some gastrointestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica* 71: 73-82.
- Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative test of parasite species richness in primates. *The American Naturalist* 162: 597-614.
- Oklander, L. I., M. M. Kowalewski, and D. Corach. 2010. Genetic consequences of habitat fragmentation in black-and-gold howler (*Alouatta caraya*) populations from northern Argentina. *International Journal of Primatology* 31: 813-832.
- Olsen, A., L. van Lieshout, H. Marti, T. Polderman, K. Polman, P. Steinmann, R. Stothard, S. Thybo, J. J. Verweij, and P. Magnussen. 2009. Strongyloidiasis—the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103: 967-972.
- Onderdonk, D. A., and C. A. Chapman. 2000. Coping with forest fragmentation: the primates of Kibale National Park, Uganda. *International Journal of Primatology* 21: 587-611.
- Ortega, Y. R., C. R. Sterling, R. H. Gilman, V. A. Cama, and F. Diaz. 1993. *Cyclospora* species—a new protozoan pathogen of humans. *New England Journal of Medicine* 328: 1308-1312.

- Ortega-Andrade, H. M., J. Bermingham, C. Aulestia, and C. Paucar. 2010. Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. *Check List* 6: 119-154.
- Pampiglione, S., and M. L. Ricciardi. 1972. Geographic distribution of *Stongyloides fulleborni* in humans in tropical Africa. *Parasitologia* 14: 329-338.
- Patz, J. A., T. K. Graczyk, N. Geller, and A. Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30: 1395-1405.
- Pedersen, A. B., and T. J. Davies. 2010. Cross-species pathogen transmission and disease emergence in primates. *EcoHealth* 6: 496-508.
- Peres, C. A. 1997. Effects of habitat quality and hunting pressure on arboreal folivore densities in neotropical forests: a case study of howler monkeys (*Alouatta* spp.). *Folia Primatologica* 68: 199-222.
- Pinto, A. C., C. Azevedo-Ramos, and O. de Carvalho Jr. 2003. Activity patterns and diet of the howler monkey *Alouatta belzebul* in areas of logged and unlogged forest in Eastern Amazonia. *Animal Biodiversity and Conservation* 26: 39-49.
- Puttker, T., Y. Meyer-Lucht, and S. Sommer. 2008. Effects of fragmentation on parasite burden (nematodes) of generalist and specialist small mammal species in secondary forest fragments of the coastal Atlantic Forest, Brazil. *Ecological Research* 23: 207-215.
- Raharivololona, B. M., and J. U. Ganzhorn. 2009. Gastrointestinal parasite infection of the Gray mouse lemur (*Microcebus murinus*) in the littoral forest of Mandena, Madagascar: Effects of forest fragmentation and degradation. *Madagascar Conservation & Development* 4: 103-112.
- Salzer, J. S., I. B. Rwego, T. L. Goldberg, M. S. Kuhlenschmidt, and T. R. Gillespie. 2007. *Giardia* sp. and *Cryptosporidium* sp. infections in primates in fragmented and undisturbed forest in western Uganda. *Journal of Parasitology* 93: 439-440.
- Sanford, R. L., H. E. Braker, and G. S. Hartshorn. 1986. Canopy openings in a primary neotropical lowland forest. *Journal of Tropical Ecology* 2: 277-282.
- Schwitzer, N., D. Clough, H. Zahner, W. Kaumanns, P. Kappeler, and C. Schwitzer. 2010. Parasite prevalence in blue-eyed black lemurs *Eulemur flavifrons* in differently degraded forest fragments. *Endangered Species Research* 12: 215-225.
- Soave, R. 1996. Cyclospora: an overview. *Clinical Infectious Diseases* 23: 429-435.
- Stoner, K. E. 1996. Prevalence and intensity of intestinal parasites in mantled howler monkeys (*Alouatta palliata*) in Northeastern Costa Rica: Implications for conservation biology. *Conservation Biology* 10: 539-546.
- Taylor, L., S. Latham, and M. Woolhouse. 2001. Risk factors for human disease emergence. *Philosophical Transactions Royal Society of London Series B* 356: 983-989.

- Teare, J. A., and M. R. Loomis. 1982. Epizootic of Balantidiasis in lowland gorillas. *Journal of the American Veterinary Medical Association* 181: 1345-1347.
- Teichroeb, J. A., S. J. Kutz, U. Parkar, R. C. Thompson, and P. Sicotte. 2009. Ecology of the gastrointestinal parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: Possible anthroponotic transmission. *American Journal of Physical Anthropology* 140: 498-507.
- Trejo-Macias, G., A. Estrada, and M. A. Cabrera. 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in Southern Mexico. *International Journal of Primatology* 28: 931-945.
- Trejo-Macias, G., and A. Estrada. 2012. Risk factors connected to gastrointestinal parasites in mantled *Alouatta palliata mexicana* and black howler monkeys *Alouatta pigra* living in continuous and in fragmented rainforests in Mexico. *Current Zoology* 58: 375-383.
- Valdespino, C., G. Rico-Hernández, and S. Mandujano. 2010. Gastrointestinal parasites of howler monkeys (*Alouatta palliata*) inhabiting the fragmented landscape of the Santa Marta mountain range, Veracruz, Mexico. *American Journal of Primatology* 72: 539-548.
- Vandergrift, K. J., T. R. Raffel, and P. J. Hudson. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. *Ecology* 89: 2251-2258.
- Vitazkova, S. K., and S. E. Wade. 2007. Effects of ecology on the gastrointestinal parasites of *Alouatta pigra*. *International Journal of Primatology* 28: 1327-1343.
- Vitazkova, S. K. 2009. Overview of parasites infecting howler monkeys, *Alouatta* sp., and human-howler interactions. *In: Primate parasite ecology: the dynamics and study of host-parasite relationships*. Huffman, M. A., and C. A. Chapman (eds.). Cambridge University Press, Cambridge. p 371-385.
- Wallis, J., and D. R. Lee. 1999. Primate conservation: the prevention of disease transmission. *International Journal of Primatology* 20: 803-826.
- Walther, B. A., and S. Morand. 1998. Comparative performance of species richness estimation methods. *Parasitology* 116: 395-405.
- Weiss, L. M., and E. M. Keohane. 1997. The uncommon gastrointestinal Protozoa: Microsporidia, *Blastocystis*, *Isospora*, *Dientamoeba*, and *Balantidium*. *Current Clinical Topics in Infectious Diseases* 17: 147-187.
- Wells, K. L., E. K. Smales, and M. Pfeiffer. 2007. Impact of rain-forest logging on helminth assemblages in small mammals (Muridae, Tupaiidae) from Borneo. *Journal of Tropical Ecology* 23: 35-43.
- Weyher, A. H., C. Ross, and S. Semple. 2006. Gastrointestinal parasites in crop raiding and wild foraging *Papio anubis* in Nigeria. *International Journal of Primatology* 27: 1519-1534.

Wilcox, B. A., and R. R. Colwell. 2005. Emerging and reemerging infectious diseases: biocomplexity as an interdisciplinary paradigm. *EcoHealth* 2: 244-257.

Wolfe, N. D., C. P. Dunavan, and J. Diamond. 2007. Origins of major human infectious diseases. *Nature* 447: 279-283.

Chapter 4

CHARACTERIZATION OF *BLASTOCYSTIS* SPECIES INFECTION IN HUMANS AND MANTELD HOWLER MONKEYS, *ALOUATTA PALLIATA AEQUATORIALIS*, PROXIMAL TO ONE ANOTHER

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ABSTRACT

This study characterizes *Blastocystis* species infections in humans and mantled howler monkeys, *Alouatta palliata aequatorialis*, living in close proximity to one another in northwestern Ecuador. *Blastocystis* species were identified from 58 of 96 (60.4%) mantled howler monkey fecal samples, and 44 of 55 human fecal samples (81.5%) by polymerase chain reaction (PCR). Using single stranded conformation polymorphism, I was able to efficiently separate and sequence subtypes (ST) within mixed samples without the need for cloning. *Blastocystis* ST1, ST2, and ST3 were found in people, and two individuals were infected with more than one subtype. All monkey samples were ST8 and could be divided into three unique sequence types. The lack of shared subtypes between humans and monkeys suggests no *Blastocystis* transmission occurs between these species in spite of close proximity in some instances. Based on analysis of demographic data from a questionnaire given to human participants, individuals who boiled their water before consumption were significantly less likely to be infected with *Blastocystis* (44.4%) compared to those who did not (93.8%). No other risk factors were significant, though hunters, females, individuals living in large families and those living closer to forested habitat tended to have a higher proportion of *Blastocystis* infections.

Key words: *Blastocystis*, *Alouatta*, Ecuador, single stranded conformation polymorphism, zoonoses, gastrointestinal

INTRODUCTION

Transmission of parasites between humans and wildlife poses a potential health risk to both groups (Polley, 2005; Thompson et al., 2009; Thompson, 2013). The spread of emerging pathogens in either human or wildlife populations places each at risk of new infections or from expansion of previously rare pathogens (Muriuki et al., 1998; Daszak et al., 2000; Pedersen et

al., 2005; Pedersen and Davies, 2010). What is troubling is that in the tropics, some forty percent of infectious diseases have been linked to non-human primate origins, likely due to the close evolutionary relatedness of anthropoids and their often close contact with each other (Wolfe, 2007; Pedersen and Davies, 2010). Coupled with this idea is the fact that the tropics are where some of the greatest ecological disturbances are occurring, creating a hotbed for future zoonoses (Morse, 1995; Patz et al., 2000; Daszak et al., 2001; Chapman et al., 2005; Gillespie et al., 2005; Laurance et al., 2006; Mosandl et al., 2008; Puttker et al., 2008).

To investigate the likelihood of parasitic exchange between humans and wildlife, I chose the mantled howler monkey, *Alouatta palliata aequatorialis*, as a study species. This primate species lives in close proximity to people, often lives in degraded habitat or areas near agricultural fields which might be conducive to parasite transmission (i.e., crop raiding), and other gastrointestinal parasite studies have been conducted with *Alouatta* species (Gilbert, 1994; Stuart et al., 1998; Cruz et al., 2000; Phillips et al., 2004; Stoner and Gonzalez, 2006; Eckert, 2006; Vitazkova and Wade, 2006; Trejo-Macias, 2007; Cristobal-Azkarate et al., 2010). Here I focused on *Blastocystis* species, namely because it is one of the most commonly encountered parasite species from my morphological work (Chapter 2). *Blastocystis* species represent a complex of genetic groups subdivided into subtypes (ST) representing phylogenetic lineages (Stensvold et al., 2007). The occurrence of 17 distinct subtypes in people and other wild and domestic animals have been summarized previously (Noel et al. 2005; Stensvold et al. 2007; Stensvold et al. 2009; Parkar et al. 2010; Fayer et al. 2012; Alfellani et al. 2013a). For convenience, I will refer to this collection of subtypes heretofore as “*Blastocystis*” - as is the convention.

There are few reports identifying *Blastocystis* in wild howler monkeys (Phillips et al., 2004; Stoner and Gonzalez, 2006; Milozzi et al., 2012; Ramirez et al., 2014), and this may

simply be due to lack of testing and reporting. However, a study of *Blastocystis* subtypes in numerous organisms found in Colombia found that two sampled howler monkeys were ST4 positive (Ramirez et al. 2014). In captive howlers, ST8 has been reported from captive *Alouatta caraya* (Alfellani et al. 2013b). In other primates, including humans, a variety of subtypes have been found. For example, in old world primates and in humans several authors have reported ST1, ST2, ST3, and ST4 (Abe et al., 2004; Noel et al., 2005; Scicluna et al., 2006; Yoshikawa et al., 2009; Parkar et al., 2010; Petrasova et al., 2011; Stensvold et al., 2012; Alfellani et al., 2013). *Blastocystis* ST5 is widely found in apes (including humans), livestock, and several species of Old World monkeys (Noel et al. 2005; Parkar et al. 2007; Yan et al. 2007; Stensvold et al. 2009; Alfellani et al. 2013b). Subtype 6 has been reported in domestic animals and people (Scicluna et al. 2006; Parkar et al. 2010; Petrasova et al. 2011), subtype 8 has been found in found in several captive primate species, their caregivers, marsupials, and in a pheasant species (Stensvold et al. 2009; Petrasova et al. 2011; Alfellani et al. 2013b; Ramirez et al. 2014), subtype 9 has been found in humans, but not in nonhuman primates (Yoshikawa 2004; Noel et al. 2005; Petrasova et al. 2011; Stensvold et al. 2012), and subtypes 10, 13 and 15 have all been found in numerous Old World primates (Alfellani et al. 2013b).

Subtyping can be conducted directly from DNA extracted from feces and is typically conducted using conventional PCR and sequencing of the small subunit ribosomal DNA, (Scicluna et al., 2006; Whipps et al., 2010). However, when more than one subtype is present in a host, expensive and time-consuming cloning techniques - coupled with sequencing - are normally required to identify subtypes. A potentially faster and less expensive technique known as single strand conformation polymorphism (SSCP) has previously been used to detect *Blastocystis* subtypes in humans (Menounos et al., 2008). The advantage of SSCP is that mixed

subtypes can be separated on a gel and these fragments can then be sequenced by conventional methods.

The aim of this study is to examine the subtype composition of *Blastocystis* in humans and monkeys living in close proximity, using the SSCP methodology where mixed infections are encountered. For humans, questionnaire data will be used to determine whether there are particular attributes of people that make them more likely to harbor *Blastocystis* and a specific subtype. If interspecies transmission was happening, or there was a common source of infection, then I would expect to find identical subtypes in both monkeys and people. Conversely, the occurrence of distinct subtypes in humans and monkeys would suggest limited transmission and lack of a common source of infection. I would also expect that if direct or indirect transmission (environmental sources or reservoir host transmission) were occurring, that people who are in closest contact with nonhuman primates - in this case researchers and hunters - would be the most likely to have the same *Blastocystis* subtypes found in howler monkeys.

METHODS

Fecal sample collections were conducted in Ecuador at the Bilsa Biological Station (0°21'N 79°44'W) for howler monkeys (N=96) and from people (N=54) living in the local reserve or in adjacent communities from June to August 2011 (Figure 4.1). This field site has both primary and secondary forest, human population expansion into a buffer zone surrounding primate populations, and areas where humans and monkeys live as little as 5m away from each other. Fecal samples were collected using sterile tongue depressors and a subsample placed in RNeasy (Qiagen Inc., Valencia, California) for subsequent *Blastocystis* PCR analysis. Location of each sample was recorded using GPS (global positioning system) and howler monkey group

demographics noted when feasible. Contamination was minimized by wearing disposable gloves. Howler monkey field methods have previously been described in further detail (Chapter 2).

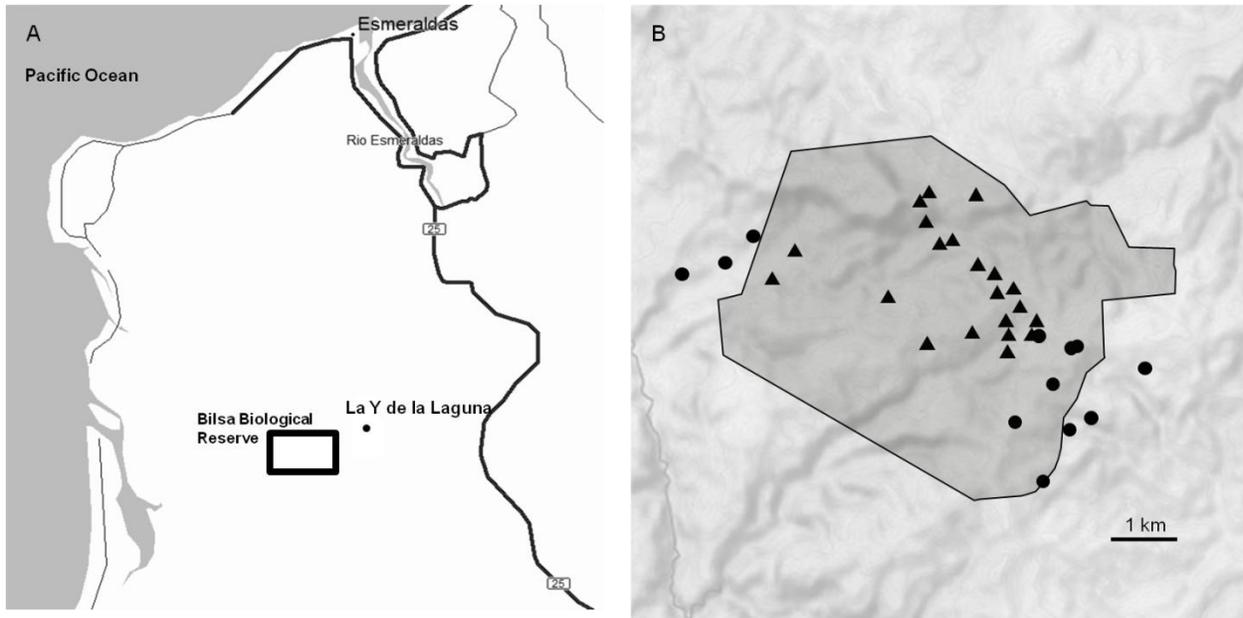


Figure 4.1 (A) Field site location in the Tumbes-Choco-Magdalena bioregion of northwestern Ecuador. (B) Ninety-six mantled howler monkeys samples were collected within the Bilsa reserve (triangles), along with 54 human samples from two surrounding communities and individuals working at the field research station (circles).

Fecal samples were collected from individuals living at the field station and in two communities outside the reserve at Dogola and La Yecita. Community meetings were advertised by word of mouth and information regarding the study was conveyed to possible participants. The research team worked through a questionnaire with each participant, asking questions about their age, size of family, occupation, interaction with animals (both domestic and wild, including non-human primates), method for sterilizing water, and whether they hunted. Information was provided on how to collect a fecal sample so as to minimize contamination using sterile surgical gloves. All samples were delivered to a central location within three hours of collections to be preserved. Each participant's house was also visited in order to gather GPS data and assess distance to nearest forested area.

Genomic DNA was extracted from 200 mg of feces using the QIAamp DNA Stool Mini Kit following the manufacturer's instructions. Partial *Blastocystis* species small subunit ribosomal DNA (SSU rDNA) was sequenced using previously published PCR based primer sets BLF and BLR (Menounos et al., 2008), BH1F (Whipps et al., 2010) and BHRDr (Scicluna et al., 2006), and bl1400ForC and bl1710RevC (Stensvold et al., 2006). DNA was amplified by PCR in 25 μ L reaction volumes in Quick-Load[®] Taq 2X Master Mix (New England Biolabs, Ipswich, Massachusetts), 0.25 μ M of each primer and 3 μ L of template DNA using a C1000 Thermal Cycler (Bio-Rad) for 40 cycles at 95°C for 30 s, 53°C for 60 s, 68°C for 60 s, preceded by an initial denaturation at 95°C for 3 min, and followed by a final extension at 68°C for 7 min. Product amplification was initially evaluated by agarose gel electrophoresis. Samples that did not amplify were run two more times before categorizing as negative. Positive samples were then run using SSCP to determine if multiple subtypes were present. For SSCP, 15 μ L of each PCR product was mixed with 28 μ L of loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol). Samples were denatured at 95°C for ten minutes and then snap-cooled by placing on ice for five minutes. Thirty-five microliters of sample were subjected to electrophoresis on a Bio-Rad Protean II xi Cell apparatus on a 0.4 mm thick 12% polyacrylamide gel in 1X TBE buffer at 300 V for 6 hours at 6-8°C. A thermostatically controlled refrigerated circulator was used to maintain a constant temperature throughout the buffer chamber. Polyacrylamide gels were post-stained with 10 mg/ml ethidium bromide and distinct amplified fragments were poked with a sterile pipette tip, which was transferred to a new PCR reaction for re-amplification. Amplified products were purified with E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Inc. Norcross, Georgia). Purified PCR samples were sequenced using forward primers BH1F, BLF, or bl1400ForC (depending on original PCR primers used) in reactions using the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1, on the

ABI3730xl Genetic Analyzer (Applied Biosystems, Foster City, California). Sequences were uploaded to BioEdit (Hall 1999), edited to remove base-calling errors and subtype determined based on highest sequence similarity from BLAST results in Genbank and checked against the phylogenetic framework of Whipps et al. (2010). Amplified nucleotide sequences obtained from both howler monkeys and people were deposited in GenBank (Table 4.1).

Table 4.1. *Blastocystis* species small subunit ribosomal DNA sequence types in howler monkeys and humans. Unique sequences within a subtype were assigned an alphabetic designator (A, B, or C) and the number (N) of each unique type is listed.

Host and subtype	Accession Number	N
Howler monkey host		
Subtype 8		
ST8-A	KM374608	29
ST8-B	KM374609	1
ST8-C	KM374610	1
Howler total		31†
Humans host		
Subtype 1		
ST1-A	KM374611	18
ST1-B	KM374612	1
ST1-C	KM374613	1
ST1-D	*	1
Subtype 2		
ST2-A	*	2
ST2-B	KM374619	4
ST2-C	KM374614	2
ST2-D	KM374615	1
ST2-E	KM374616	1
Subtype 3		
ST3-A	KM374617	11
ST3-B	KM374618	1
Human total		43‡

†DNA sequences generated solely from primer sets BLF/BLR (~260bp) and BH1F and BHRDr (~600bp). Sixteen howler monkey samples were amplified using bl1400ForC and bl1710RevC, yielding subtype 8; however, these samples are not included in this table because of incongruence between sequences. ‡Similarly, only human sequences amplified with BLF/BLR and BH1F and BHRDr are included. Two other samples could only be amplified using bl1400ForC and bl1710RevC. *Accession numbers are not included for ST1-D and ST2-A because the sequences are less than 200bp.

Data were described using mean and standard deviation for continuous variables and proportions for categorical variables. Mann-Whitney U test was used to analyze univariate association of each potential risk factor between those who harbored *Blastocystis* versus those who did not. I compared binomial dichotomous data using Fisher's exact test. Comparison of infected and uninfected individuals were calculated using Kruskal-Wallis nonparametric analysis of variance across three or more categorical responses. All effects were considered significant at the level of $p < 0.05$ using STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, USA).

RESULTS

Fifty-eight out of 96 (60.4%) howler monkey fecal samples tested positive for *Blastocystis* by PCR of SSU rDNA sequence. Eighteen out of nineteen groups (94.7%) harbored at least one positive individual. In seventy-four percent (73.7) of groups over half of the individuals ($\geq 50\%$) were positive. Eleven of the 58 howler monkey samples were consistently positive for *Blastocystis* using two different primer sets; however, sufficient SSU ribosomal sequence could not be obtained to determine subtype, likely due to either low quality DNA or insufficient DNA to visualize using SSCP. Of those that were sequenced, BLAST results revealed that all 47 samples were ST8. Sixteen of these samples could only be amplified using primer set b11400ForC/b11710RevC and were not included in Table 4.1 as the sequence was not congruent with those amplified using BLF/BLR and BH1F/BHRDr. ST8 sequences were not all identical, and could be divided into three unique sequence types.

Forty-four out of 54 (81.5%) human samples were positive, of which one was positive for *Blastocystis* but could not be subtyped likely due to low quality DNA. Human samples contained ST1, ST2, and ST3 (Table 4.1; Figure 4.2). Two human samples harbored mixed subtypes: ST1

and ST3 (N=1), and ST1 and ST2 (N=2). Subtypes 1, 2, and 3 were further categorized into unique sequence types that varied by as little as a single base pair in some cases (Table 4.1).

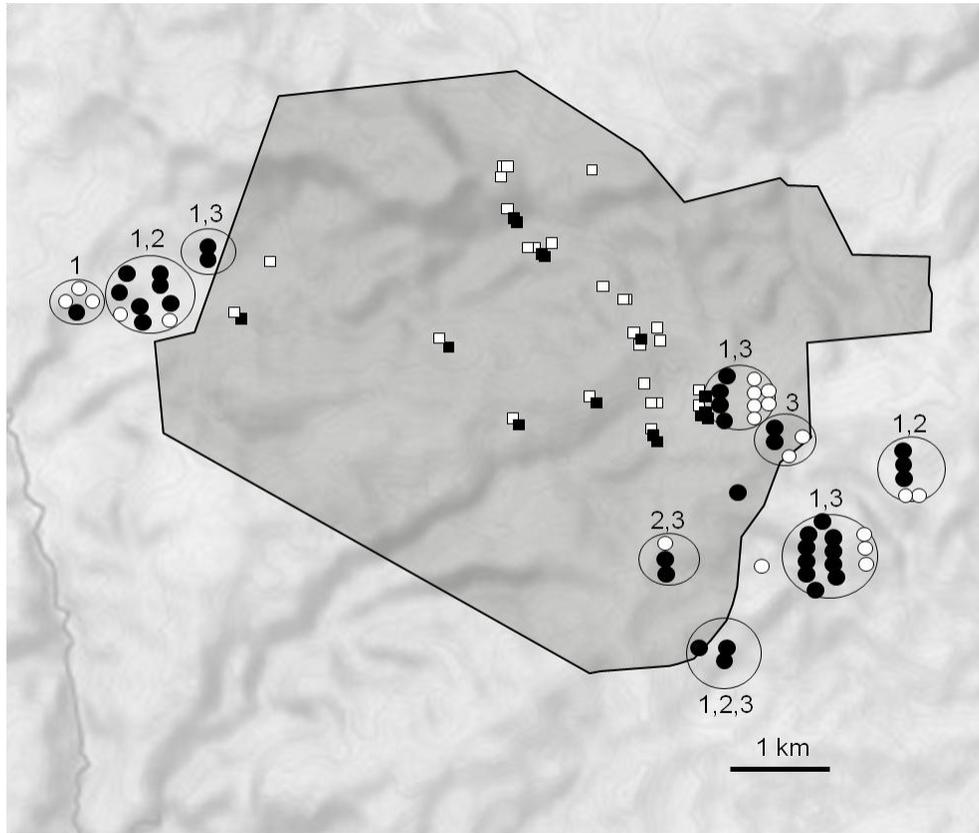


Figure 4.2. Fifty-eight out of ninety six monkey samples from 19 groups contained *Blastocystis* ST8 (black squares). Those samples which tested negative for *Blastocystis* are reported as white squares. Forty-four out of 55 humans were infected (dark circles) with either ST1, ST2, ST3, or a combination of two subtypes (N=3). Small white circles are individuals which tested negative for *Blastocystis*. Larger circles surrounding human samples represent individual households.

Attributes and potential risk factors of people living in two communities surrounding the biological reserve were compared with the presence of *Blastocystis* species and *Blastocystis* subtypes (Table 4.2). The presence of *Blastocystis* was significantly higher in those who did not boil their water ($p=0.002$). Ninety-four percent (93.8%) of people who didn't boil their water had *Blastocystis*, while only 44.4% of those who did had a detectable infection. Comparisons among all other risk factors (i.e., age, sex, family size, anti-parasitic treatment in last year, or monkeys within 1 km of home) were not statistically significant; however, several trends were observed.

All self-described hunters (N=6) were infected, while 80.5% of those who did not hunt were infected with *Blastocystis*. Individuals who were infected tended to be younger ($M = 26.5$ years) versus those who were uninfected ($M = 31.3$ years; $p=0.68$). A greater proportion of females ($M = 89.7\%$) were infected with *Blastocystis* sp. compared to males ($M = 26.5\%$; $p=0.09$). Smaller family size was associated with reduced prevalence ($M = 6.7$ versus 8.1 ; $p=0.35$), and those who were infected with *Blastocystis* reported living much closer to rainforests ($M = 420.1\text{m}$) than those who were not infected ($M = 797.5\text{m}$; $p=0.38$).

Table 4.2. Potential risk factors associated with overall prevalence of *Blastocystis* and subtypes. Categories ST1-Mixed represent number and percentage of total *Blastocystis* positive infections.

Characteristics	N (%)	<i>Blastocystis</i> N (%)†	ST1 N (%)	ST2 N (%)	ST3 N (%)	Mixed N (%)
Age						
0-15	17 (31.5)	14 (82.4)	7 (50.0)	2 (14.3)	3 (21.4)	1 (7.1)
16-30	13 (24.1)	13 (100.0)	7 (53.8)	4 (30.8)	3 (23.1)	1 (7.7)
31-45	11 (20.4)	7 (63.6)	2 (28.6)	2 (28.6)	3 (42.9)	0 (0)
46-60	9 (16.7)	6 (66.7)	3 (42.9)	1 (14.3)	2 (28.6)	0 (0)
60+	1 (1.9)	1 (100.0)	1 (100.0)	0 (0)	0 (0)	0 (0)
Unreported	4 (7.3)	3 (75.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)
Gender						
Female	29 (53.7)	26 (89.7)	13 (50.0)	5 (19.2)	6 (23.1)	1 (3.8)
Male	25 (46.3)	17 (68.0)	7 (41.2)	5 (29.4)	6 (35.3)	1 (5.9)
Unreported	1 (1.8)	1 (100.0)	1 (100.0)	-	-	-
Family Size						
1-3	13 (24.1)	10 (76.9)	3 (30.0)	2 (20.0)	4 (40.0)	0 (0)
4-6	9 (16.7)	6 (66.7)	1 (16.7)	3 (50.0)	2 (33.3)	0 (0)
7-10	19 (35.2)	14 (73.7)	9 (64.3)	4 (28.6)	2 (14.3)	1 (7.1)
11-13	13 (24.1)	13 (100.0)	7 (53.8)	1 (7.7)	4 (30.8)	1 (7.7)
Unreported	1 (1.8)	1 (100.0)	1 (100.0)	-	-	-
Treated with antiparasitic drugs in last year						
Yes	19 (35.2)	13 (68.4)	4 (30.8)	6 (42.9)	3 (21.4)	0 (0)
No	27 (50.0)	24 (88.9)	12 (50.0)	3 (12.5)	7 (29.2)	1 (4.2)
Unreported	9 (16.4)	7 (77.8)	5 (71.4)	1 (14.3)	2 (28.6)	1 (14.3)
Boil water						
Yes	9 (16.7)	4 (44.4)*	2 (50.0)	0 (0)	2 (50.0)	0 (0)
No	32 (59.3)	30 (93.8)*	13 (43.3)	7 (23.3)	9 (30.0)	2 (6.7)
Unreported	14 (25.5)	10 (71.4)*	6 (60.0)	3 (30.0)	1 (10.0)	0 (0.0)
Method of water treatment						
No treatment	34 (63.0)	30 (88.2)	13 (43.3)	7 (23.3)	9 (30.0)	2 (6.7)
Boil	7 (13.0)	4 (57.1)	2 (50.0)	0 (0)	2 (50.0)	0 (0)
Filtered	-	-	-	-	-	-
Rain water	-	-	-	-	-	-
Multiple	-	-	-	-	-	-
Unreported	14 (25.5)	10 (71.4)	6 (60.0)	3 (30.0)	1 (10.0)	0 (0.0)

Monkeys within 1k of house						
Yes	45 (83.3)	37 (82.2)	16 (43.2)	7 (18.9)	12 (32.4)	1 (2.7)
No	4 (7.4)	3 (75.0)	3 (100.0)	1 (33.3)	0 (0)	1 (33.3)
Unreported	6 (11.0)	4 (67.0)	2 (50.0)	2 (50.0)	0 (0)	0 (0)
Monkeys within 1k of farm						
Yes	37 (68.5)	30 (81.0)	15 (50.0)	5 (16.1)	10 (33.3)	2 (7.0)
No	8 (14.8)	7 (87.5)	3 (42.9)	3 (42.9)	0 (0)	0 (0)
No farm	7 (13.0)	5 (71.4)	1 (20.0)	2 (40.0)	2 (40.0)	0 (0)
Unreported	3 (5.0)	2 (66.7)	2 (100.0)	0 (0)	0 (0)	0 (0)
Hunt wildlife						
Yes	6 (11.1)	6 (100)	1 (16.7)	1 (16.7)	4 (66.7)	0 (0)
No	41 (75.9)	33 (80.5)	18 (54.5)	7 (21.2)	7 (21.2)	2 (6.1)
Unreported	8 (14.5)	5 (62.5)	2 (40.0)	2 (40.0)	1 (20.0)	0 (0)
How often do you hunt?						
Never	41 (75.9)	33 (80.5)	18 (54.5)	7 (21.2)	7 (21.2)	2 (6.1)
Everyday	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2-3 times per week	3 (5.6)	3 (100.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)
1 time per week	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 time per month	1 (1.9)	1 (100.0)	0 (0)	0 (0)	1 (100.0)	0 (0)
Unreported	10 (16.7)	7 (77.8)	2 (28.6)	2 (28.6)	3 (42.9)	0 (0)
Hunt or eat monkey?						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	45 (83.3)	37 (82.2)	17 (45.9)	8 (21.6)	11 (29.7)	2 (5.4)
Unreported	10 (16.7)	7 (70.0)	4 (40.0)	2 (20.0)	1 (10.0)	0 (0)

* p -value <0.05; ** p -value <0.10. †Includes positive PCR-based samples which did not provide sufficient quality sequence to determine subtype.

DISCUSSION

I found no evidence of *Blastocystis* transmission between howler monkeys and people living in close proximity to one another. Specifically, ST8 was the only *Blastocystis* subtype found in monkeys, whereas ST1, ST2, and ST3 were detected in people living in the surrounding community. Two people were found with mixed *Blastocystis* subtypes, yet none harbored ST8. A lack of identical *Blastocystis* subtypes in sampled howler monkeys and people living in close proximity suggests zoonotic transmission of this parasite is not occurring between humans and howler monkeys. It also suggests there is no common source of infection, or perhaps that there are differences in host specificity in these particular subtypes. Subtype 8 has primarily been reported in non-human primates and in some cases, has been found in zookeepers working with captive primates (Stensvold et al. 2009). The presence of ST8 in sampled wild howler monkeys

suggests that captive howlers with ST8 also harbor a wild form of *Blastocystis*, as opposed to acquiring the ST8 infection from handlers.

The primary mode of gastrointestinal zoonotic transmission occurs through contaminated food or water (Slifko et al. 2000; Ithoi et al. 2011; Leelayoova et al. 2008). In *Blastocystis*, production of thick walled cysts are believed to provide protection in an external environment once transmitted via a fecal-oral route (Garcia, 1999). In the case of wild howler monkeys, they have rarely been seen drinking from river water. Instead, drinking water is obtained from arboreal cisterns or standing bodies of water (Glander, 1978; Guidice and Mudry, 1999), suggesting little opportunity for humans to transmit *Blastocystis* to howler monkeys via rivers. The only other real opportunity for transmission from people to howlers would be if monkeys came to the ground and happened upon human fecal material. Howlers are known to traverse on the ground, especially in disturbed habitats (Pozo and Serio-Silva, 2007).

Conversely, the main source of drinking water in people living near these sampled howler monkeys is rivers. Because howler monkeys are routinely found living near rivers and in close proximity to people, this raises the possibility that howler fecal matter could wash off leaves and rooftops into rivers where people retrieve their drinking water. In this scenario, people could conceivably be infected with howler monkey ST8 if they drank contaminated water, yet this was not observed. Those who didn't boil their water were much more likely to harbor *Blastocystis*, which suggests that the river is a potential source of *Blastocystis*. However, without testing the river water directly, there is no way to know if ST8 is present.

In 78% of reported cases this drinking water is left untreated before consumption. By boiling water, the threat of ingesting these infectious cysts would likely be reduced, as has been recorded in other areas throughout the world (Taamasri et al. 2000; Leelayoova et al. 2008; Abdulsalam et al. 2012). I did find that individuals who boiled their water were significantly less

likely to have *Blastocystis* ($p=0.002$) than those who didn't boil drinking water. Ninety-three percent of those who reportedly did not boil their water before consumption were infected with *Blastocystis*, while only 44% of those who did were positive. Based on multivariate logistic regression analysis, those who didn't boil their water were 3.5 times more likely to be infected ($p=0.02$). The benefits of simple water treatment are likely not limited to *Blastocystis*, but also other gastrointestinal macroparasites, protozoa, bacteria and viruses (Levy et al., 2008).

Other risk factors may be associated with the presence of *Blastocystis*; however, several variables were answered uniformly or there simply were not enough responses in a particular category. For example, all but four participants lived within relative close proximity (<1 km) to howler monkeys based on survey results. All participants also had some contact with domesticated animals and nobody reported eating monkeys, precluding us from testing the effect of wildlife proximity on presence or absence of *Blastocystis*. Several studies have found similar *Blastocystis* subtypes in people and their domestic animals, which makes this a worthy avenue of exploration (Lee et al. 2012). In another example, only six people reportedly hunted which is likely an underestimate based on personal communications with local community members. Nevertheless, all reported hunters were positive for *Blastocystis* while only 80.5% of non-hunters were infected.

The effect of *Blastocystis* infection on human and wildlife health is rather contentious and still remains poorly understood (Boorom et al., 2008). Infection with *Blastocystis* species does not necessarily indicate that an individual will indeed have gastrointestinal disease, though *Blastocystis* infections have been associated with various gastrointestinal symptoms as well as headache, fatigue, and depression (Boorom et al., 2008; Denoed et al., 2011). The inconsistent gastrointestinal response to infection may be a product of antibiotic resistance, host immune

response and overall health, variability in subtype infection, or any combination of these factors (Stensvold et al., 2007; Dogruman et al., 2008; Whipps et al., 2010).

Only one other study has described *Blastocystis* subtypes in wild South American primates, in which two sampled howler monkeys were positive for ST4 (Ramirez et al. 2014). Other subtypes, including ST1, ST2, ST3, ST4, ST5, and ST8, have previously been reported in Old World primates and captive howler monkeys (Abe et al., 2004; Noel et al., 2005; Yoshikawa et al., 2009; Petrasova et al., 2011; Alfellani et al., 2013); however, with only two Colombian howler monkey samples reported by Ramirez et al. (2014), we are unable to ascertain whether our results are common and applicable to other howler populations or species.

I anticipate that the use of SSCP to uniquely identify multiple subtypes within a single sample may play a particularly useful role in understanding the interactions of particular *Blastocystis* subtypes throughout domestic animals, wildlife, and people. These methods also provide an inexpensive and relatively quick way to diagnose mixed subtypes in individual samples obtained from either people or nonhuman primates. Though we did not find direct evidence of zoonotic transmission, this does not preclude the possibility that people living in close contact with domestic animals and wildlife could still be at risk of obtaining *Blastocystis* under certain conditions, as evidenced from shared subtypes of infected primate zookeepers reported in other studies.

LITERATURE CITED

- Abdulsalam, A. M, I. Ithoi, H. M. Al-Mekhlafi, A. Ahmed, J. Surin, and J. W. Mak. 2012. Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary schoolchildren. *Parasitology* 139: 1-7.
- Abe, N. 2004. Molecular and phylogenetic analysis of *Blastocystis* isolates from various hosts. *Veterinary Parasitology* 120: 235-242.

- Alfellani, M. A., D. Taner-Mulla, A. S. Jacob, C. A. Imeede, H. Yoshikawa, C. R. Stensvold, and C. G. Clark. 2013a. Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist* 164: 497-509.
- Alfellani, M. A., A. S. Jacob, N. O. Perea, R. C. Krecek, D. Taner-Mulla, J. J. Verweij, B. Levecke, E. Tannich, C. G. Clark, and C. R. Stensvold. 2013b. Diversity and distribution of *Blastocystis* sp. subtypes in non-human primates. *Parasitology* 140: 966-971.
- Alfellani, M. A., C. R. Stensvold, A. Vidal-Lapiedra, E. S. U. Onuoha, A. F. Fagbenro-Beyioku, and C. G. Clark. 2013c. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Tropica* 126: 11-18.
- Boorom, K. F., H. Smith, L. Nimri, E. Viscogliosi, G. Spanakos, U. Parkar, L. H. Li, X. N. Zhou, U. Z. Ok, S. Leelayoova, and M. S. Jones. 2008. Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. *Parasite Vector* 1: 40.
- Chapman, C. A., T. R. Gillespie, and T. L. Goldberg. 2005. Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology* 14: 134-144.
- Cristobal-Azkarate, J., B. Hervier, S. Vegas-Carrillo, D. Osorio-Sarabia, E. Rodriguez-Luna, and J. J. Veá. 2010. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. *Primates* 51: 231-239.
- Cruz, A. C., J. T. Borda, E. M. Patino, L. Gomez, and G. E. Zunino. 2000. Habitat fragmentation and parasitism in howler monkeys (*Alouatta caraya*). *Neotropical Primates* 8: 146-148.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science* 287: 443-449.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* 78: 103-116.
- Denoëud, F., M. Roussel, B. Noel, I. Wawrzyniak, C. Da Silva, M. Diogon, E. Viscogliosi, C. Brochier-Armanet, A. Couloux, J. Poulain, B. Segurens, V. Anthouard, C. Texier, N. Blot, P. Poirier, G. C. Ng, K. S. Tan, F. Artiguenave, O. Jaillon, J. M. Aury, F. Dogruman-Al, S. Kustimur, and H. Yoshikawa. 2011. Genome sequence of the stramenopile *Blastocystis*, a human anaerobic parasite. *Genome Biology* 12: 1-16.
- Eckert, K. A., N. E. Hahn, A. Genz, D. M. Kitchen, M. D. Stuart, G. A. Averbeck, B. E. Stromberg, and H. Markowitz. 2006. Coprological surveys of *Alouatta pigra* at two sites in Belize. *International Journal of Primatology* 27: 227-238.
- Garcia, L. S. 1999. Practical guide to diagnostic parasitology. American Society of Microbiology, Washington, D. C.

Gilbert, K. A. 1994. Endoparasitic infection in red howling monkeys (*Alouatta seniculus*) in the Central Amazonian basin: a cost of sociality? Dissertation, Rutgers University.

Gillespie, T. R., C. C. Chapman, and E. C. Greiner. 2005. Effects of logging on gastrointestinal parasite infections and infection risk in African primates. *Journal of Applied Ecology* 42: 699-707.

Glander, K. E. 1978. Drinking from arboreal water sources by mantled howling monkeys (*Alouatta palliata* Gray). *Folia Primatologica* 29: 206-217.

Hall, T. A. 2001. BioEdit: a user-friendly biological sequence alignment editor and analysis, version 5.09. Department of Microbiology, North Carolina State University, Raleigh, NC.

Ithoi, I., A. Jali, J. W. Mak, W. Y. Wan Sulaiman, and R. Mahmud. 2011. Occurrence of *Blastocystis* in water of two rivers from recreational areas in Malaysia. *Journal of Parasitology Research* 1-8.

Laurance, W. F., B. M. Croes, L. Tchignoumba, S. A. Lahm, A. Alonso, M. E. Lee, P. Campbell, and C. Ondzeano. 2006. *Conservation Biology* 20: 1251-1261.

Lee, L. I., T. T. Chye, B. M. Karmacharya, and S. K. Govind. 2012. *Blastocystis* sp.: waterborne zoonotic organism, a possibility. *Parasite Vectors* 5: 130.

Leelayoova, S., S. Siripattanapipong, U. Thathaisong, T. Naaglor, P. Taamasri, P. Piyaraj, and M. Mungthin. 2008. Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. *American Journal of Tropical Medicine and Hygiene* 79: 401-406.

Levy, K., K. L. Nelson, A. Hubbard, and J. N. Eisenberg. 2008. Following the water: a controlled study of drinking water storage in northern coastal Ecuador. *Environmental Health Perspective* 116: 1533-1540.

Menounos, P. G., G. Spanakos, N. Tegos, C. M. Vassalos, C. Papadopoulou, and N. C. Vakalis. 2008. Direct detection of *Blastocystis* sp. in human faecal samples and subtype assignment using single strand conformational polymorphism and sequencing. *Molecular and Cellular Probes* 22: 24-29.

Milozzi, C., G. Bruno, E. Cundom, M. D. Mudry, and G. T. Navone. 2012. Intestinal parasites of *Alouatta caraya* (Primates, Ceboidea): Preliminary study in semi-captivity and in the wild in Argentina. *Mastozoologia Neotropical* 19: 271-278.

Morse, S. S. 1995. Factors in the emergence of infectious diseases. *Emerging Infectious Diseases* 1: 7-15.

Mosandl, R., S. Gunter, B. Stimm, and M. Weber. 2008. Ecuador suffers the highest deforestation rate in South America. *In: Gradients in a tropical mountain ecosystem of Ecuador*. Beck, E. (ed.). Verlag Berlin Heidelberg, p. 37-40.

- Muriuki, S. M., R. K. Murugu, E. Munene, G. M. Karere, and D. C. Chai. 1998. Some gastrointestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta tropica* 71: 73-82.
- Noel, C., F. Dufernez, D. Gerbod, V. P. Edgcomb, P. Delgado-Viscogliosi, L. C. Ho, M. Singh, R. Wintjens, M. L. Sogin, M. Capron, R. Pierce, L. Zenner, and E. Viscogliosi. 2005. Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonoses. *Journal of Clinical Microbiology* 43: 348-355.
- Parkar, U., R. J. Traub, S. Vitali, A. Elliot, B. Levecke, I. Robertson, T. Geurden, J. Steele, B. Drake, and R. C. Thompson. 2010. Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Veterinary Parasitology* 169: 8-17.
- Patz, J. A., T. K. Graczyk, N. Geller, and A. Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal of Parasitology* 30: 1395-1405.
- Pedersen, A. B., S. Altizer, M. Poss, A. A. Cunningham, and C. L. Nunn. 2005. Patterns of host specificity and transmission among parasites of wild primates. *International Journal of Parasitology* 35: 647-657.
- Pedersen, A. B., and T. J. Davies. 2010. Cross-species pathogen transmission and disease emergence in primates. *EcoHealth* 6: 496-508.
- Petrasova, J., M. Uzlikova, M. Kostka, K. J. Petrzekova, M. A. Huffman, and D. Modry. 2011. Diversity and host specificity of *Blastocystis* in syntopic primates on Rubondo Island, Tanzania. *International Journal of Parasitology* 41: 1113-1120.
- Phillips, K. A., M. E. Haas, B. W. Grafton, and M. Yrivarren. 2004. Survey of the gastrointestinal parasites of the primate community at Tambopata National Reserve, Peru. *Journal of Zoology* 264: 149-151.
- Polly, L. 2005. Navigating parasite webs and parasite flow: emerging and re-emerging parasitic zoonoses of wildlife origin. *International Journal Parasitology* 35: 1279-1294.
- Püttker, T., Y. Meyer-Lucht, and S. Sommer. 2008. Effects of fragmentation on parasite burden (nematodes) of generalist and specialist small mammal species in secondary forest fragments of the coastal Atlantic Forest, Brazil. *Ecological Research* 23: 207-215.
- Ramirez, J. D., L. V. Sanchez, D. C. Bautista, A. F. Corredor, A. C. Florez, and C. R. Stensvold. 2013. *Blastocystis* subtypes detected in humans and animals from Colombia. *Infection, Genetics, and Evolution* 22: 223-228.
- Scicluna, S. M., B. Tawari, and C. G. Clark. 2006. DNA barcoding of *Blastocystis*. *Protist* 157: 77-85.
- Slifko, T. R., H. V. Smith, and J. B. Rose. 2000. Emerging parasite zoonoses associated with water and food. *International Journal of Parasitology* 30: 1379-1393.

Stensvold, R., A. Brillowska-Dabrowska, H. V. Nielsen, and M. C. Arendrup. 2006. Detection of *Blastocystis hominis* in unpreserved stool specimens by using polymerase chain reaction. *Journal of Parasitology* 92: 1081–1087.

Stensvold, C. R., G. K. Suresh, K. S. Tan, R. C. Thompson, R. J. Traub, E. Viscogliosi, H. Yoshikawa, and C. G. Clark. 2007. Terminology for *Blastocystis* subtypes – a consensus. *TRENDS Parasitology* 23: 93-96.

Stensvold, C.R., M. A. Alfellani, S. Nørskov-Lauritsen, K. Prip, E. L. Victory, C. Maddox, H. V. Nielsen, and C. G. Clark. 2009. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *International Journal of Parasitology* 39: 473-479.

Stensvold, C. R., M. Alfellani, and C. G. Clark. 2012. Levels of genetic diversity vary dramatically between *Blastocystis* subtypes. *Infection, Genetics, and Evolution* 12: 263-273.

Stoner, K., and A. M. Gonzalez. 2006. Intestinal parasitic infections in *Alouatta pigra* in tropical rainforest in Lacandona, Chiapas, Mexico: Implications for behavioral ecology and conservation. *In: New perspectives in the study of mesoamerican primates. Distribution, ecology and conservation.* Estrada, A., P. A. Garber, M. Pavelka, and L. Luecke. (eds.). New York: Springer, p. 215–240.

Stuart, M., V. Pendergast, S. Rumfelt, S. Pierberg, L. Greenspan, K. Glander, and M. Clarke. 1998. Parasites of wild howlers *Alouatta* spp. *International Journal of Primatology* 19: 493-512.

Taamasri, P., M. Mungthin, R. Rangsin, B. Tongupprakarn, W. Areekul, and S. Leelayoova. 2000. Transmission of intestinal blastocystosis related to the quality of drinking water. *Journal of Tropical Medicine and Public Health* 31: 112-117.

Thompson, R. C. 2013. Parasite zoonoses and wildlife: one health, spillover and human activity. *International Journal of Parasitology* 43: 1079-1088.

Trejo-Macías, G., A. Estrada, and M. A. Cabrera. 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in Southern Mexico. *International Journal of Primatology* 28: 931-945.

Vitazkova, S. K., and S. E. Wade. 2006. Parasites of free ranging black howler monkeys (*Alouatta pigra*) from Belize and Mexico. *American Journal of Primatology* 68: 1089-1097.

Whipps, C. M., K. Boorom, L. E. Bermudez, and M.L. Kent. 2010. Molecular characterization of *Blastocystis* species in Oregon identifies multiple subtypes. *Parasitology Research* 106: 827-832.

Wolfe, N., C. Dunavan, and J. Diamond. 2007. Origins of major human infectious disease. *Nature* 447: 279-283.

Yan, Y., S. Su, J. Ye, X. Lai, R. Lai, H. Liao, G. Chen, R. Zhang, Z. Hou, X. Luo. 2007. *Blastocystis* sp. subtype 5: a possibly zoonotic genotype. *Parasitology Research* 101: 1527-1532.

Yoshikawa, H., Z. Wu, I. Kimata, M. Iseki, I. Hossain, V. Zaman, R. Haque, and Y. Takahasi. 2004. Polymerase chain reaction-based genotype classification among human *Blastocystis hominis* populations isolated from different countries. *Parasitology Research* 92: 22-29.

Yoshikawa, H., Z. Wu, K. Pandey, B. D. Pandey, J. B. Sherchand, T. Yanagi, and H. Kanbara. 2009. Molecular characterization of *Blastocystis* isolates from children and rhesus monkeys in Kathmandu, Nepal. *Veterinary Parasitology* 160: 295-300.

Chapter 5

RISK FACTORS ASSOCIATED WITH ENDOPARASITISM IN TWO RURAL ECUADORIAN COMMUNITIES

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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, 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. Results from this study demonstrate that people who don't boil their water, are relatively young, live in larger families, hunt wildlife, 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

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 (Montresor et al., 1999; Wilson et al., 1999; Asaolu and Ofoezie, 2003; De Silava et al., 2003; Horton, 2003; Mara, 2003). In particular, infants, children, and

pregnant women living in developing countries show an increased risk of infection (Haque et al., 2007; Miguel and Kremer, 2004). 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 (Sackey et al., 2003).

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) (Mara, 2003; Haque et al., 2007; Garcia et al., 1991; Schantz et al., 1993; Ali and Hill, 2003; Hotez et al., 2008; Olsen et al., 2009; Wu et al., 2012). The impact these infections have on tropical communities has been well documented (Table 5.1). Helminth infections may impair appetite, leading to varying degrees of malnourishment (Stephenson et al., 2000; Capello, 2004). Gastrointestinal malabsorption may arise from *Ascaris* species infections and hookworms (Crompton and Nesheim, 2002). Decline in cognitive function has been associated with hookworm infection (Jardim-Botelho et al., 2008). 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 Wilson et al., (1999). Results from an Ecuadorian community suggest that *A. lumbricoides* infections were correlated with verbal impairment (Levav et al., 1995). When anti-helminthic treatments are implemented, a positive association with weight gain, height and other anthropometric measurements has been found (Dickson et al., 2000).

Table 5.1. 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.

Parasite	Route of Infection	Risk Factors				
		Age	Family Size	Water	Anti-parasitic regime	Other reported associations
<i>Entamoeba</i> spp. (specifically <i>E. histolytica</i>)	Ingestion of mature cysts.	Higher in 5-15 years, ¹ and 9-10 years. ²	No effect. ³	Chlorine Treatment ⁴ ; Source (river) has no effect. ⁶	Increased prevalence within 1 month after treatment. ⁴ Chlorine. ⁴	Dirt floors. ⁵ Sanitation ⁴ ; Wash Hands Before Eating ⁴ ; Wash Vegetables ⁴ ; Number domestic animals ⁶ ; Adequate garbage disposal. ⁶
<i>Balantidium</i> sp.	Passed in feces. Infection by ingestion of mature cysts.	No affect. ⁷	ND	ND	ND	ND
<i>Capillaria</i> spp.	Dependent on species; no reports in S. America.	ND	ND	ND	ND	ND
<i>Ascaris</i> sp.	Passed in feces. Infection by ingestion of eggs.	Highest in teenagers. ⁸ Increased with child's age. ⁹ No effect. ¹⁰	No effect. ^{10,11}	Chlorine Treatment. ⁴	Rapidly increased prevalence within 6 months after treatment. ⁴	Race ¹² ; Sanitation ¹³ ; Health education ^{10,14} ; Wash Hands Before Eating ⁴ ; Wash Vegetables ⁴ ; Mother's Level of Education ^{15,16} ; Socioeconomic status. ¹⁵
<i>Blastocystis</i> sp.	Passed in feces. Infection by ingestion of mature cysts. ^{17,18}	Highest in 9-10 years. ² No effect. ¹⁹	No effect. ¹⁹	Highest in untreated water compared to boiling or filtered. ³	75% still negative after 6 months.	ND
<i>Strongyloides</i> sp.	Roundworm enters host through exposed skin.	Highest in 3-24 months. ⁹ In adults, highest in 21-30 years of age.	Higher in smaller families. ⁹	ND	ND	Socioeconomic status. ¹²

1. Mengistu et al., 2007; 2. Boeke et al., 2010; 3. Cooper et al., 2003; 4. Rinne et al., 2005; 5. Jacobsen et al., 2007; 6. Sackey et al., 2003; 7. Esteban et al., 1998; 8. Hotez et al., 2008; 9. King and Mascie-Taylor, 2004; 10. Hadidjaja et al., 1998; 11. Jardim-Botelho et al., 2008; 12. Cooper et al., 1993; 13. Henry, 1988; 14. Albonico et al., 1996; 15. Holland et al., 1989; 16. Huat et al., 2012; 17. Stensvold et al., 2009; 18. Coyle et al., 2012; 19. Abdulsalam et al., 2012. ND: no data available.

Numerous studies and agencies have investigated effects of control and treatment options including chemotherapy, broad-spectrum anti-helminthics, vaccines, improved water treatment and sanitation, health education, and overall improved economic development (Asaolu and Ofoezie, 2003; Miguel and Kremer, 2004; Sackey et al., 2003; Bundy et al., 1998; Rinne et al., 2005; WHO, 2005). 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 (Asaolu and Ofoezie, 2003; Albonico et al., 1996). However, long-term control of parasitic infections is highly dependent on routine behavioral changes such as hygiene education (Albonico et al., 1996). 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 (De Silva et al., 2003; WHO, 1990).

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 I 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.

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 5.1). 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.

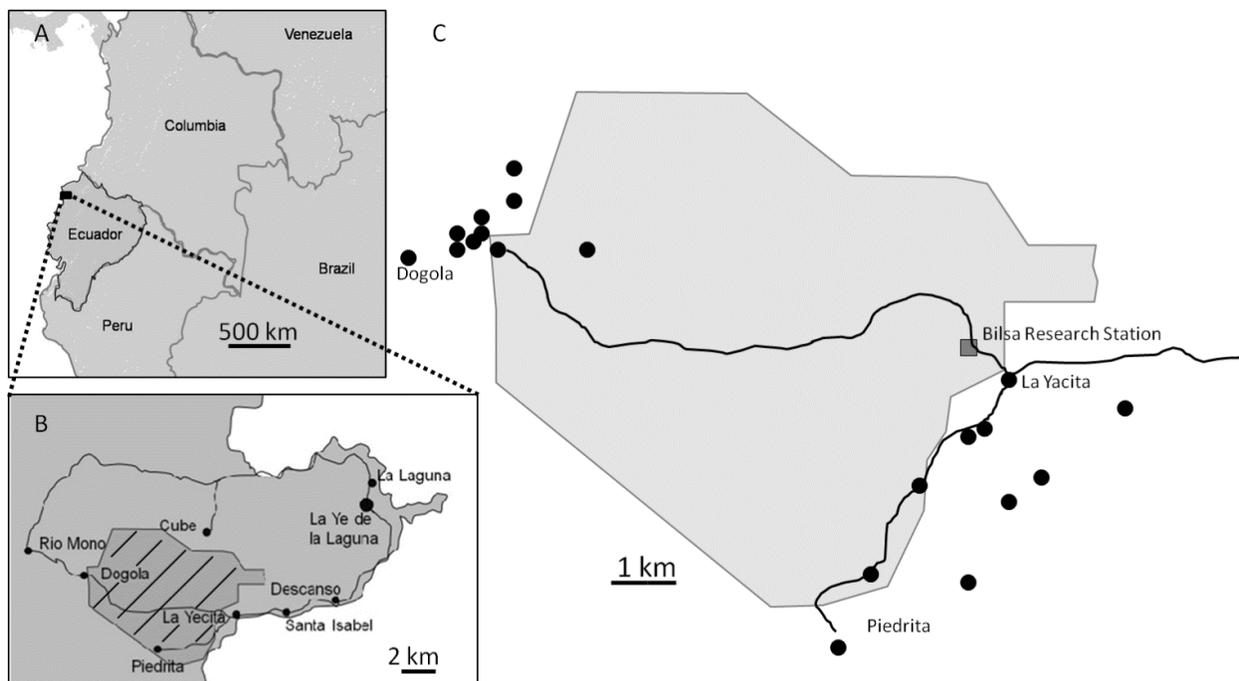


Figure 5.1. Field research took place in Northwestern Ecuador (A) at the Bilsa Biological Station - highlighted with diagonal lines (B). Several small communities surround the ecological reserve, containing numerous families (black dots) (C). A single main road runs loops from La Ye de la Laguna to the local communities and back.

A total of 54 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 Garcia et al., (1993) and Hendrix and Robinson (2006) with the following modifications. A NaNO_3 solution (SG 1.2) was used for optimal retrieval of parasite eggs in flotations. 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. Fecal smears were also used in order to obtain protozoan parasites. 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 with a 20x objective lens using a Nikon 80i compound microscope with Nomarski and phase objectives. Images were captured with a 40x objective lens using 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.

In the case of *Blastocystis* sp., PCR-based detection was used because of the difficulty of identification based solely on morphology (Stensvold et al., 2006). 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 (Whipps et al., 2010) and BLF and BLR primers (Menounos et al., 2008). Because finding a *Capillaria* species was unexpected, the small subunit ribosomal DNA sequence was amplified using primer combinations F-573M (5'-CGCGGTAATYCCAGCTCCA-3') and 18S1330R (5'-GTACGCGCCGTCACTATTTA-3') to confirm the morphological assessment. Both PCR-based regimes, coupled with single strand conformation polymorphism (SSCP) and DNA sequencing utilized the same protocols previously reported (Chapter 4).

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 (e.g., Gillespie and Chapman, 2006). Effects were considered significant if $p < 0.05$. All statistical analyses were done with STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, USA).

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 ascarid species. These eggs were mammillated (48-57 μm long), consistent with an *Ascaris* sp., but I 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 5.2). 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 5.3). Individuals who 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 5.3).

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 ($p=0.04$).

Table 5.2. Univariate analysis of risk factors associated with gastrointestinal parasites in two Ecuadorian communities.

Risk Factor	N	<i>Entamoeba</i> spp. N (%)	<i>Balantidium</i> sp. N (%)	<i>Capillaria</i> sp. N (%)	Ascarididae <i>gen. sp.</i> N (%)	<i>Blastocystis</i> sp. N (%)	<i>Strongyloides</i> 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) $p=0.36$	1 (1.9) NA	11 (20.4) $p=0.004^*$	7 (13.0) $p=0.89$	44 (81.5) $p=0.68$	1 (1.9) 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)							
Yes	37	4 (10.8)	0 (0)	9 (24.3)	5 (13.5)	31 (83.8)	0 (0)
No	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 5.3. 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
<i>Entamoeba</i> 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
<i>Capillaria</i> sp.						
	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.						
	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
<i>Blastocystis</i> sp.						
	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.

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. Five of these parasite species have been previously described in Ecuadorian communities, with the exception of *Capillaria* sp. (Table 5.1; Esteban et

al., 1998; San Sabastian and Santi, 2000; Jacobsen et al., 2007; Minvielle et al., 2008). No cases of *Capillaria* sp. have been described in South American communities, though numerous studies have described *Trichuris* spp. infections (San Sabastian and Santi, 2000; Jacobsen et al., 2007). For this reason I amplified and sequenced a portion of the small unit ribosomal DNA for analysis and found that the closest genetically related species was *Capillaria xenopi* (92% 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% (Boeke et al., 2010; Gamboa et al., 1998; Ramirez et al., 2013). I surmise that higher prevalence of *Blastocystis* sp. in this study is likely due to the 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 (Levy et al., 2008), and is rarely boiled or treated with chlorine (Sackey et al., 2003). Source waters have been shown to harbor gastrointestinal parasites due to poor sanitation efforts from surrounding communities, and domestic and wildlife fecal contamination (Levy et al., 2008). 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 (Clasen and Bastable, 2003).

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 (Clasen and Bastable, 2003; Lantagne et al., 2006; Levy et al., 2008).

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 5.2). 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 (Nematian et al., 2004). 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 (Udonsi et al., 1996; Quihui et al., 2006).

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 (Okay et al., 2004; King and Mascie-Taylor, 2004).

In this study, individuals 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 (Rinne et al., 2005), 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 (Ehrenberg and Ault, 2005). As a specific example, *A. lumbricoides* was found to persist at high levels despite anti-parasitic treatment (Henry, 1988).

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, I 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 (Fewtrell et al., 2005). I am 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 (Goldberg et al., 2007).

Several risk factors were not significantly associated with parasite prevalence, including gender, hunting activity, or distance to the forest (Table 5.2). Based on previous research, I did not expect a difference between the sexes (Henry, 1988). I did expect people who were in contact with wildlife, such as hunters, to carry more parasites (Wolfe et al., 1998). All reported hunters were infected with *Blastocystis* sp. (N=6) while 82.9% of those who did not hunt were infected, but these differences were not significant ($p=0.40$). The number of hunters was rather small, which likely affected 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 5.1). On average, people with *Entamoeba* sp. were slightly older (though not significantly), which is in contrast to two other studies which found younger children had higher infection rates (Boeke et al., 2010; Mengistu et al., 2007). Secondly, the presence of *Capillaria* sp. was associated with younger participants (<28 yrs) 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 (Mehraj et al., 2008). Most people in our study (87%) were infected with either Ascarididae gen. sp., *Capillaria* sp., *Strongyloides* sp, or *Entamoeba* spp. which means 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 children infected with gastrointestinal parasites experience reductions in growth (Oberhelman et al., 1998; Mondal et al., 2006; Jardim-Botelho et al., 2008), malnutrition (Jardim-Botelho et al., 2008) and reduced cognitive function (Hadidjaja et al., 1998; Tarleton et al., 2006; Jardim-Botelho et al., 2008). The developmental impact can influence everything from physical fitness, school performance, absenteeism at school (or work), decreased work capacity and productivity (Stephenson et al., 2000) – all factors equating to an economic impact as well as a social impediment to an already disadvantaged populace (Miguel and Kremer, 2004; Ehrenberg and Ault, 2005). 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, 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. The results can be applied in two ways. First, information on intrinsic factors (e.g., age and family size) can help the health community 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.

LITERATURE CITED

Abdulsalam, A. M., I. Ithoi, H. M. Al-Mekhlafi, A. Ahmed, J. Surin, and J. W. Mak. 2012. Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary schoolchildren. *Parasitology* 139: 1014-1020.

Albonico, M., N. Shamlaye, C. Shamlaye, and L. Savioli. 1996. Control of intestinal parasitic infections in Seychelles: a comprehensive and sustainable approach. *Bulletin of the World Health Organization* 74: 577-586.

Ali, S. A. and D. R. Hill. 2003. *Giardia intestinalis*. *Current Opinion in Infectious Diseases* 16: 453-460.

Asaolu, S. O. and I. E. Ofoezie. 2003. The role of health education and sanitation in the control of helminth infections. *Acta Tropica* 86: 283-294.

Boeke, C. E., M. Mora-Plazas, Y. Forero, and E. Villamor. 2010. Intestinal protozoan infections in relation to nutritional status and gastrointestinal morbidity in Colombian school children. *Journal of Tropical Pediatrics* 56: 299-306.

Bundy, D. A., M. S. Wong, L. L. Lewis, and J. Horton. 1990. Control of geohelminths by delivery of targeted chemotherapy through schools. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 84: 115-120.

Cappello, M. 2004. Global health impact of soil-transmitted nematodes. *The Pediatric Infectious Disease Journal* 23: 663-664.

Clasen, T. and A. Bastable. 2003. Faecal contamination of drinking water during collection and household storage: the need to extend protection to the point of use. *Journal of Water and Health* 1: 109-115.

Cooper, P. J., E. A. Guevara, and R. H. Guderian. 1993. Intestinal helminthiasis in Ecuador: the relationship between prevalence, genetic and socioeconomic factors. *Revista-sociedade Brasileira de Medicina Tropical* 26: 175-180.

Coyle, C. M., J. Varughese, L. M. Weiss, and H. B. Tanowitz. 2012. *Blastocystis*: To treat or not to treat... *Clinical Infectious Diseases* 54: 105-110.

Crompton, D. W. and M. C. Nesheim. 2002. Nutritional impact of intestinal helminthiasis during the human life cycle. *Annual Review of Nutrition* 22: 35-59.

De Silva, N. R., S. Brooker, P. J. Hotez, A. Montresor, D. Engels, and L. Savioli. 2003. Soil-transmitted helminth infections: updating the global picture. *Trends in Parasitology* 19: 547-551.

Dickson, R., S. Awasthi, P. Williamson, C. Demellweek, and P. Garner. 2000. Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. *British Medical Journal* 320: 1697-1701.

Ehrenberg, J. P. and S. K. Ault. 2005. Neglected diseases of neglected populations: thinking to reshape the determinants of health in Latin America and the Caribbean. *BMC Public Health* 5: 119.

Esteban, J. G., C. Aguirre, R. Angles, L. R. Ash, and S. Mas-Coma. 1998. Balantidiasis in Aymara children from the northern Bolivian Altiplano. *American Journal of Tropical Medicine and Hygiene* 59: 922-927.

Fewtrell, L., R. B. Kaufmann, D. Kay, W. Enanoria, L. Haller, and J. M. Colford Jr. 2005. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *The Lancet Infectious Diseases* 5: 42-52.

Gamboa, M. I., J. A. Basualdo, L. Kozubsky, E. Costas, E. C. Rua, and H. B. Lahitte. 1998. Prevalence of intestinal parasitosis within three population groups in La Plata, Argentina. *European Journal of Epidemiology* 14: 55-61.

Garcia, H. H., M. Martinez, R. Gilman, G. Herrera, V. C. Tsang, J. B. Pilcher, F. Diaz, M. Verastegui, C. Gallo, M. Porras, M. Alvarado, J. Naranjo, and E. Miranda. 1991. Diagnosis of cysticercosis in endemic regions. *The Lancet* 338: 549-551.

Garcia, L. S., R. Y. Shimizu, A. Shum, and D. A. Bruckner. 1993. Evaluation of intestinal protozoan morphology in polyvinyl alcohol preservative: Comparison of zinc sulfate- and mercuric chloride-based compounds for use in Schaudinn's fixative. *Journal of Clinical Microbiology* 31: 307-310.

Gillespie, T. R., and C. A. Chapman. 2006. Prediction of parasite infection dynamics in primate metapopulations based on attributes of forest fragmentation. *Conservation Biology* 20: 441-448.

Goldberg, T. L., T. R. Gillespie, I. B. Rwego, E. Wheeler, E. L. Estoff, and C. A. Chapman. 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation* 135: 511-517.

Hadidjaja, P., E. Bowang, M.A. Suyardi, A.N. Abidin, I.S. Ismid, and S.S. Margono. 1998. The effect of intervention methods on nutritional status and cognitive function of primary school children infected with *Ascaris lumbricoides*. *American Journal of Tropical Medicine and Hygiene* 59: 791-795.

Haque, R., D. Mondal, P. Duggal, M. Kabir, S. Roy, B. M. Farr, R. B. Sack, and W. A. Petri. 2006. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. *Infection and Immunity* 74: 904-909.

Hendrix, C. M., and E. Robinson. 2006. *Diagnostic Parasitology for Veterinary Technicians*, 3rd ed. Mosby Inc., St. Louis, Missouri, p. 416.

Henry, F. J. 1988. Reinfection with *Ascaris lumbricoides* after chemotherapy: a comparative study in three villages with varying sanitation. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 82: 460-464.

Holland, C. V., S. O. Asaolu, D. W. T. Crompton, R. C. Stoddart, R. Macdonald, and S. E. A. Torimiro. 1989. The epidemiology of *Ascaris lumbricoides* and other soil-transmitted helminths in primary school children from Ile-Ife, Nigeria. *Parasitology* 99: 275-285.

Horton, J. 2003. Human gastrointestinal helminth infections: are they now neglected diseases? *Trends in Parasitology* 19: 527-531.

Hotez, P. J., P. J. Brindley, J. M. Bethony, C. H. King, E. J. Pearce, and J. Jacobson. 2008. Helminth infections: the great neglected tropical diseases. *The Journal of Clinical Investigation* 118: 1311-1321.

Huat, L. B., A. K. Mitra, N. I. N. Jamil, P. C. Dam, H. J. J. Mohamed, and W. A. M. W. Muda. 2012. Prevalence and risk factors of intestinal helminth infection among rural Malay children. *Journal of Global Infectious Diseases* 4: 10-14.

Jacobsen, K. H., P. S. Ribeiro, B. K. Quist, and B. V. Rydbeck. 2007. Prevalence of intestinal parasites in young Quichua children in the highlands of rural Ecuador. *Journal of Health, Population, and Nutrition* 25: 399-405.

Jardim-Botelho, A., S. Raff, R. DeÁvila Rodrigues, H. J. Hoffman, D. J. Diemert, R. Corrêa-Oliveira, J. M. Bethony, and M. F. Gazzinelli. 2008. Hookworm, *Ascaris lumbricoides* infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. *Tropical Medicine and International Health* 13: 994-1004.

- King, S. E., and C. Mascie-Taylor. 2004. *Strongyloides fuelleborni kellyi* and other intestinal helminths in children from Papua New Guinea: associations with nutritional status and socioeconomic factors. *Papua New Guinea Medical Journal* 47: 181-191.
- Lantagne, D. S., R. Quick, and E. D. Mintz. 2006. Household water treatment and safe storage options in developing countries: a review of current implementation practices. *Wilson Quarterly*, Woodrow Wilson International Center for Scholars Environmental Change and Security Program 99: 11-37.
- Levav, M., A. F. Mirsky, P. M. Schantz, S. Castro, and M. E. Cruz. 1995. Parasitic infection in malnourished school children: effects on behaviour and EEG. *Parasitology* 110: 103-111.
- Levy, K., K. L. Nelson, A. Hubbard, and J. N. Eisenberg. 2008. Following the water: a controlled study of drinking water storage in northern coastal Ecuador. *Environmental Health Perspectives* 116: 1533-1540.
- Mara, D. D. 2003. Water, sanitation and hygiene for the health of developing nations. *Public Health* 117: 452-456.
- Mehraj, V., J. Hatcher, S. Akhtar, G. Rafique, and M. A. Beg. 2008. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PLoS One* 3: 1-6.
- Mengistu, A., S. Gebre-Selassie, and T. Kassa. 2007. Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethiopia. *Ethiopian Journal of Health Development* 21: 12-17.
- Menounos, P. G., G. Spanakos, N. Tegos, C. M. Vassalos, C. Papadopoulou, and N. C. Vakalis. 2008. Direct detection of *Blastocystis* sp. in human faecal samples and subtype assignment using single strand conformational polymorphism and sequencing. *Molecular and Cellular Probes* 22: 24-29.
- Miguel, E. and M. Kremer. 2004. Worms: identifying impacts on education and health in the presence of treatment externalities. *Econometrica* 72: 159-217.
- Minvielle, M. C., N. B. Molina, D. Polverino, and J. A. Basualdo. 2008. First genotyping of *Giardia lamblia* from human and animal feces in Argentina, South America. *Memórias do Instituto Oswaldo Cruz* 103: 98-103.
- Mondal, D., W. A. Petri Jr., R. B. Sack, B. D. Kirkpatrick, and R. Haque. 2006. *Entamoeba histolytica* associated diarrheal illness is negatively associated with the growth of preschool children: evidence from a prospective study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 100: 1032-1038.
- Montresor, A., D. W. T. Crompton, A. Hall, D. A. Bundy, and L. Savioli. 1998. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. Geneva: World Health Organization 1-49.

Nematian, J., E. Nematian, A. Gholamrezanezhad, and A. A. Asgari. 2004. Prevalence of intestinal parasitic infections and their relation with socio-economic factors and hygienic habits in Tehran primary school students. *Acta tropica* 92: 179-186.

Oberhelman, R. A., E. S. Guerrero, M. L. Fernandez, M. Silio, D. Mercado, N. Comiskey, G. Ihenacho, and R. Mera. 1998. Correlations between intestinal parasitosis, physical growth, and psychomotor development among infants and children from rural Nicaragua. *The American Journal of Tropical Medicine and Hygiene* 58: 470-475.

Okuy, P., S. Ertug, B. Gultekin, O. Onen, and E. Beser. 2004. Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. *BMC Public Health* 4: 1-6.

Olsen, A., L. van Lieshout, M. Hanspeter, T. Polderman, K. Polman, P. Steinmann, R. Stothard, S. Thybo, J. J. Verweij, and P. Magnussen. 2009. Strongyloidiasis—the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103: 967-972.

Quihui, L., M. E. Valencia, D. W. Crompton, S. Phillips, P. Hagan, G. Morales, and S. P. Díaz-Camacho. 2006. Role of the employment status and education of mothers in the prevalence of intestinal parasitic infections in Mexican rural schoolchildren. *BMC Public Health* 6: 1-8.

Ramírez, J. D., L. V. Sánchez, D. C. Bautista, A. F. Corredor, A. C. Flórez, and C. R. Stensvold. 2013. *Blastocystis* subtypes detected in humans and animals from Colombia. *Infection, Genetics and Evolution* 22: 238-228.

Rinne, S., E. J. Rodas, R. Galer-Unti, N. Glickman, and L. T. Glickman. 2005. Prevalence and risk factors for protozoan and nematode infections among children in an Ecuadorian highland community. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 99: 585-592.

Sackey, M. E., M. M. Weigel, and R. X. Armijos. 2003. Predictors and nutritional consequences of intestinal parasitic infections in rural Ecuadorian children. *Journal of Tropical Pediatrics* 49, 17-23.

San Sebastián, M. and S. Santi. 2000. Control of intestinal helminths in schoolchildren in Low-Napo, Ecuador: impact of a two-year chemotherapy program. *Revista da Sociedade Brasileira de Medicina Tropical* 33: 69-73.

Schantz, P. M., M. Cruz, E. Sarti, and Z. Pawlowski. 1993. Potential eradicability of taeniasis and cysticercosis. *Bulletin of the Pan American Health Organization* 27: 397-403.

Stensvold, R., A. Brillowska-Dabrowska, H. V. Nielsen, and M. C. Arendrup. 2006. Detection of *Blastocystis hominis* in unpreserved stool specimens by using polymerase chain reaction. *Journal of Parasitology* 92: 1081-1087.

Stensvold, C. R., H. C. Lewis, A. M. Hammerum, L. J. Porsbo, S. S. Nielsen, K. E. P. Olsen, M. C. Arendrup, H. V. Nielsen, and K. Mølbak. 2009. *Blastocystis*: unravelling potential risk factors

and clinical significance of a common but neglected parasite. *Epidemiology and Infection* 137: 1655-1663.

Stephenson, L. S., M. C. Latham, and E. A. Ottesen. 2000. Malnutrition and parasitic helminth infections. *Parasitology* 121: 23-38.

Taamasri, P., M. Mungthin, R. Rangsin, B. Tongupprakarn, W. Areekul, and S. Leelayoova. 2000. Transmission of intestinal blastocystosis related to the quality of drinking water. *Southeast Asian Journal of Tropical Medicine and Public Health* 31: 112-117.

Tarleton, J. L., R. Haque, D. Mondal, J. Shu, B. M. Farr, and W. A. Petri, Jr. 2006. Cognitive effects of diarrhea, malnutrition, and *Entamoeba histolytica* infection on school age children in Dhaka, Bangladesh. *The American Journal of Tropical Medicine and Hygiene* 74: 475-481.

Udonsi, J. K., J. M. Behnke, and F. S. Gilbert. 1996. Analysis of the prevalence of infection and associations between human gastrointestinal nematodes among different age classes living in the urban and suburban communities of Port Harcourt, Nigeria. *Journal of Helminthology* 70: 75-84.

Wade, S. E. 1982. *Capillaria xenopodis* sp. n. (Nematoda: Trichuroidea) from the epidermis of the South African clawed frog (*Xenopus laevis* Daudin). *Proceedings Helminthological Society of Washington* 49: 86-92.

Whipps, C. M., K. Boorum, L. E. Bermudez, and M. L. Kent. 2010. Molecular characterization of *Blastocystis* species in Oregon identifies multiple subtypes. *Parasitology Research* 106: 827-832.

Wilson, W. M., D. L. Dufour, L. K. Staten, M. Barac-Nieto, J. C. Reina, and G. B. Spurr. 1999. Gastrointestinal parasitic infection, anthropometrics, nutritional status, and physical work capacity in Colombian boys. *American Journal of Human Biology* 11: 763-771.

Wolfe, N. D., A. A. Escalante, W. B. Karesh, A. Kilbourn, A. Spielman, and A. A. Lal. 1998. Wild primate populations in emerging infectious disease research: the missing link? *Emerging Infectious Diseases* 4: 149-158.

World Health Organization (1990). WHO model prescribing information: drug use in parasitic diseases, Geneva, Switzerland.

World Health Organization (2005). Deworming for health and development: report of the third global meeting of the partners for parasite control. World Health Organization, Geneva, Switzerland.

Wu, M., H. Bridle, and M. Bradley. 2012. Targeting *Cryptosporidium parvum* capture. *Water research* 46: 1715-1722.

Chapter 6

RISK FACTORS ASSOCIATED WITH CHANGES IN GASTROINTESTINAL PARASITE COMMUNITIES IN MANTLED HOWLER MONKEYS AND PEOPLE LIVING IN ECUADOR – EVIDENCE OF TRANSMISSION?

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ABSTRACT

Parasitism and associated infectious diseases play a critical role in both non-human primate conservation and human health. Infectious parasitic disease in humans affects billions of people worldwide and in some cases is likely to have originated in wild tropical primates. Several studies have shown anthropogenic disturbances such as increasing human encroachment of tropical forests, logging (and subsequent fragmentation, habitat degradation, and habitat loss), ecotourism, livestock introduction, and agriculture are all likely drivers of changing parasite communities and emerging infectious diseases. Modifications of primate habitat can lead to changes in parasite communities, a potential cause of increased spill-over into human populations and subsequent associative infectious disease. Structural equation modeling was used to better understand the dynamic relationship between changing ecological systems and gastrointestinal parasite communities in mantled howler monkeys, and to assess risk factors in people living in close proximity to these same monkey populations. Sequence data of morphologically similar parasites was compared from both host species to gather evidence of zoonotic transmission. My hypothesized causal models fit the data well, helping explain overall parasite species richness and the presence of specific parasite species in howler monkeys. Human encroachment and forest complexity were both factors strongly associated with gastrointestinal parasite communities in howler monkeys. Models for parasitism in human communities found that age, family size, water treatment, previous chemotherapeutic treatment, gender, and distance to forests and monkeys were all significantly associated with individual parasitism. I also present evidence that *Capillaria* sp. may be transmitted between monkeys and people living in close proximity to one another, the first genetic evidence of macroparasite transmission between wild non-human primates and people.

Keyword: Zoonotic transmission, *Alouatta*, epidemiology, gastrointestinal parasites

INTRODUCTION

Humans and non-human primates have both faced similar infectious diseases throughout evolutionary history (Dobson and Carper, 1996; Barrett et al., 1998; Wolfe et al., 1998; Morens et al., 2004; Wolfe et al., 2007). Some of the greatest threats to the survival of both groups have included both highly virulent, intermittent, pathogenic outbreaks (Morens et al., 2004; Chapman et al., 2005; Nunn and Altizer, 2006), and chronic gastrointestinal parasitism (Dobson and Carper, 1996; Hudson et al., 1998; Scott, 1998; Tompkins and Begon, 1999; Marcogliese, 2004). These infectious diseases account for over 13 million human deaths per year - approximately 25% of annual global deaths (WHO, 1999). Numerous pathogenic organisms are responsible, 175 of which are associated with emerging diseases and 132 of these parasites are zoonotic, with transmission from wildlife to people, and vice versa (Wolfe et al., 1998; Daszak et al., 2000; Taylor, 2001; Wolfe et al., 2007; Jones et al., 2008). Zoonotic parasite transmission involving primates is of particular concern because they are often reservoirs for human diseases (Wolfe et al., 1998). Specific to the tropics, home to the vast majority of primates, 40% of infectious tropical diseases in humans have been linked to non-human primate origins (Wolfe, 2007).

For much of the past century, biologists have focused on predation and carrying capacity as the selection force shaping populations; however, Anderson and May (1979) outlined the effects that pathogens play regulating host survival, fecundity, behavior, and population growth. With new found focus on effects of parasitism and associated infectious disease on short-term and long-term survival of individuals, groups, and species, there is a need to better understand how and why parasite communities change, what effects they have on host behavior, and what environmental changes might exacerbate changes in prevalence, abundance, and range (Tompkins and Begon, 1999).

Anthropogenic disturbance

Parasite communities are naturally dynamic; however, various lines of evidence suggest anthropogenic disturbances influence changes in parasite communities (Barrett et al., 1998; Daszak et al., 2000; Daszak et al., 2001; McCallum, 2002; Patz et al., 2004; Chapman et al., 2005; Goldberg et al., 2007; Wells et al., 2007; Smith et al., 2008; Mborera et al., 2009; Valdespino et al., 2010). Logging, human encroachment, and livestock introduction, and larger global phenomena such as climate change and globalization are likely drivers of changing parasite communities and resulting emerging infectious diseases (Daszak et al., 2000; Daszak et al., 2001). Other factors responsible for changes in parasite communities include ecological modifications, introduction of new parasites via zoonotic transmission associated with encroachment of humans and their domestic animals into previously uninhabited areas (Daszak et al., 2000), host modifications (e.g., changes to grouping patterns, ranging patterns, range use intensity) (Nunn et al., 2003; Gillespie et al., 2005; Macpherson, 2005), or simply as a result of parasite expansion into areas more conducive to growth (e.g., wet climates, higher host densities, new intermediate hosts) (Harvell et al., 2002). One potential outcome of altered parasite communities is the spread of associated infectious diseases in both human and wildlife populations. Understanding these causal pathways, from anthropogenic disturbance to changing parasite communities and zoonotic transmission, is difficult due to the dynamic interactions and responses of parasites to changes in their environment (Figure 6.1).

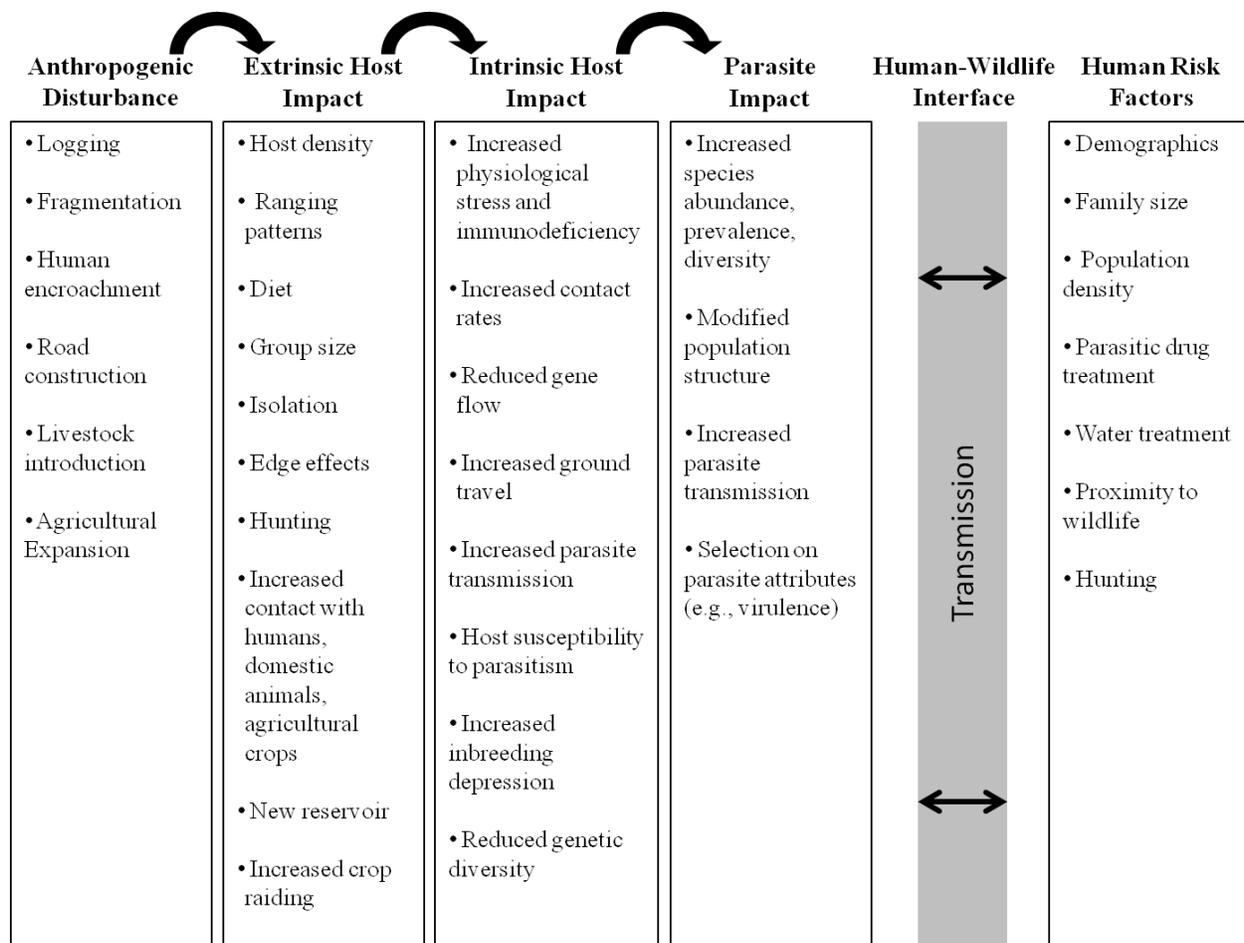


Figure 6.1: Hypothetical causal model linking anthropogenic disturbances with changes in gastrointestinal parasite communities. Coupled with this are potential risk factors in humans that increase their probability of becoming infected with gastrointestinal parasites, some of which originate in wildlife living in close proximity.

Forest Degradation

Habitat loss and degradation associated with logging is one of the biggest anthropogenic disturbances, affecting wildlife and forests worldwide (Brooks et al., 2002). Nearly 10% of forests have been lost in Latin America alone between 1980 and 1995 (Chapman and Peres, 2001). Estimated forest loss throughout Central and South America averaged 0.50% annually from 1990-2000 and accelerated over the following years (0.61%/yr.) (Eva et al., 2012). Selective logging and forest fragmentation have the potential to influence parasite abundance, prevalence, and species richness in wildlife in several ways. One of the main effects of logging

on hosts is changing ranging patterns which can lead to increased densities of individuals and groups as they are forced into new territories or into closer contact with neighboring groups as resource distribution is altered (Banks et al., 2007; Arroyo-Rodriguez and Dias, 2010). As individuals and groups become more crowded, transmission rates increase with density dependent parasite species, resulting in higher parasite prevalence and diversity (Arneberg et al., 1998; Churcher et al., 2005). Forest fragmentation has also been shown to have several other effects on host populations. Monkeys travel more often on the ground in patchy areas, bringing them into contact with various pathogens they wouldn't normally encounter (Gillespie et al., 2005; Pozo-Montuy and Serio-Silva, 2007). In fragmented landscapes, groups are also forced into "genetic islands," limiting their ability to recruit new members and ultimately leading to inbreeding depression (Arroyo-Rodriguez and Dias, 2010). These individuals would be more prone to immunodeficiency and subsequent parasitic disease (Charpentier et al., 2008). Individuals forced into smaller areas from forest fragmentation are also likely to see an increasing number of agonistic interactions between hosts, which can increase long-term stress (Martinez-Mota et al., 2007). As a result, cortisol levels, an indicator of stress, have been positively correlated with increased parasitism in primates (Chapman et al., 2006; Muehlenbein and Watts, 2010).

Documented effects of logging also include the elimination of high value food sources, causing individuals to consume new and often less than ideal food items (Cristobal-Azkarate and Arroyo-Rodriguez, 2007; Martinez-Mota et al., 2007). Modified diets can reduce nutritional health, potentially affecting immunological competence and cortisol levels (Roecker et al., 1996; Pride, 2005), ultimately increasing the likelihood of parasitic infection (Chapman et al., 2005). Feeding on lower quality food sources found in degraded habitat can also lead to more time spent foraging and subsequent increased exposure to gastrointestinal parasites (Harris et al., 2010).

Environmental degradation in turn has been shown to influence nutrition, immune response and parasitic infection in colobus monkeys (Chapman, 2006b).

Changes in forest structure can also affect group size and host densities (Chapman et al., 1995; Clarke et al., 2002; Gillespie et al., 2005). Secondary forests support smaller groups and higher densities in most primate studies (Plumptre and Reynolds, 1994; Chapman et al., 1995; Clarke et al., 2002; Gillespie et al., 2005). However, Peres (1997) reported that primary, terra firme forests with closed canopies harbor some of the lowest numbers of howler monkeys. Important to this study, some of the highest densities of howlers were found in late successional forests. Heterogeneous landscapes with varying forest types might provide benefits of both primary and secondary forests.

Human encroachment

Primates forced into new areas not normally considered ideal habitat, specifically areas bordering edges or human settlements, may also be at risk of acquiring gastrointestinal parasites. Monkeys in forest edges have been shown to harbor up to ten times the number of parasite eggs of those living in undisturbed forest, while individuals living in edge habitats near agricultural plots actually show lower intensity infections attributed to increased nutritional status from crop raiding (Elay et al., 1989; Chapman et al., 2006). Reports of similar gastrointestinal parasites in people and non-human primates living in close proximity to one another exist, though few provide any direct evidence aside from similar morphological appearance in both hosts (e.g., Muriuki et al., 1998; Legesse and Erko, 2004; Salzer et al., 2007; Teichroeb et al., 2009). One example of direct evidence involves *Escherichia coli* - people living near non-human primates are more likely to share genetically similar bacteria (Goldberg et al., 2007; Goldberg et al., 2008).

Human risk factors

Aside from changes in primate parasite communities as a result of anthropogenic disturbance, there are human intrinsic factors and behaviors that also have the potential to increase susceptibility to various gastrointestinal parasite communities. Particularly vulnerable groups include children, those with inadequate clean water, and poor sanitation (Albonico et al. 1996; Asaolu and Ofoezie, 2003; Rinne et al. 2005; Haque, 2007). Similar to non-human primates, proximity to other species – whether they be wild animals, domesticated or livestock – also has the potential to increase zoonotic transmission (Sackey et al. 2003; Goldberg et al., 2007).

Predictive modeling

This study was designed around three main objectives. I first tested the relationship between two indicators of anthropogenic disturbance – human encroachment and forest degradation - with howler monkey group size and gastrointestinal parasite communities. Structural Equation Modeling (SEM) has several advantages for analysis: ability to incorporate both continuous and binary data, greater statistical power than conventional multiple regression analyses, and the use of latent variables - multiple measurements for a single conceptual variable - which allows for estimates and removal of measurement error (Grace and Pugesek, 1997; Beran and Violato, 2010). Initial exploratory analysis found the following relationships which were used to create structural equation models (SEM): 1) forest complexity (e.g., basal area) was positively associated with parasite species richness while indicators of human proximity to mantled howler monkeys was negatively associated with parasite species richness, 2) human proximity to monkeys was negatively associated with group size while forest complexity had little or no relationship to group size, 3) and group size was positively associated with gastrointestinal parasite species richness and the presence of specific parasite species. For the

second objective, I tested the relationship between several potential risk factors in humans with the presence of gastrointestinal parasite parasites. I hypothesize that age, treatment of drinking water, family size, previous anti-parasitic treatment, proximity of people to forests and wildlife, along with hunting have the potential to influence gastrointestinal parasite communities in people. The final objective was to compare two gastrointestinal parasite species found in both howler monkeys and people living in close proximity to one another. If the presence of morphologically similar gastrointestinal parasite species in both monkeys and people is due to zoonotic transmission, then we might expect monkeys living nearest to people to have more genetically similar gastrointestinal parasites of people than those further away. Likewise, we would expect that humans living closest to monkey populations to have more genetically similar gastrointestinal parasites of monkeys than those further way.

METHODS

The Bilsa Biological Station, a private reserve of 3,300 hectares in northwestern Ecuador (35 km west of Quininde, 0°21'N, 79°44'W), is located in a pre-montane tropical forest along the Pacific coast (Figure 6.2A). This area is part of the Tumbes-Choco-Magdalena bioregion and is currently under threat due to increased logging pressure, but still maintains a mixture of disturbed and undisturbed forest (Ortega-Andrade et al., 2010). At Bilsa, an estimated 20% of the reserve is comprised of secondary forest, while only 4% of primary rainforest remains throughout the rest of Northwest Ecuador (Ortega-Andrade et al., 2010).

The Ecuadorian mantled howler monkey, *Alouatta palliata aequatorialis*, is found throughout the reserve at Bilsa, in both primary and secondary forest. Along portions of the east and west perimeter of the reserve, two main communities reside, with roughly 150 people total (pers. comm.). Because these communities sit on the periphery of the reserve, there are some

homes in close proximity to howler groups. The biological station, Bilsa, itself harbors tourists and volunteers and lies within the boundaries of the reserve (Figure 5.2B). A dirt road connects the communities and runs through the middle of Bilsa, meaning that large tracts of forest bordering the road are highly degraded.

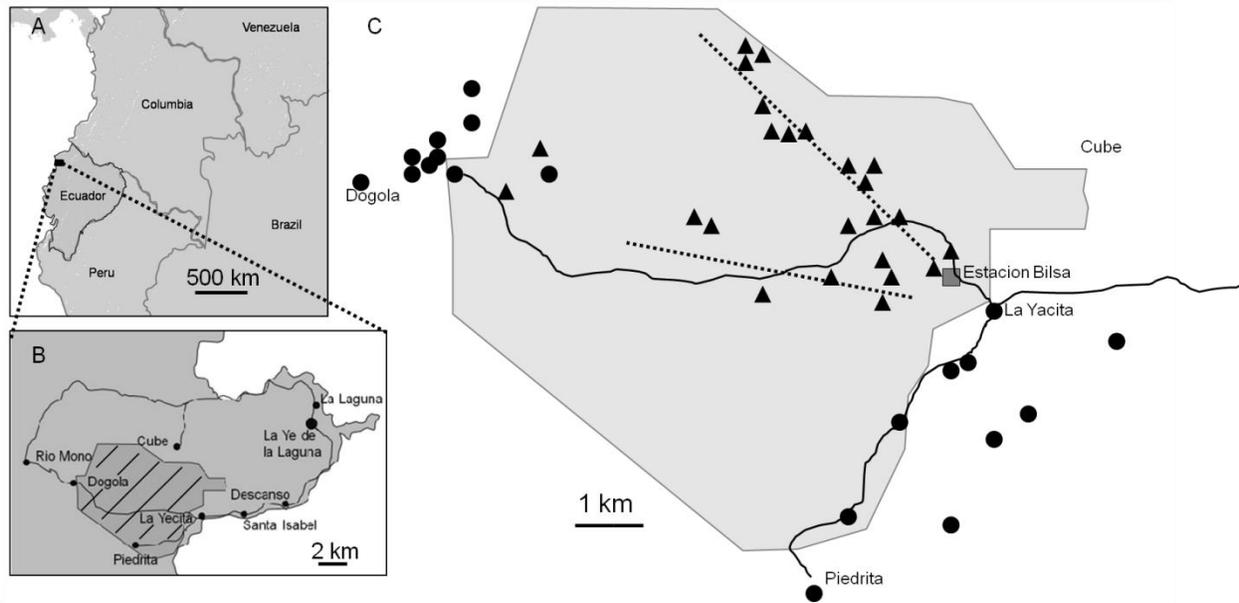


Figure 6.2. Field research took place in Northwestern Ecuador (A) at the Bilsa Ecological Station - highlighted with diagonal lines (B). Two transects are indicated, running through both secondary and primary forest, along which mantled howler monkey groups were sampled (triangles) (C). Several small villages are found surrounding the ecological reserve.

Howler monkey fecal sampling

Field researchers collected 96 fecal samples from 19 primate groups starting June until August 2010 along two 5-km transects at the Bilsa Biological Reserve as previously described (Chapter 2) (Figure 6.2C). Location of each sample was recorded using GPS and manipulated into the 3 containers - zinc polyvinyl alcohol (Zn-PVA) was used to preserve the feces for parasite recovery, RNAlater® (Qiagen Inc., Valencia, California) was used to preserve parasite DNA for *Blastocystis* and *Capillaria* PCR analysis, and a third preservation solution of 50% ethanol was used for a separate study.

Forest structure was estimated from basal area, calculated using data from point-centered quarter methods (Cottam and Curtis, 1956). Basal area has been used to indicate the extent to which forest has been disturbed (Arroyo-Rodríguez and Mandujano, 2006). The center of each 10m circle plot was established using the location of the first sample collected in a group and recorded using GPS (Phillips et al., 1998). Location of human settlements, agricultural plots and roads was calculated using a combination of field-collected GPS points and satellite imagery. Proximity of howlers to people and their houses, farms, and roads was calculated using straight line distance.

Human field collections

A total of fifty-four people from local communities on the edge of the Bilsa biological reserve in northeastern Ecuador were administered questionnaires and asked to provide a fecal sample which was collected within 3 hours or less of defecation. Detailed field methods have previously been described in (Chapter 5). A survey was given to participants and included questions on name, age, gender, family size, previous anti-parasitic treatment, method of water treatment, wildlife proximity, and distance to forest. 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. RNeasy (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.

Laboratory

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 previously described (Chapter 2). 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. PCR-based sequencing was used to determine whether similar gastrointestinal parasites in both hosts using morphological techniques were in fact the same species, and to establish whether there is evidence of transmission. PCR-based analysis was used as the gold standard for detection of *Blastocystis* (Chapter 4). The identity and genetic similarity of *Capillaria* sp. in people and monkeys was done using similar PCR-based sequencing methods found in Chapter 5 with the following modifications. DNA was amplified from *Capillaria* species using overlapping PCR-based primers F-573M with 18S1330R, and 18S87F with 18S462R. PCR amplified samples were run using single strand conformation polymorphism (SSCP), purified, sequenced, and compared using BLAST results. Thirteen gastrointestinal parasite species were previously reported among 96 howler monkeys (Chapter 1) and six parasite species found among 54 people (Table 6.1; Chapter 5). Five gastrointestinal parasites at the genus level were common to people and monkeys: *Blastocystis* sp., *Capillaria* sp., *Entamoeba* spp., *Balantidium* sp., and *Strongyloides* sp.

Table 6.1: Previously reported prevalence of gastrointestinal parasites of Ecuadorian mantled howler monkeys (N=96) and people (N=54) living sympatrically in Ecuador.^{1,2}

		Mantled howler monkeys P % (CI)	Humans P % (CI)
Apicomplexa	<i>Cyclospora</i> sp.	17.7 (11-27)	-
	<i>Isospora</i> sp.	3.1 (1-9)	-
Other Protozoa	<i>Balantidium</i> sp.	9.4 (5-17)	1.9
	<i>Blastocystis</i> sp.	60.4* (60-78)	81.5*
	<i>Chilomastix</i> sp.	4.2 (1-11)	-
	<i>Dientamoeba</i> sp.	3.1 (1-9)	-
	<i>Entamoeba</i> sp.	56.3 (46-66)	14.8
	<i>Iodamoeba</i> sp.	5.2 (2-11)	-
Nematoda	<i>Enterobius</i> sp.	3.1 (1-9)	-
	<i>Capillaria</i> sp.	78.1* (69-85)	20.4*
	<i>Strongyloides</i> sp.	87.5 (79-93)	1.9
	<i>Trypanoxyuris</i> sp.	11.5 (6-20)	-
	Ascarididae gen. sp.	-	13.0
Platyhelminth	<i>Controrchis</i> sp.	14.6 (9-23)	-

* *Blastocystis* confirmation using PCR-based detection. ¹Chapter 1; ²Chapter 5.

Data Analysis

Structural equation modeling (SEM) was used to test relationships between factors using linked regression equations (Mbora and McPeck, 2009; Grace et al., 2010). Several sets of structural equation models (SEM) were created based on univariate analyses ($p < 0.10$) (data not shown). One set of models was created to test the relationship between ecological disturbances and parasite species richness in monkeys, while the other was developed to test the relationship of these same ecological indices with the presence of specific parasite species. Empirical data was tested against hypothesized path models to determine path coefficients and their standard errors using generalized least squares, a method particularly conducive to smaller sample sizes (Beran and Violato, 2010). Chi-square statistic was used to assess the fit of the model, along with the Akaike Information Criterion (AIC), root mean square error of approximation (RMSEA), Schwarz's Bayesian Criterion (Schwarz), and Browne-Cudeck Cross Validation Index (Browne). Lower AIC values are associated with best fit models, along with RMSEA values < 0.05 . The AIC penalizes model complexity, weighting those most parsimonious models the highest. Models with the lowest Schwarz and Browne values are considered best. All variables were tested for normality and univariate Box-Cox transformations used where necessary. Unless otherwise specified, all statistical analyses were conducted using STATISTICA 10 for Windows (StatSoft, Inc., Tulsa, OK).

RESULTS

Howler monkey models

Human encroachment, forest degradation and group size were included in model development based on their significant association with parasite species richness and presence of several key parasite species using univariate analysis. Several causal structures were tested. The

first two models tested relationships between measured anthropogenic disturbances group size, and parasitism (Figure 6.3A: $\chi^2=12.75$, d.f.=14, $p=0.55$; measured parasite species richness and Figure 6.3B: $\chi^2=12.34$, d.f.=14, $p=0.58$; estimated parasite species richness which takes into account sampling effort). Note that a non-significant goodness of fit test indicates that the data match the model well. Both models fit based on RMSEA and AIC values, while Schwarz and Brown measurements suggest that observed parasite species richness was a better model fit than any of the adjusted species richness estimates.

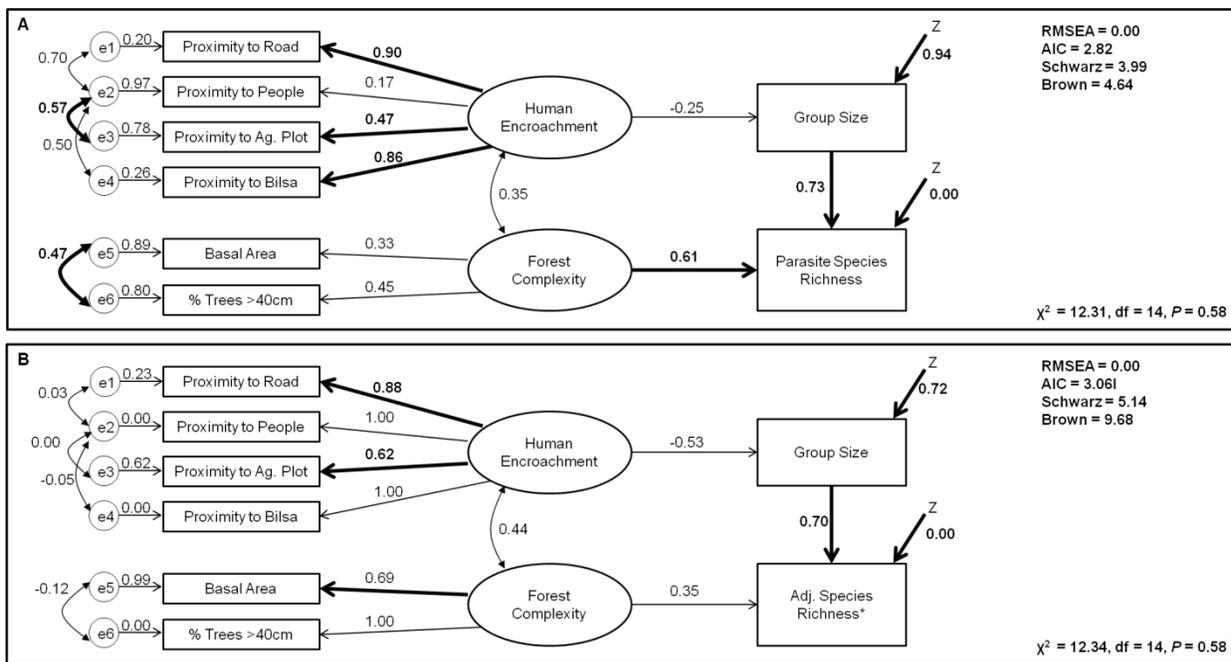


Figure 6.3 (A) Structural equation model (SEM) linking anthropogenic disturbance with group size and parasite species richness in mantled howler monkeys. (B) The Jackknife species richness estimator was used to account for sampling effort which varied with group size. Significance tests are of unstandardized parameters, representing predictive coefficients. Ovals represent latent variables (multiple measurements for a single conceptual variable). Boxes represent observed variables. Double-headed arrows represent covariances. Bolded arrows and numbers signify $p < 0.05$. Note non-significant goodness of fit test indicates theoretical model fits empirical data well. Approximate root mean square error (RMSEA) below 0.05 considered significant. Smaller values for Akaike Bayesian information criteria (AIC), Schwarz Bayesian Criterion, and Brown-Cudeck Single Sample Cross-Validation Index indicate preferable models.

Variance in group size could not be significantly explained by the latent variable human encroachment ($R^2=0.25$; $F=1.4$; d.f. = 4,17; $p < 0.27$), though all indices except distance to agricultural plots were negatively correlated. Relative contribution of human encroachment

indices towards group size variance was compared using standardized coefficients since all measured distance variables use the same units – meters (data not shown). Distance to roads and Bilsa both contributed nearly equally to group size variance regardless of whether standardized coefficients or partial regression coefficients were compared. The contribution of distance to agricultural plots was comparatively lower, yet significantly more important than the general measured variable, human distance.

Group size was positively associated with observed parasite species richness ($r=0.75$, $p=0.00$) and all three species richness adjusted indices (e.g. Jackknife species richness estimator: $r=0.75$, $p=0.00$). Note, forest structure was not correlated with group size (basal area: $r=-0.07$, $p=0.77$; percent trees greater than 40cm DBM: $r=-0.05$, $p=0.82$), which is reflected in the causal model. Forest structure was significantly correlated with observed species richness – as forest complexity increased, so too did measured and adjusted parasite species richness. Univariate analysis results were similar for all combinations of forest structure and species richness estimates.

Forest structure and human proximity were correlated with one another – groups further from people (all indicators) tended to also be found in areas with slightly higher forest structure (both indicators) – though not significantly. The combined variance of parasite species richness uniquely explained by human distance variables was 0.27 - compared to 0.04 for forest structure variables - suggesting that proximity of people, roads, agricultural plots, and the research station play a more important role than forest complexity.

A second set of models were developed based on the presence of specific gastrointestinal parasite species: Figure 6.4A: $\chi^2=2.69$, d.f.=5, $p=0.75$; *Entamoeba* sp., and Figure 6.4B: $\chi^2=11.05$, d.f.=14, $p=0.68$; *Controrchis* sp. Groups found with *Entamoeba* sp. were larger and closer to roads and Bilsa than uninfected groups. The presence of *Controrchis* sp. was dependent

upon a combination of both human proximity and forest structure variables. Infected groups tended to be larger than uninfected groups and found in areas with higher forest complexity. Every group was infected with *Strongyloides* sp., *Capillaria* sp. and *Blastocystis* sp., making this approach uninformative.

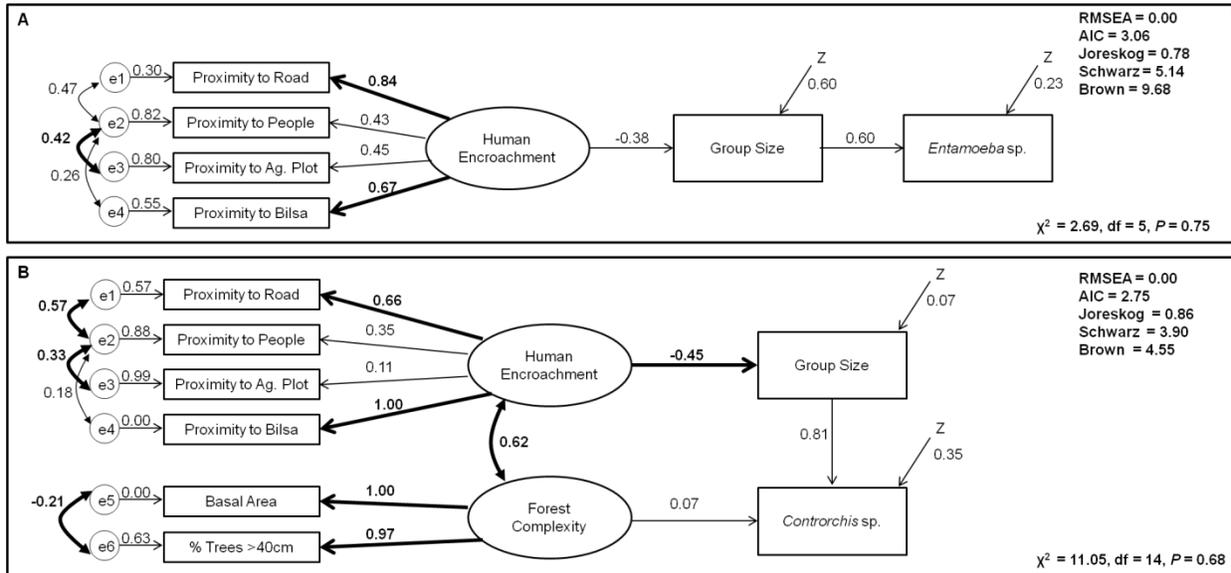


Figure 6.4. Significant structural equation models (SEM) based on the presence of (A) *Entamoeba* spp., and (B) *Controrchis* sp. Significance tests are of unstandardized parameters, representing predictive coefficients. Bolded arrows and numbers signify $p < 0.05$.

Human models

Overall species richness could best be explained by the inclusion of several risk factors (Figure 6.5A). Younger individuals, females, those living in larger families, who had not been treated for any gastrointestinal parasites in the last year, and who didn't boil their water before consumption showed higher levels of parasite species richness. Gender and boiling of water both contributed significantly to the presence of *Blastocystis* (Figure 6.5B). All females were infected with *Blastocystis* sp. while only 72% of males were positive. The unique model could not be tested for goodness of fit ($df=0$). The presence of *Entamoeba* sp. was significantly more likely in those who didn't boil their water and those who lived further from forests (Figure 6.5C). Every individual had domestic or wild animals within 100m of their home and could not be included as

a variable in the model. The presence of *Capillaria* sp. was significantly associated with the same risk factors found in the general parasite species richness model. No significant inhibitory or mutualistic relationships were found between parasites.

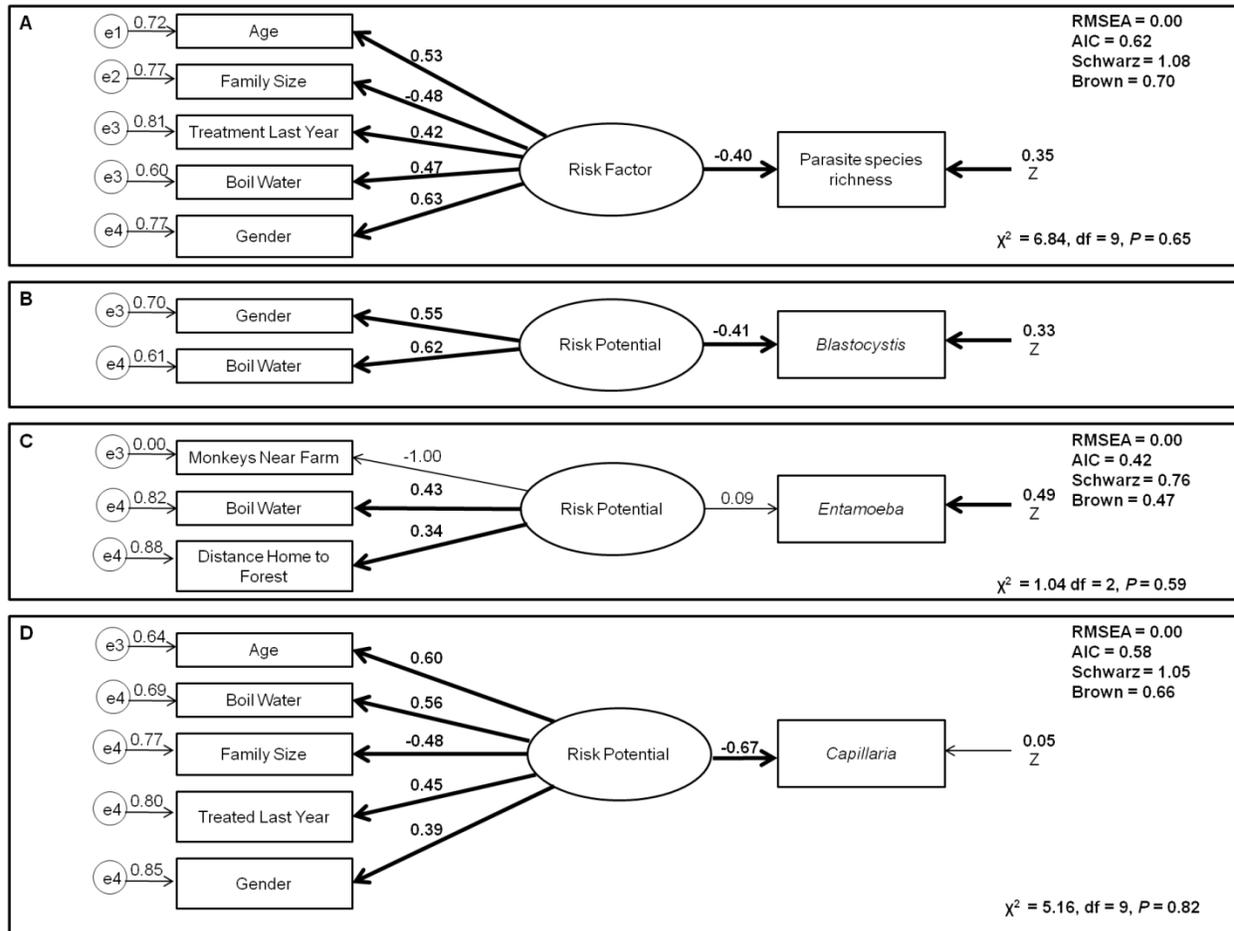


Figure 6.5. Structural equation model (SEM) developed for human risk in communities living sympatrically with mantled howler monkeys. Intrinsic and extrinsic human factors were compared with (A) parasite species richness in individuals, (B) presence of *Blastocystis* sp., (C) *Entamoeba* spp., and (D) *Capillaria* sp. Bolded arrows and numbers signify $p < 0.05$.

Zoonotic transmission

Five out of six parasites groups found in humans were also found in monkeys living nearby (Table 6.1). Of these five similar parasite species, I amplified the small ribosomal subunit of two parasites from both monkeys and people – *Blastocystis* sp. and *Capillaria* sp. In the case of *Blastocystis* sp. howler monkeys only harbored Subtype 8, while people living less than 10m

away in some cases, harbored either Subtype 1, 2, 3, or 5. Approximately 977 bp of small subunit ribosomal DNA from *Capillaria* sp. was nearly identical from both human and monkey hosts. A single base pair difference was found in a small subset of monkey samples (herein referred to as subtype 2), while the majority of sequences obtained from other monkeys and humans were identical (subtype 1). The closest sequence match in GenBank was *Capillaria xenopi* (91% similarity). Monkeys with *Capillaria* sp. subtype 1 were found an average of 378m from Bilsa and 420m from peoples' homes, while subtype 2 was found an average of 1123m from Bilsa and 1415m from homes.

DISCUSSION

Anthropogenic disturbance

Our research supports the premise that indicators of anthropogenic disturbance are associated with parasite communities. In the case of human encroachment, howler monkey groups increased in size the closer they were to people. In turn, larger groups were associated with a significantly higher number of parasite species. The presence of larger groups closer to people is antithetical to many previous primate studies, as these areas are generally quite disturbed (Gonzalez-Kirchner 1998). However, groups living on the edge of agricultural plots or near fruit-bearing tree plantations may actually benefit from increased quality and quantity of food (McCann et al., 2003). The result of a better diet would coincide with increased reproductive rates and carrying capacity (Chapman et al., 1990; Thompson and Wrangham 2008).

Surprisingly, forest complexity was not associated with changes in group size which may be a result of our previously discussion – areas near people, which are often degraded, can actually benefit individuals and groups by providing accessible, high quality food sources.

Primary forest normally harbor larger groups because of the increased carrying capacity associated with more available food types and better quality food sources, whereas in disturbed forests primates are limited to a lower quality and quantity of food (Cristobal-Azkarate and Arroyo-Rodriguez, 2007; Martinez-Mota et al., 2007). However, given ample time to recover, secondary forests might actually provide an ideal heterogenous habitat for primates – particularly for those monkey species such as howlers which are quite adaptable to a broad array of food types and environmental conditions (Peres, 1997).

Other factors could play a role in gastrointestinal parasite communities, such as host density and subsequent contact rates, stress levels, and immune susceptibility (Martinez-Mota et al., 2007; Chapman et al., 2006; Muehlenbein and Watts, 2010). Fragmentation can reportedly drive changes in primate densities; however, there was limited evidence of restricted dispersal aside from a single road that ran through the biological reserve and clear-cutting outside of one community, Dogola, in which monkeys were entirely absent. Logging has mostly ceased throughout the rest of the reserve, though evidence of limited selective logging exists in some areas. In these heterogeneous landscapes, fragmentation was absent and thus, group ranges were likely only limited by the presence of other howler groups.

When human encroachment and forest complexity were factored into parasite species richness models, both indicators of anthropogenic disturbance were critical in explaining parasite distribution patterns. Similarly, the presence of *Controchis* sp. was also associated with both measures of anthropogenic disturbance, while *Entamoeba* sp. was only linked to human proximity indices such as roads and a local research station. No other single parasite SEM models were significant, though individuals with *Strongyloides* sp. were found closer to people, agricultural plots, and Bilsa using univariate analysis. Comparisons of *Blastocystis* sp.,

Strongyloides sp., and *Capillaria* sp. were not conducted because they were ubiquitous throughout sampled monkey groups.

Human models

Parasite species richness at an individual level was significantly associated with several host attributes and risk factors. Parasite species richness decreased with age of individuals, was higher in females and larger families, and individuals who didn't boil their water or have any type of gastrointestinal parasite medication in the previous year were more likely to be parasitized. Previously, using univariate analyses, only individuals who boiled their water were found to have reduced parasite species richness (Chapter 4). By controlling for covariance between measured variables, previous trends were significant using SEM. Additional statistically significant risk factors were also found when looking at specific parasite species (Figure 6.5). For instance, gender was significantly associated with the presence of *Blastocystis* sp. and *Capillaria* sp. in developed models.

Zoonotic transmission

The finding of distinct *Blastocystis* species subtypes in humans and monkeys suggests that there is no transmission of this protozoan between these two hosts. However, with *Capillaria* sp., there are two genetic subtypes that occur in monkeys (subtype 1 and 2), one of which was shared with humans (subtype 1). Our results should not be considered proof of transmission, but several pieces of evidence suggest possible transmission or a common source of infection. First, the *Capillaria* sp. ribosomal sequence match is identical in several samples from both hosts (subtype 1). A second subtype is present (subtype 2) but is only found in howler samples further away from people. Few cases of any *Capillaria* in humans or wildlife have been described in South America with the exception of an archaeological site in Patagonia (Fugassa et al., 2008), possibly in a Brazilian howler group (Godoy et al., 2004), and in wild pigs living in

the Andes (Dittmar, 2002). Shared *Capillaria* sp. sequence across several hundred base pairs is interesting considering the closest related match in GenBank is *Capillaria xenopi* (91% similar), obtained from South African frogs.

The rarity of *Capillaria* species in the South American literature could be due to several reasons. Misidentification is likely as this species is quite similar morphologically to *Trichuris* spp. Few studies have looked genetically at wildlife gastrointestinal parasites, in which case these parasites could easily go undetected or be misidentified. The parasite could simply be rare in humans and other non-human primates, simply going unnoticed up until now. This seems unlikely though as *Capillaria* sp. is quite prevalent in both of our study species.

If zoonotic transmission was occurring, similar subtypes were expected in monkeys and people living closest to each other. Subtype 2 was found in monkeys further away from people and that subtype 1 – found also in humans – was in monkeys living closer to people. Additional *Capillaria* sp. sequencing is needed to confirm whether this trend continues. There is the possibility that the positive human samples are a result of transmission from wildlife or domestic animals, or both monkeys and people could be infected from a common source. Besides *Blastocystis* species, for which we are fairly certain no transmission is occurring between sampled host species, there are three other gastrointestinal parasite species that are morphologically similar in monkeys and people. Additional genetic testing is needed to confirm whether they are the same species and whether distribution patterns of subtypes are indicative of zoonotic transmission.

LITERATURE CITED

Albonico, M., N. Shamlaye, C. Shamlaye, and L. Savioli. 1996. Control of intestinal parasitic infections in Seychelles: a comprehensive and sustainable approach. *Bulletin of the World Health Organization* 74: 577-586.

- Anderson, R. M., and R. M. May. 1979. Population biology of infectious diseases: Part I. *Nature* 280: 361-367.
- Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London Series B: Biological Sciences* 265: 1283-1289.
- Asaolu, S. O. and I. E. Ofoezie. 2003. The role of health education and sanitation in the control of helminth infections. *Acta Tropica* 86: 283-294.
- Barrett, R., C. W. Kuzawa, T. McDade, and G. J. Armelagos. 1998. Emerging and re-emerging infectious diseases: the third epidemiologic transition. *Annual Review of Anthropology* 247-271.
- Brooks, T. M., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, A. B. Rylands, W. R. Konstant, P. Flick, J. Pilgram, S. Oldfield, G. Magin, and C. Hilton-Taylor. 2002. Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* 16: 909-923.
- Chapman, C. A., S. Walker, and L. Lefebvre. 1990. Reproductive strategies of primates: the influence of body size and diet on litter size. *Primates* 31: 1-13.
- Chapman, C., T. Gillespie, and T. Goldberg. 2005. Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology* 14: 134-144.
- Chapman, C. A., M. D. Wasserman, T. R. Gillespie, M. L. Speirs, M. J. Lawes, T. L. Saj, and T. E. Ziegler. 2006a. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology* 131: 525-534.
- Chapman, C. A., M. L. Speirs, T. R. Gillespie, T. Holland, and K. M. Austad. 2006b. Life on the edge: gastrointestinal parasites from the forest edge and interior primate groups. *American Journal of Primatology* 68: 397-409.
- Charpentier, M. J., C. V. Williams, and C. M. Drea. 2008. Inbreeding depression in ring-tailed lemurs (*Lemur catta*): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics* 9: 1605-1615.
- Churcher, T. S., N. M. Ferguson, and M. G. Basanez. 2005. Density dependence and overdispersion in the transmission of helminth parasites. *Parasitology* 131: 121-132.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science* 287: 443-449.
- Daszak, P., A. Cunningham, and A. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* 78: 103-116.

- Dittmar, K. 2002. Arthropod and helminth parasites of the wild guinea pig, *Cavia aperea*, from the Andes and the cordillera in Peru, South America. *Journal of Parasitology* 88: 409-411.
- Dobson, A. P., and E. R. Carper. 1996. Infectious diseases and human population history. *Bioscience* 115-126.
- Eley, R. M., S. C. Strum, G. Muchemi, and G. D. F. Reid. 1989. Nutrition, body condition, activity patterns, and parasitism of free-ranging troops of olive baboons (*Papio anubis*) in Kenya. *American Journal of Primatology* 18: 209-219.
- Eva, H. D., F. Achard, R. Beuchle, E. de Miranda, S. Carboni, R. Seliger, M. Vollmar, W. A. Holler, O. T. Oshiro, V. B Arroyo, and J. Gallego. 2012. Forest cover changes in tropical South and Central America from 1990 to 2005 and related carbon emissions and removals. *Remote Sensing* 4: 1369-1391.
- Fugassa, M. H., V. Taglioretti, M. L. Gonçalves, A. Araújo, N. H. Sardella, and G. M. Denegri. 2008. *Capillaria* spp. eggs in Patagonian archaeological sites: statistical analysis of morphometric data. *Memórias do Instituto Oswaldo Cruz* 103: 104-105.
- Godoy, K. C. I., A. Odalia-Rímoli, and J. Rímoli. 2004. Infecção por endoparasitas em um grupo de busios-pretos (*Alouatta caraya*) em um fragment florestal no Estado Do Mato Grosso Do Sul, Brasil. *Neotropical Primates* 12: 63-68.
- Goldberg, T., T. Gillespie, I. Rwego, E. Wheeler, E. Estoff, and C. Chapman. 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation* 135: 511-517.
- Pedro Gonzalez-Kirchner, J. 1998. Group size and population density of the black howler monkey (*Alouatta pigra*) in Muchukux forest, Quintana Roo, Mexico. *Folia Primatologica* 69: 260-265.
- Grace, J. B., and B. H. Pugsek. 1997. A structural equation model of plant species richness and its application to a coastal wetland. *American Naturalist* 436-460.
- Grace, J. B., and K. A. Bollen. 2005. Interpreting the results from multiple regression and structural equation models. *Bulletin of the Ecological Society of America* 86: 283-295.
- Grace, J. B., T. M. Anderson, H. Olf, and S. M. Scheiner. 2010. On the specification of structural equation models for ecological systems. *Ecological Monographs* 80: 67-87.
- Harris, T. R., C. A. Chapman, and S. L. Monfort. 2010. Small folivorous primate groups exhibit behavioral and physiological effects of food scarcity. *Behavioral Ecology* 21: 46-56.
- Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158-2162.

- Hudson, P. J., A. P. Dobson, and D. Newborn. 1998. Prevention of population cycles by parasite removal. *Science* 282: 2256-2258.
- Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451: 990-993.
- Legesse, M., and B. Erko. 2004. Zoonotic intestinal parasites in *Papio anubis* (baboon) and *Cercopithecus aethiops* (vervet) from four localities in Ethiopia. *Acta Tropica* 90: 231-236.
- Marcogliese, D. J. 2004. Parasites: small players with crucial roles in the ecological theater. *EcoHealth* 1: 151-164.
- Mbora, D. N., J. Wiczowski, and E. Munene. 2009. Links between habitat degradation, and social group size, ranging, fecundity, and parasite prevalence in the Tana River mangabey (*Cercocebus galerritus*). *American Journal of Physical Anthropology* 140: 562-571.
- McCallum, H., and A. Dobson. 2002. Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London Series B: Biological Sciences*: 269: 2041-2049.
- McCann, C., K. Williams-Guillén, F. Koontz, A. A. R. Espinoza, J. C. M. Sánchez, and C. Koontz. 2003. Shade coffee plantations as wildlife refuge for mantled howler monkeys (*Alouatta palliata*) in Nicaragua. In *Primates in fragments: Ecology and Conservation*. Marsh, L. K. (ed.). Springer US. P. 321-341.
- Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. *Nature* 430: 242-249.
- Muehlenbein, M. P., and D. P. Watts. 2010. The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. *BioPsychoSocial Medicine* 4: 1-12.
- Muriuki, S. M. K., R. K. Murugu, E. Munene, G. M. Karere, and D. C. Chai. 1998. Some gastrointestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica* 71: 73-82.
- Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative tests of parasite species richness in primates. *The American Naturalist* 162: 597-614.
- Patz, J. A., T. K. Graczyk, N. Geller, and A. Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30: 1395-1405.
- Patz, J., P. Daszak, G. Tabor, A. Aguirre, M. Pearl, J. Epstein, N. Wolfe, A. Kilpatrick, J. Fofopoulos, D. Molyneux, D. Bradley, and Members of the Working Group on Land Use Change and Disease Emergence. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. *Environmental Health Perspectives* 112: 1092-1098.
- Pride, R. E. 2005. Optimal group size and seasonal stress in ring-tailed lemurs (*Lemur catta*). *Behavioral Ecology* 16: 550-560.

- Rinne, S., E. J. Rodas, R. Galer-Unti, N. Glickman, and L. T. Glickman. 2005. Prevalence and risk factors for protozoan and nematode infections among children in an Ecuadorian highland community. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 99: 585-592.
- Roecker, E. B., J. W. Kemnitz, W. B. Ershler, and R. Weindruch. 1996. Reduced immune responses in rhesus monkeys subjected to dietary restriction. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 51: B276-B279.
- Scott, M. E. 1988. The impact of infection and disease on animal populations: implications for conservation biology. *Conservation Biology* 2: 40-56.
- Thompson, M. E., and R. W. Wrangham. 2008. Diet and reproductive function in wild female chimpanzees (*Pan troglodytes schweinfurthii*) at Kibale National Park, Uganda. *American Journal of Physical Anthropology* 135: 171-181.
- Valdespino, C., G. Rico-Hernández, and S. Mandujano. 2010. Gastrointestinal parasites of howler monkeys (*Alouatta palliata*) inhabiting the fragmented landscape of the Santa Marta mountain range, Veracruz, Mexico. *American Journal of Primatology* 72: 539-548.
- Wells, K., L. Smales, E. Kalko, and M. Pfeiffer 2007. Impact of rain-forest logging on helminth assemblages in small mammals (Muridae, Tupaiidae) from Borneo. *Journal of Tropical Ecology* 23: 35-43.
- Wolfe, N. D., A. A. Escalante, W. B. Karesh, A. Kilbourn, A. Spielman, and A. A. Lal. 1998. Wild primate populations in emerging infectious disease research: the missing link?. *Emerging Infectious Diseases* 4: 149-158.
- Wolfe, N. D., C. P. Dunavan, and J. Diamond. 2007. Origins of major human infectious diseases. *Nature* 447: 279-283.
- World Health Organization. 1999. Removing obstacles to healthy development: report on infectious diseases.

CHAPTER 7: DISCUSSION

OVERVIEW

Large scale rain-forest destruction, intensified land conversion for biofuel crops and monocultured palm plantations, invasive species introduction, exponential population growth, and the bushmeat trade are some of the most serious human impacts which have been tied inextricably with globalization, exponential population growth, and increased resource use (Rosa et al., 2004). These ecological disturbances have placed wildlife worldwide in peril, driving many to extinction (Cowlshaw, 1999; Lopes and Ferrari, 2000; Harvell et al., 2002; Michalski and Peres, 2005), or affecting their health, fitness, and behavior (Daszak et al., 2000; Cristobal-Azkarate and Arroyo-Rodriguez, 2007; Acevedo-Whitehouse and Duffus, 2009). Yet a largely ignored impact of mass conversion of forests into agricultural plots or monoculture crops, particularly in tropical areas, involves changes in infectious disease risk to both people and wildlife living in these areas (Schrag and Weiner, 1995; Daszak et al., 2000; Patz et al., 2000; Daszak et al., 2001; Pavelka et al., 2003; Chapman et al., 2004; Wells et al., 2007; Puttker et al., 2008; Cristobal-Azkarate et al., 2010; Schwitzer et al., 2010; Trejo-Macias and Estrada, 2012).

A majority of newly emerging diseases in humans are caused by pathogens considered zoonotic (Taylor et al., 2001; Bengis et al., 2004), often spilling-over from wildlife populations into surrounding communities (Daszak et al., 2001; Anita et al., 2003; Eisenberg et al., 2007). Specific to the tropics, forty percent of infectious diseases originate in wild primates (Wolfe et al., 2007). These zoonotic pathogens are more likely to be transmitted as humans expand into areas previously uninhabited and as forests are modified, placing both wildlife and human populations at greater risk (Chapman et al., 2005; Wells et al., 2007; Gillespie et al., 2005; Daszak and Cunningham, 2003; Puttker et al., 2008).

This study was designed to help better understand the dynamic relationship between the environment, host, and parasite communities. Several research objectives were addressed in this study: 1) the gastrointestinal parasite communities of 96 Ecuadorian mantled howler monkeys, *Alouatta palliata aequatorialis*, and 54 people living adjacent to one another in a tropical habitat were assessed; 2) parasite species richness in howlers was compared with group size, while patterns of gastrointestinal parasitism distribution were analyzed; 3) the association between anthropogenic disturbance and gastrointestinal parasite communities in howler monkeys was examined; 4) risk factors in people were compared to their gastrointestinal parasite communities; and 5) two morphologically similar gastrointestinal parasites found in both people and monkeys were compared genetically to investigate possibility of transmission. Distinct parasite genotypes in a host would be evidence for absence of transmission, and shared genotypes adds weight of evidence for transmission or common source of infection. Furthermore, if zoonotic transmission was occurring and genetic variation great enough, then parasite genotypes from monkeys living nearest to people would be most similar to parasite genotypes recovered from people.

Gastrointestinal parasite distribution

Thirteen gastrointestinal parasites were found in mantled howler monkeys, along with six parasite species in two Ecuadorian communities. No other howler monkey studies have found as many gastrointestinal parasite species, which could be a result of environmental differences associated with this unique region or the methods of parasite recovery I used. The Tumbes-Choco-Magdalena region which runs along the Pacific Coast from Panama down to Peru is a biodiversity hotspot, containing the Western Ecuador moist forests. This area is known for high rainfall and is considered one of the wettest places on Earth. Other studies have found that gastrointestinal parasitism is strongly associated with the wet season, which might explain why this area has higher parasite species richness (Teichroeb et al., 2009; Valdespino et al., 2010;

Cristobal-Azkarate et al., 2010; Trejo-Macias and Estrada, 2012). This area west of the Andes has also been isolated from the Amazon for millions of years, contributing to a unique diversity of flora and fauna, including some of the highest plant, bird, reptile, and amphibian endemism in the world. The unique diversity found in other plant and wildlife assemblages could very well apply to parasite communities as well.

This study also used multiple methods to extract gastrointestinal parasites – fecal smears, flotations, and sedimentations, along with PCR-based amplification for a select few species. In many howler monkeys studies, extractions were limited to one or two methods. There is the possibility that a multi-pronged approach helped yield a greater number of parasites, particularly species that are more difficult to recover or identify (e.g., *Entamoeba* spp.). The fecal flotation method is one of the most widely used extraction methods and this study would have only recovered 9 out of 13 parasite species simply using this method. The use of multiple methods is more time consuming, but would appear to increase recovery efficiency.

Co-infection patterns

Four parasites were found to be non-randomly distributed throughout sampled howler monkeys relative to other parasite species. Individuals infected with *Balantidium* sp. were more likely to also be infected with *Isospora* sp., while individuals harboring *Chilomastix* sp. were less likely to harbor *Capillaria* sp. There are other studies demonstrating both mutualistic and competitive interactions between parasite species (Petney and Andrews, 1998; Cox, 2001; Poulin, 2001; Ekanayake et al., 2006; Pedersen and Fenton, 2007; Graham, 2008; Chapman et al., 2011), which might explain our observed associations. However, in either case there have been no empirical studies comparing these specific parasite interactions, making it difficult to ascertain any meaningful relationship. Testing whether similar relationships exist in other howler monkeys would be a logical first step. Based on univariate analyses, no other significant parasite

patterns were found in sampled monkeys, though some individuals were more heavily parasitized than others. Individual monkeys averaged 3.6 parasite species per individual (± 1.4 SD). One individual harbored as many as 7 gastrointestinal parasites, while two had no observable infection – all three individuals fell outside two standard deviations.

Group size

Most studies looking at associations between forest type and group size have often found that secondary forests support smaller groups (Chapman et al., 1995; Clarke et al., 2002; Gillespie et al., 2005; Gillespie and Chapman, 2008b). Group size was not related to any indicator of forest degradation in this study; however, monkeys living a local research station, Bilsa, tended to be slightly larger than those further away. The latent variable human encroachment was also negatively correlated with group size after controlling for forest complexity (Figure 6.3).

The presence of larger groups in areas near people could hypothetically be explained by the quality and availability of food. Environmental factors associated with group size include size, density, quality, phenology, productivity and distribution of food patches (Chapman et al., 1995; Peres, 1997). In howler monkeys, their ability to persist in areas where other primates cannot is partially tempered by adjustments in diet (i.e., increased or decreased frugivory), and improved foraging efficiency through group fission or changes in activity patterns (Schwitzer et al., 2011). Much of the secondary forest in this study has had nearly 15 years to recover from logging, and areas near Bilsa include patches of fruit tree plantations. Basal area would not necessarily increase in these areas but quality of food would improve, potentially supporting larger groups than expected solely on the fact that these areas were previously logged. Areas surrounding the research station are also heterogeneous to some extent, meaning that monkeys aren't solely restricted to primary or secondary forest. In some instances, the same monkey

groups would traverse through areas considered secondary forest and older growth, which again might mitigate any statistical trends between forest complexity and group size.

Larger groups increase contact rates which I would expect to correlate with higher parasite prevalence of gastrointestinal parasite species and subsequently, greater group species richness (Cote and Poulin, 1995; Arneberg et al., 1998; McCallum et al., 2001; Cross et al., 2009). If smaller group size did limit frequency dependent transmission, then I would expect reduced prevalence of certain parasite species and that presence/absence would be associated with forest structure. Group size was positively associated with overall species richness and the presence of several parasite species, including *Entamoeba* spp., *Controrchis* sp., and *Balantidium* sp. (Table 3.4 and Figure 6.4). The presence of *Isospora* sp. ($p=0.06$) and *Cyclospora* sp. ($p=0.06$) were also positively associated with group size, though not statistically significant.

Evidence of wild howler monkey morbidity and mortality associated with specific macro-parasitic infections is limited, likely due to a lack of autopsies on wild primates (e.g., *Trypanoxyuris minutis* and *Controrchis biliophilus*) (Villanueva-Jimenez, 1988; Amato et al., 2002). However, evidence from other primates would suggest that many macroparasites can cause an array of host diseases (Table 1.1). The synergistic effects of multiple infections are likely to play a crucial role in long-term health and fitness, similar to that found in humans (Pullan and Brooker, 2008).

Anthropogenic disturbance

The presence of several key parasite species was associated with forest disturbance and human encroachment (Tables 3.3 and 3.4; Figure 6.4); supporting the idea that anthropogenic disturbance may be placing primate populations at risk of select gastrointestinal parasites. Logging could affect primate parasites through any number of direct and indirect pathways previously discussed (Figure 1.1), while zoonotic transmission would be greater between

monkeys and people living in close contact to one another. I've tested collected field data against hypothesized pathways and found that the data support my hypotheses. Yet to confirm causality, empirical testing of planned logging sites could be monitored before, during, and after to better understand the composition of gastrointestinal parasites of monkeys long-term. Forested areas with experimental plots and varying levels of anthropogenic disturbance could also be used to compare parasite communities and group dynamics of monkeys. In the second hypothesis, I tested whether two gastrointestinal parasite species – *Blastocystis* sp. and *Capillaria* sp. – exhibited evidence of zoonotic transmission. Humans and monkeys living in close proximity to one another did not share the same subtypes of *Blastocystis* sp. Yet in the case of *Capillaria* sp. I found preliminary evidence that monkeys and people living in close proximity to one another share the same genotype, while monkeys living further from people shared a separate subtype. Though this is not absolute evidence of zoonotic transmission, it is the first report of *Capillaria* sp. in either host species living in South America. Additional testing of other genetic markers, preferably ones with greater sequence variation, would help establish whether spill-over was occurring from people to howlers, or vice versa.

Humans

Six gastrointestinal parasite species were found in 54 individuals, all of which had a congener found in howler monkeys, with the exception of an Ascarididae gen. sp. All these parasite species, except for *Capillaria* sp., have been previously described extensively in Ecuadorian communities. From a human health perspective, there appear to be several intrinsic and extrinsic factors that are associated with reduced parasite risk. People who don't boil their water, are relatively young, live in larger families, hunt wildlife, and haven't been treated with some form of chemotherapy or anti-helminthic drug are at increased risk of acquiring certain gastrointestinal parasites. Clearly, widespread treatment for gastrointestinal parasites would be

ideal. Yet due to expense and inconvenience for rural communities, treatment is usually not an option, or only after an individual has already started experiencing severe complications. The results from my findings would suggest that even simple, cost-effective, treatment of water is effective at minimizing *Blastocystis* sp. and *Trichuris* sp. infections. Other intrinsic factors such as age, gender and family size can't be controlled, though it does suggest which people are at greatest risk of acquiring certain gastrointestinal parasites.

Humans living near rainforest and monkeys were expected to have higher parasite prevalence and species richness because of possible zoonotic transmission. I did find that all hunters, for instance, had *Blastocystis*. Surprisingly though, the exact opposite was found for other parasite species; a lower percentage of people who described themselves as living within 1km of rainforest had *Ascarididae* gen. sp. and *Entamoeba* spp. compared to those who lived further away. If these particular gastrointestinal parasites are dependent on transmission of cysts and eggs via ingestion of contaminated soil or water by hosts, there is a possibility that people who live further from forests have a different source of water and increased chance of eating contaminated soils or food if they live on farms. Both *Ascarid lumbricoides* and *Entamoeba histolytica* have been described in numerous Ecuadorian communities where infections correlate well with sanitation efforts, hand washing, garbage disposal, washing food, and health education (Table 1.1), supporting the idea that extrinsic risk factors might be more important than proximity to wildlife or forests.

Future directions

Studies focused on zoonotic transmission involving non-human primates have predominately focused on viral and bacterial infections in African apes. This study centered on gastrointestinal macroparasites – protozoans, nematodes, and platyhelminths. Expanding this type of study to include microparasites might prove useful, especially considering that a large

portion of emerging infectious diseases involve viruses and bacteria. Likewise, viral infections have been shown to play a large role in regulating howler monkey populations.

Expanding morphological and genetic analysis to include domestic animals, livestock, and other wildlife living near rural communities would add other possible reservoirs that were not considered in this study. Examining these parasite communities long-term might also help us understand how these complex interactions change with other broad-scale anthropogenic disturbances, such as climate change.

CONCLUSIONS

My research examines the gastrointestinal parasite communities of both a mantled howler monkey population and two communities of people. This study found a diverse assemblage of parasites representing protozoan, nematodes and platyhelminths in both host species. I examined the effects of anthropogenic disturbance on gastrointestinal parasite communities in mantled howler monkeys and found that parasite species richness at the group and individual level was significantly associated with indicators of both forest disturbance and human encroachment. Host group size was an important component in helping explain variance in species richness and was a key component in structural equation models that I developed.

Several morphologically similar gastrointestinal parasite species appeared in both monkey and human populations. Using small subunit ribosomal gene sequence data from *Blastocystis* and *Capillaria* species, I found two patterns. For *Blastocystis* sp., unique subtypes were found in each host species, suggesting no transmission or common source of infection for this parasite. In contrast, two subtypes of *Capillaria* sp. were found, but both hosts were infected with subtype 1. The other subtype was only found in monkeys living further away from people (>0.5km). Despite the small subunit gene being considered a rather conservative evolutionary

marker, the finding of identical subtypes in both monkeys and people is compelling and doesn't allow me to rule out zoonotic transmission or a common source of infection. If the proximity of monkeys and people living next to one another is associated with an increased chance of *Capillaria* sp. transmission, this would clearly have larger implications for other areas where people and wildlife overlap.

Lastly, I report on human risk factors associated with increased gastrointestinal parasitism. Intrinsic and extrinsic factors of people were strongly associated with gastrointestinal parasite communities. With ever-increasing human encroachment on wild animal populations and continued habitat degradation of rainforests, continued research on parasite ecology and evolution as it relates to emerging parasitic diseases is critical to the health of local human and wildlife populations.

LITERATURE CITED

Acevedo-Whitehouse, K., and A. L. Duffus. 2009. Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B Biological Sciences*: 364: 3429-3438.

Almeida, M. A. B. D., E. D. Santos, J. D. C. Cardoso, D. F. D. Fonseca, C. A. Noll, V. R. Silveira, A. Y. Maeda, R. P. de Souza, C. Kanamura, and R. A. Brasil. 2012. Yellow fever outbreak affecting *Alouatta* populations in southern Brazil (Rio Grande do Sul State), 2008–2009. *American Journal of Primatology* 74: 68-76.

Bicca-Marques, J. C., and D. S. de Freitas. 2010. The role of monkeys, mosquitoes and humans in the occurrence of a yellow fever outbreak in a fragmented landscape in south Brazil: protecting howler monkeys is a matter of public health. *Tropical Conservation Science* 78-89.

Chammartin, F., R. G. Scholte, L. H. Guimarães, M. Tanner, J. Utzinger, and P. Vounatsou. 2013. Soil-transmitted helminth infection in South America: a systematic review and geostatistical meta-analysis. *The Lancet Infectious Diseases* 13: 507-518.

Cowlishaw, G. 1999. Predicting the pattern of decline of African primate diversity: an extinction debt from historical deforestation. *Conservation Biology* 13: 1183-1193.

- Cristóbal-Azkarate, J., and V. Arroyo-Rodríguez. 2007. Diet and activity pattern of howler monkeys (*Alouatta palliata*) in Los Tuxtlas, Mexico: effects of habitat fragmentation and implications for conservation. *American Journal of Primatology* 69: 1013-1029.
- Danielsen, F., H. Beukema, N. D. Burgess, F. Parish, C. A. Bruehl, P. F. Donald, and E. B. Fitzherbert. 2009. Biofuel plantations on forested lands: double jeopardy for biodiversity and climate. *Conservation Biology* 23: 348-358.
- Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5: 735-748.
- FAO. 2010. Global Forest Resources Assessment. Main Report.
- Fitzherbert, E. B., M. J. Struebig, A. Morel, F. Danielsen, C. A. Brühl, P. F. Donald, and B. Phalan. 2008. How will oil palm expansion affect biodiversity?. *Trends in Ecology and Evolution* 23: 538-545.
- Galindo, P., and S. Srihongse. 1967. Evidence of recent jungle yellow-fever activity in eastern Panama. *Bulletin of the World Health Organization* 36: 151-161.
- Gibson, L., T. M. Lee, L. P. Koh, B. W. Brook, T. A. Gardner, J. Barlow, C. A. Peres, C. J. A. Bradshaw, W. F. Laurance, T. E. Lovejoy, and N. S. Sodhi. 2011. Primary forests are irreplaceable for sustaining tropical biodiversity. *Nature* 478: 378-381.
- González, A. H., V. C. Regalado, and J. Ende. 2001. Non-invasive management of *Ascaris lumbricoides* biliary tract migration: a prospective study in 69 patients from Ecuador. *Tropical Medicine and International Health* 6: 146-150.
- Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158-2162.
- Holzmann, I., I. Agostini, J. I. Areta, H. Ferreyra, P. Beldomenico, and M. S. Di Bitetti. 2010. Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *American Journal of Primatology* 72: 475-480.
- Koh, L. P., and D. S. Wilcove. 2008. Is oil palm agriculture really destroying tropical biodiversity?. *Conservation Letters* 1: 60-64.
- Laemmert Jr, H. W., and H. W. Kumm. 1950. The susceptibility of howler monkeys to yellow fever virus. *American Journal of Tropical Medicine* 30: 723-731.
- Loomis, M. R., J. O. Britt Jr., A. P. Gendron, H. J. Holshuh, and E. B. Howard. 1983. Hepatic and gastric amebiasis in black and white colobus monkeys. *Journal of the American Veterinary Medical Association* 183: 1188-1191.

- Lopes, M. A., and S. F. Ferrari. 2000. Effects of human colonization on the abundance and diversity of mammals in eastern Brazilian Amazonia. *Conservation Biology* 14: 1658-1665.
- Michalski, F., and C. A. Peres. 2005. Anthropogenic determinants of primate and carnivore local extinctions in a fragmented forest landscape of southern Amazonia. *Biological Conservation* 124: 383-396.
- Milton, K. 1996. Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. *Journal of Zoology* 239: 39-63.
- Okulewicz, A., and G. Zalesny. 2004. Biodiversity of Capillariinae. *Wiadomosci Parazytologiczne* 51: 9-14.
- Patz, J. A., T. K. Graczyk, N. Geller, and Y. Vittor. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30: 1395-1405.
- Pullan, R., and S. Brooker. 2008. The health impact of polyparasitism in humans: are we underestimating the burden of parasitic diseases?. *Parasitology* 135: 783-794.
- Reid, H., and K. Swiderska. 2008. Biodiversity, climate change and poverty: exploring the links. IIED Briefing Paper.
- Rosa, E. A., R. York, and T. Dietz. 2004. Tracking the anthropogenic drivers of ecological impacts. *AMBIO: A Journal of the Human Environment* 33: 509-512.
- Sallis, E. S. V., V. L. R. S. de Barros, S. L. Garmatz, R. A. Figuera, and D. L. Graça. 2003. A case of yellow fever in a brown howler (*Alouatta fusca*) in Southern Brazil. *Journal of Veterinary Diagnostic Investigation* 15: 574-576.
- San Sebastián, M., and S. Santi. 2000. Control of intestinal helminths in schoolchildren in Low-Napo, Ecuador: impact of a two-year chemotherapy program. *Revista da Sociedade Brasileira de Medicina Tropical* 33: 69-73.
- Slansky, F. 2007. Insect/mammal associations: effects of Cuterebrid botfly parasites on their hosts. *Annual Review of Entomology* 52: 17-36.
- Villanueva-Jiménez, E. A. 1988. Identificación de helmintos del tracto digestivo del mono aullador (*Alouatta palliata*) en poblaciones silvestres. BSc Thesis. Universidad Veracruzana, Xalapa.

APPENDIX 1: DESCRIPTION OF PARASITES

Thirteen gastrointestinal parasites were identified in mantled howler monkeys and six parasite species were found in humans. Five of the parasites found in humans had a monkey equivalent. Only an Ascarididae gen. sp. was not found in sampled monkeys. The following appendix contains information on the classification, identification, life cycle, geographical distribution, zoonotic potential, pathogenicity, and host specificity of recovered parasites from this study.

Cyclospora sp.

Classification

Phylum: Apicomplexa

Class: Conoidasida

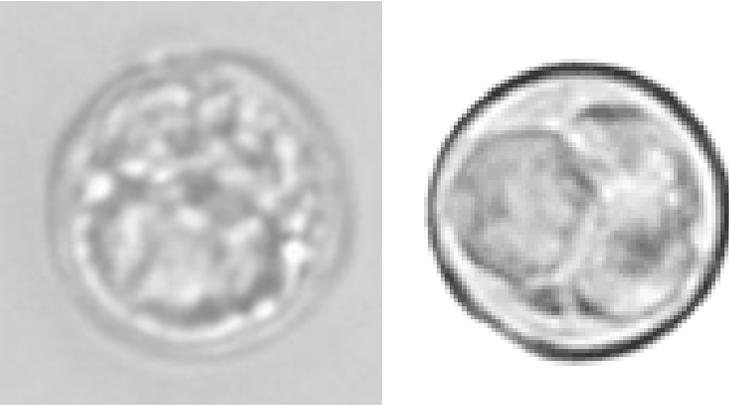
Order: Eucoccidiorida

Family: Eimeriidae

Genus: *Cyclospora*

Identification

Cyclospora sp. oocysts measure 8 to 10 µm in diameter with a distinct oocyst wall (Strausbaugh and Herwaldt, 2000). Oocysts tends to be smaller and more spherical in people and non-human primates compared to other hosts (e.g., rodents) (Chacin-Bonilla, 2010). *Cyclospora cayetanensis* is primarily restricted to humans, while *C. cercopithecii*, *C. colobi*, and *C. papionis* are found in non-human primates (Eberhard et al., 2001; Olivier et al., 2001; Chacin-Bonilla, 2010; Zhao et al., 2013). *Cyclospora cayetanensis* has been reported in rhesus monkeys (*Macaca mulatta*), suggesting that transmission is possible (Chu et al., 2004).



Cyclospora spp. oocysts. 11 μ m (Right: CDC, 2014).

Biology

Infection occurs as a result of ingesting sporulated oocysts through food, water, or soil contaminated with feces (Chacin-Bonilla, 2010; CDC, 2014). Infected individuals excrete unsporulated oocysts which require several days to weeks to develop into the infective sporulated stage. Therefore, direct transmission between hosts is unlikely. Once ingested, oocysts excyst in gastrointestinal tract and release sporozoites that invade epithelial cells of small intestine (CDC, 2014). Replication occurs through asexual reproduction, developing into mature oocysts which can then be shed in feces.

Geographical distribution

Cyclosporiasis occurs mostly in tropical and subtropical climates, following a seasonal pattern of infection (Chacin-Bonilla, 2010; CDC, 2014).

Zoonotic potential

An obligate intracellular parasite, *C. cayetanensis* is restricted to a single host species, making interspecies transmission unlikely. Likewise, it would appear that strains are host specific based on sequence variability in the internal transcribed spacer (ITS) region (Adam et al., 2000; Olivier et al., 2001). The presence of *C. cayetanensis* in rhesus monkeys does suggest that zoonotic transmission may be possible.

Disease

Cyclosporiasis occurs, on average, 7 days after ingestion of sporulated oocysts. Symptoms can include watery diarrhea, loss of appetite, weight loss, nausea, and fatigue (CDC, 2014). Left untreated, symptoms can persist for more than a month, causing morbidity and mortality, particularly in immunocompromised individuals (Chacin-Bonilla, 2010). In some areas where *Cyclospora* sp. is widespread, people may not develop symptoms.

***Isospora* sp.**

Classification

Phylum: Apicomplexa

Class: Conoidasida

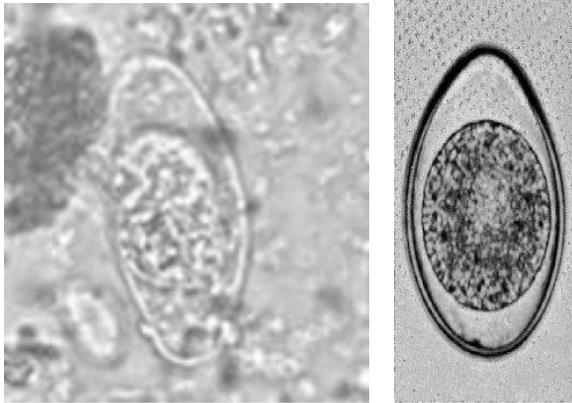
Order: Eucoccidiorida

Family: Eimeriidae

Genera: (*Cysto*)*Isospora*

Identification

Isospora is a coccidian protozoan which includes approximately 248 species across the animal kingdom, though humans are primarily infected by *Isospora belli*, also known as *Cystoisospora belli* (Frenkel, 1997; Lindsay et al., 1997; Barta et al., 2005). The oocysts are most often used for diagnostic identification from fecal flotations, measuring 22-33 μm in length by 10-15 μm in width (Soave and Weikel, 1990). The oocysts are unsporulated in fresh feces. Sporulated oocysts contain two sporocysts and four sporozoites.



Isospora felis unsporulated oocyst (Right: CDC, 2014).

Biology

The immature *Isospora* spp. oocyst stage is excreted in stool and is environmentally resistant. Immature oocyst contains just one sporoblast which divides and matures into sporocysts containing four sporozoites each. Infection occurs by ingestion of mature oocysts (Soave and Weikel, 1990; CDC, 2014). Sporocysts divide yet again and produce four sporozoites. Fully sporulated oocysts are then ingested via the fecal-oral route. The sporozoites are ruptured in the small intestine and invade the epithelial cells and begin producing schizonts. Schizonts release merozoites, invade epithelial cells and asexual replication continues. Trophozoites develop into schizonts, which contain merozoites. Male and female gametocytes develop and eventually fertilization occurs, forming oocytes which are then excreted in stool and the process repeats (CDC, 2012).

Geographical distribution

Most common in tropical and subtropical climates.

Zoonotic potential

Considered to be highly host specific and most often only parasitizes a single species, though exceptions exist. In some cases, a paratenic host is used to distribute oocysts (Lindsay et al.,

1997). *Isospora belli* is primarily a human parasite, though Chapman et al., (2012) suspect *I. belli* infection in several African primates. Several other parasite species have been described in primates, though specific to New World primates, *I. arctopitheci* has been described in *Alouatta villosa* (Hendricks, 1977).

Disease

Most species are only mildly pathogenic, but can cause diarrhoeal disease (coccidiosis), colic, weight loss, fever, and even death in immunocompromised individuals (Lindsay et al., 1997; CDC, 2014). Severity of infections can be exacerbated by viruses or other immunosuppressant agents, and is generally more severe in infants and children as adults develop protective immunity (Lindsay et al., 1997).

Balantidium sp.

Classification

Phylum: Ciliophora

Class: Litostomatea

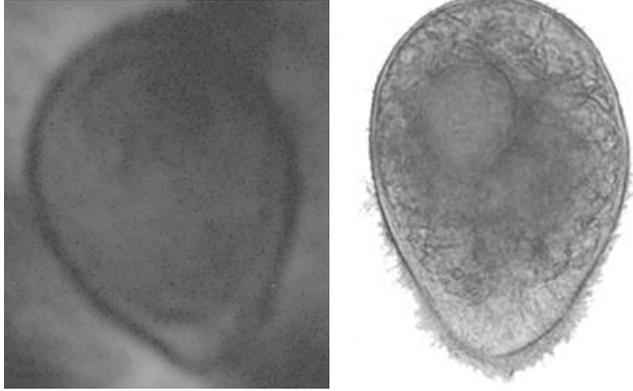
Order: Vestibuliferida

Family: Balantididae

Genera: *Balantidium*

Identification

A large ciliated intestinal protozoan parasite with upwards of 50 species described, *Balantidium coli* is the only known ciliated protozoan species to be pathogenic to humans (Schuster and Ramirez-Avila, 2008). The trophozoite varies from 25 to 120 µm in width, and 30 to 150 µm in length; while the cyst is spherical and measures 40-60 µm in diameter (Schuster and Ramirez-Avila, 2008).



Trophozoite measuring 35 μm in length. Recovered from fecal smear (Left). (Right: CDC, 2014).

Life cycle

Cysts are the infectious stage responsible for transmission and are capable of surviving in the environment. Most commonly the cyst is ingested via the fecal-oral route through contaminated food and water, though direct transmission is possible. Once ingested, excystation occurs in the small intestine and trophozoites are released and colonize in the small intestine. Replication occurs via binary fission. Trophozoites encyst and are infective. Multiplication may occur in the cell wall or mature cysts are released in stool.

Geographical distribution

Found throughout the world.

Zoonotic potential

The only ciliated protozoan to infect humans. Also found in domestic and wild hogs, rats, and other mammals, including numerous tropical primates (Garcia, 1999; Nakauchi, 1999).

Considered an emerging pathogen (Schuster and Ramirez-Avila, 2008).

Disease

Mostly asymptomatic, though cases of dysentery may develop. Diarrhea and dysentery are rare, causing ulcers in the large intestine (Nakauchi, 1999).

Blastocystis spp.

Classification

Phylum: Heterokontophyta

Class: Blastocystae

Order: Blastocystida

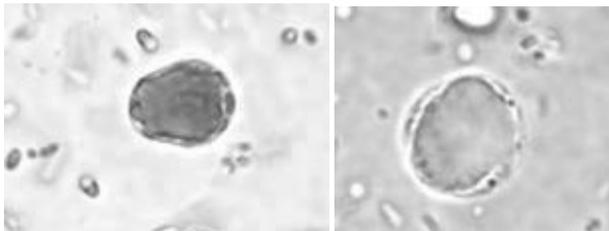
Family: Blastocystidae

Genera: *Blastocystis*

Note: Now placed among the group stramenopiles (Arisue et al., 2002).

Identification

Morphological identification is primarily dependent on determination of cysts which vary in size from 6 to 40 μm . Four commonly forms are normally found: 1) a vacuolar form (2 μm and 200 μm diameter); 2) a granular form (similar in appearance to vacuolar form); 3) an amoeba form that is non-motile; and 4) cyst form (smaller and thick multi-layered wall). The cyst form is the most resistant and able to persist in the environment. For the purposes of this study, I relied on PCR-based amplification of the small subunit gene (18S) (Chapter 4). Nine subtypes are found in primates (including humans) based on ribosomal analysis (Coyle, 2011).



Cyst dyed with iodine. Unstained cyst (right: CDC, 2014).

Biology

The life cycle for *Blastocystis* spp. is still under investigation (CDC, 2014). Consumed cysts found in stool are likely responsible for external transmission via contaminated food or water.

Cysts infect epithelial cells of digestive tract and multiple asexually (CDC, 2014). Vacuolar forms of the parasite rise to multi-vacuolar walled cysts. This particular form is likely responsible for autoinfection. The vacuolar form also develops into an amoeboid form and then into a thick-walled cyst by schizogony which is passed via stool (CDC, 2014).

Geographical distribution

Found throughout the world. Often one of the most common infections found fecal samples from the U.S. (Coyle, 2011).

Common throughout the world.

Zoonotic potential

Various subtypes found in humans are also found in primates, other mammals and birds.

Disease

There is some controversy as to the effect *Blastocystis* has on its host. Disease may be associated with any number of factors, including age, intensity of infection, parasite subtype, host genotype, and health of host (Boorum et al., 2008; Coyle, 2011). Symptoms include chronic diarrhea, nausea, weight loss, and abdominal pain (CDC, 2014).

Chilomastix sp.

Classification

Phylum: Retortamonada

Class: Retortamonadea

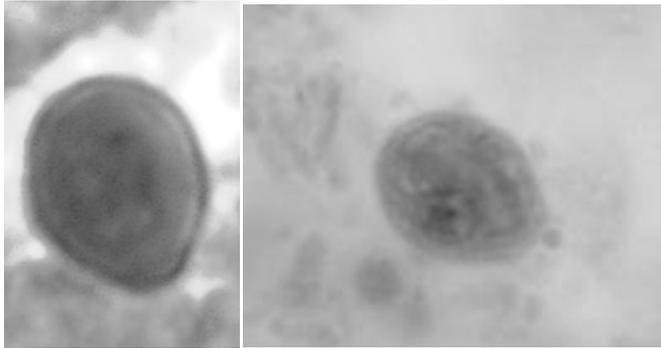
Order: Retortamonadida

Family: Tetramitidae

Genera: *Chilomastix*

Identification

Both cysts and trophozoites can be found in feces and are used for diagnosis. Cysts are lemon-shaped and measure 6-10 μm in length. They contain a large single nucleus. *Chilomastix mesnili* is found in humans and non-human primates



Cysts of *C. mesnili* from stool specimens, stained with trichrome (Right: CDC, 2014).

Biology

The cyst stage is environmentally resistant and responsible for transmission (CDC, 2014). Ingestion of cysts occurs via contaminated water, food, or because of poor hygiene. Cysts migrate to the large intestine, excystation releases trophozoites.

Geographical distribution

Chilomastix mesnili is cosmopolitan in distribution although found more frequently in warm climates. This is the largest flagellate found in man with an incidence of 1-10% being in the large intestine.

Zoonotic potential

Animals serve as a reservoir. Previously described in primates, including New World monkeys (Stuart et al., 1998; Gillespie et al., 2005).

Disease

It is thought to be non-pathogenic and commensal, although the trophozoite has been associated with diarrhea (Kulda and Nohynkova, 1978).

Dientamoeba sp.

Classification

Phylum: Metamonada

Class: Parabasalia

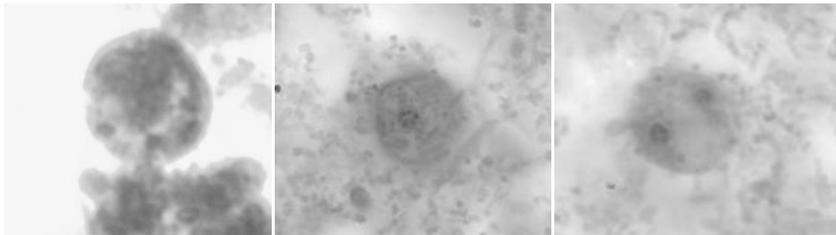
Order: Trichomonadida

Family: Monocercomonadidae

Genera: *Dientamoeba*

Identification

Dientamoeba fragilis, a flagellate protozoan, has no known cyst stage, and its trophozoites measure 5 to 15 μm . While most trophozoites are typically binucleate, some have only one nucleus.



Uninnucleate and binucleate trophozoites of *D. fragilis* stained with trichrome (Middle and Right: CDC, 2014).

Biology

The life cycle is still disputed. No cysts have been found in feces, and only trophozoites.

Transmission is likely via the fecal-oral route, possibly gaining passage on helminth eggs such as *Ascaris* sp. or *Enterobius* spp. Degrades quickly in the environment.

Geographical distribution

Worldwide distribution.

Zoonotic potential

Little support for any other natural host aside from humans (Johnson et al., 2004); however, in a more recent study testing for *D. fragilis* across a broad spectrum of animals, only lowland gorillas harbored trophozoites (Stark et al., 2008). Zoonotic disease is unlikely and transmission might be highly host specific.

Disease

Intestinal symptoms may be asymptomatic or include diarrhea and abdominal pain, nausea, anorexia, fatigue, malaise and weight loss (Peek et al., 2004; CDC, 2014). The possibility exists that distinct genetic forms are associated with different pathogenicities (Johnson et al., 2004; Peek et al., 2004). More likely to be found in individuals younger than 20 years and females (Yang and Scholten, 1977).

Entamoeba spp.

Classification

Phylum: Amoebozoa

Class: Archamoebae

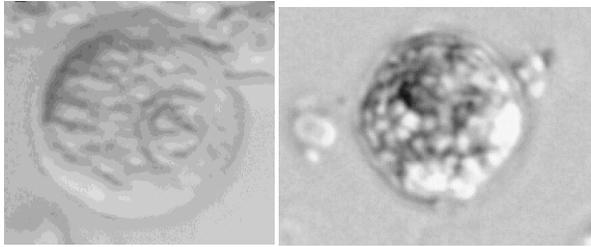
Order: Amoebida

Family: Endamoebidae

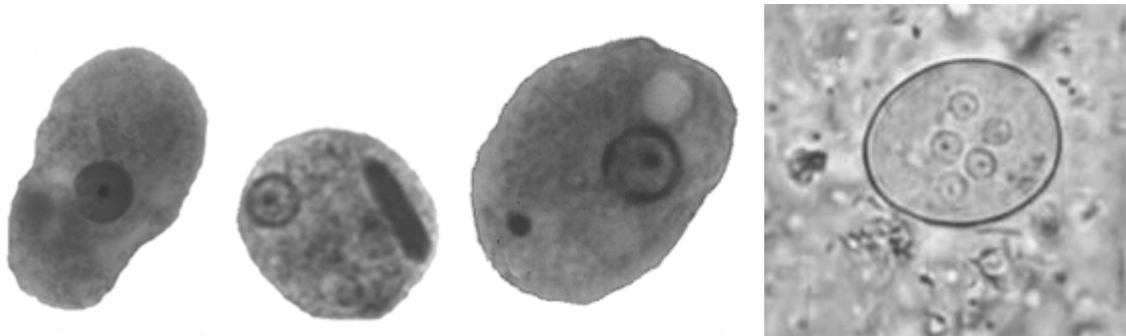
Genera: *Entamoeba*

Identification

Several species of *Entamoeba* are found in people. Almost all species form cysts which are involved in transmission (CDC, 2014). Depending on the species, these can have one, four or eight nuclei and are variable in size. For example, *E. histolytica* cysts vary from 10-20 μm , while trophozoites (feeding-dividing form) is approximately 15-60 μm in diameter and feeds primarily on bacteria.



Entamoeba spp. cysts. 15µm.



Entamoeba histolytica/E. dispar trophozoite, *E. histolytica/E. dispar* cysts, and *E. coli* trophozoites (CDC, 2014).

Trophozoites of *E. coli* usually measure 15 to 50 µm. *E. coli* cysts are usually spherical, but may be elongated, and measure 10 to 35 µm. Mature cysts typically have 8 nuclei but may have as many as 16 or more. *E. coli* is the only species in the genus encountered in humans with more than four nuclei in the cyst stage.

Biology

Cysts and trophozoites are passed primarily in feces. Cysts are often associated with normally-formed stool while trophozoites are found in loose stool (CDC, 2014). Infection occurs through ingestion of mature cysts found in contaminated food, water, and via the fecal-oral route.

Excystation occurs in the small intestine. Trophozoites are released, migrating to the large intestine, and undergo binary fission. Both cysts and trophozoites are then passed in feces. Only cysts can withstand environment degradation and are responsible for transmission.

Geographical distribution

E. histolytica is found in people and non-human primates throughout the world. Nearly 50 million people worldwide are expected to be infected. Ten percent of people are likely infected with *E. dispar* (CDC, 2014).

Zoonotic distribution

Predominantly infecting humans and other non-human primates.

Disease

Pathogenicity dependent upon parasite species. In the case of *Entamoeba histolytica*, the parasite can be either symptomatic or asymptomatic. *E. dispar* and *E. coli* are generally considered non-pathogenic or commensals of their host.

Iodamoeba sp.

Classification

Phylum: Amoebozoa

Class: Archamoebae

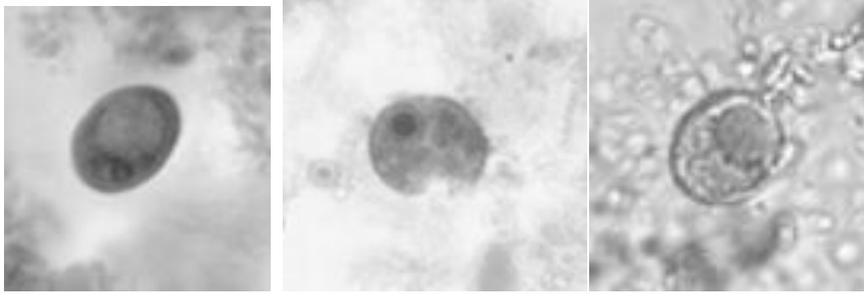
Order: Amoebida

Family: Entamoebidae

Genera: *Iodamoeba*

Identification

Iodamoeba buetschlii trophozoites are 9-14 μm in diameter with a single nucleus. Cysts average 10 μm in diameter and irregularly shaped.



Iodamoeba buetschlii cyst using trichrome stain (cyst). *Iodamoeba buetschlii* (cyst with iodine). *Iodamoeba buetschlii* (cyst) (Middle and Left: CDC, 2014).

Life cycle

Non-pathogenic amoebic cysts colonize the small intestine after ingestion of contaminated food, water, or via fecal-oral route (CDC, 2014). Excystation occurs in the small intestine, where trophozoites are released and migrate to the large intestine. Trophozoites multiply by binary fission and produce cysts, which are both passed in stool. Cysts can survive for weeks in the environment, while trophozoites rapidly decay.

Geographical distribution

Worldwide distribution.

Zoonotic potential

Obligate parasite found in the large intestines in people, pigs, and other mammals including nonhuman primates.

Disease

Non-pathogenic intestinal protozoan.

Enterobius sp.

Classification

Phylum: Nematoda

Class: Secernentea

Order: Oxyurida

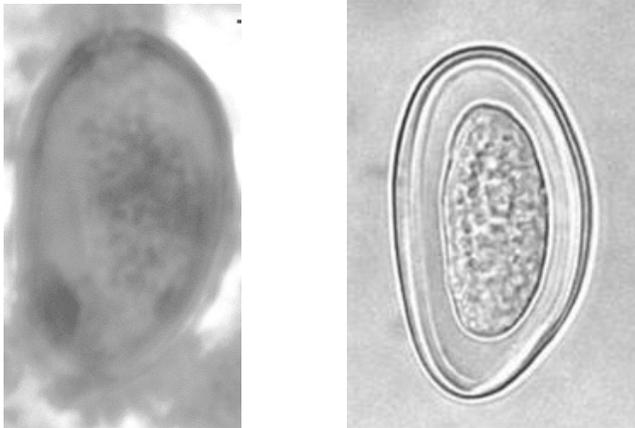
Family: Oxyuridae

Genera: *Enterobius*

Identification

A nematode, *Enterobius vermicularis* eggs average 50-60 μm in length and are 20-30 μm wide.

The eggs are oval shaped, with one side flattened.



Enterobius vermicularis egg (45 μm) (Right: CDC, 2014).

Biology

Infection begins with ingestion of pinworm eggs. Eggs take 1-2 months to mature in the small intestine where mature females hatch and migrate to the colon and lay eggs around the anus (CDC, 2014). Larvae develop inside the egg and become infective within 4-6 hours. Pinworm eggs can survive in the environment for 2-3 weeks or autoinfect the host. Evidence of pinworm infection by breathing in eggs.

Geographical distribution

Worldwide distribution.

Zoonotic potential

E. vermicularis is primarily described as a parasite specific to humans (CDC, 2014). However, *E. vermicularis* has been described in other primates – mostly captive (Brooks and Glen, 1982). In the wild, other *Enterobius* spp. are found in primates, including *E. bipapillatus* (Old world monkeys), *E. buckleyi* and *E. lerouxi* (great apes). The pinworm *Enterobius* spp. is primarily described in the Catarrhini (Old world monkeys), while *Trypanoxyuris* spp. is found in Platyrrhini (New world monkeys) (Hugot, 1999). However, Holsback et al. (2013) describes *Enterobius* sp. in red howler monkeys (*Alouatta seniculus*).

Disease

The human pinworm is most likely to infect children under 18 and is generally asymptomatic aside from itching around the anus (CDC, 2014). *Enterobius vermicularis* is the most common nematode infection in the US.

Capillaria sp.

Classification

Phylum: Nematoda

Class: Adenophorea

Order: Trichurida

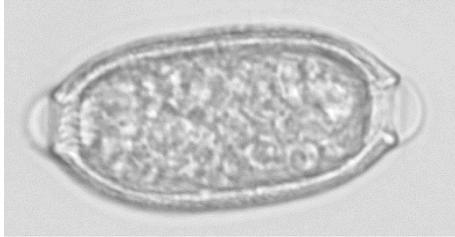
Family: Capillariidae

Genera: *Capillaria*

Identification

Hundreds of species encompass the genus *Capillaria*. Three main species infect humans, *Capillaria hepatica*, *Capillaria philippinensis*, and *Capillaria aerophilia* (McCarthy and Moore,

2000). However, *C. philippinensis* is unlikely to be found in South America and will not be discussed further. *C. hepatica* and *Capillaria brochieri* have also been reported in primates (Brack et al., 1994; Graczyk et al., 1999); however, *C. aerophila* has not been described in non-human primates. *C. hepatica* eggs range from 50 μm (Brack et al., 1994) to $54.3 \pm 0.5 \mu\text{m}$ (Graczyk et al., 1999). *C. brochieri* eggs in chimpanzees were documented from 45-55 μm .



Capillaria sp. egg (45 μm).

Biology

C. hepatica eggs are not normally passed in the feces but rather colonizes in the liver and remain until the death of the host (CDC, 2014). Upon ingestion of the host by scavengers or predators, the eggs are passed on. These eggs are unembryonated and do not infect the host. Instead, eggs passed in feces embryonate in the environment under damp soil conditions until they are re-ingested by a mammalian host (CDC, 2014). Infective eggs release larvae in the intestines and migrate to the liver where they develop for roughly four weeks until maturity. Humans are infected usually as a result of fecal-contaminated food, water, or soil.

C. aerophila is a respiratory nematode that infects its host once larvated eggs or earthworms (a facultative intermediate host) are ingested (Traversa et al., 2011). Adult nematodes live under the epithelium of the trachea, bronchi, and bronchioles where females produce non-larvated eggs which are coughed up, swallowed, and finally defecated into the environment (Traversa et al., 2011). Once ingested by a new host, the embryonated eggs hatch

and the larvae penetrate the intestinal wall. From there they migrate via the blood stream to the lungs, reaching maturity after three to six weeks.

Geographical distribution

Representatives of *Capillaria* are distributed throughout the world. In Ecuador, *Capillaria* spp. have only been reported to genus.

Zoonotic potential

Specific to *Alouatta* species, *Capillaria* have only been found in *A. caray*, but not *A. palliata* (Godoy et al., 2004).

Disease

C. hepatica can cause hepatitis, while *C. philippinensis* is ingested via infected small freshwater fish – resulting in diarrhea and emaciation (CDC, 2014). *C. aerophila* can cause pulmonary capillariasis.

Strongyloides sp.

Classification

Phylum: Nematoda

Class: Secernentea

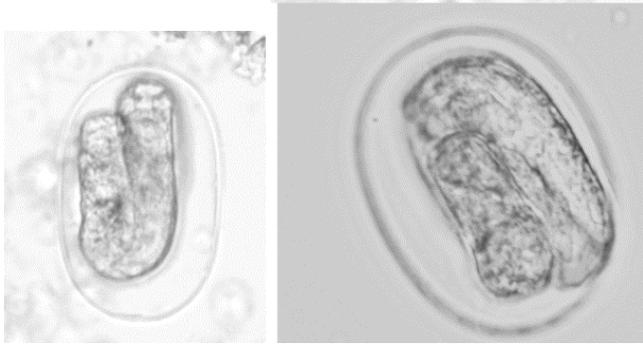
Order: Rhabditida

Family: Strongyloididae

Genera: *Strongyloides*

Identification

Identification is primarily confirmed using larvae. Few larvae were recovered in this study, and so identification was based of prolific egg recovery. Eggs are oval and thin shelled, 50-58µm long by 30-34µm wide (Garcia, 1999).



Strongyloides sp. Two morphotypes averaging 36 µm and at 52.8 µm.

Biology

Infection begins by skin penetration of the filariform larvae from soil (Garcia, 1999). Larvae are then carried via cutaneous blood vessels to the lungs. From there they migrate out of pulmonary capillaries into alveoli and eventually into trachea and pharynx where they are swallowed and end in the small intestine (Garcia, 1999; CDC, 2014). Continued development occurs for about 2 weeks until the females are adults. Parthenogenesis occurs, producing eggs which hatch into rhabditiform larvae and then are passed in feces. In warmer climates environment, eggs mature into free-living male and female worms. Females produce eggs which mature into rhabditiform larvae, and then develop into the infective filariform. In temperate climates, the free-living males and females do not develop. Instead, rhabditiform larvae passed in the stool develop into filariform larvae, which begin the cycle again by entering the skin of the host (CDC, 2014).

Geographical distribution

Both *Strongyloides stercoralis* and *Strongyloides fuelleborni* are found throughout the tropics.

Zoonotic potential

Strongyloides stercoralis is most commonly found in humans. Less common, *Strongyloides fuelleborni* also may produce infections in humans, and also nonhuman primates (CDC, 2014).

Disease

Initially asymptomatic; however, dermatitis, wheezing and coughing, and intestinal tissue damage resulting in edema are possible. Hyperinfective syndrome also possible in immunocompromised individuals.

Trypanoxyuris sp.

Classification

Phylum: Nematoda

Class: Chromadorea

Order: Oxyurida

Family: Oxyuridae

Genera: *Trypanoxyuris*

Identification

Eighteen species of *Trypanoxyuris* have been described (Pinto et al., 2013). *Trypanoxyuris lagothricis* eggs found in *Lagothrix cana* averaged 40 µm in length by 21 µm in width (Pinto et al., 2013).



Trypanoxyuris sp. egg (40 µm) found in fecal sample of *A. palliata*.

Biology

Similar life cycle patterns to *Enterobius* spp. though a detailed understanding is not available.

Infection begins with ingestion of pinworm eggs. Eggs develop and migrate to the small intestine

where mature females hatch and migrate to the colon and lay eggs around the anus. Larvae develop inside the egg and become infective within 4-6 hours. Pinworm eggs can survive in the environment for 2-3 weeks or autoinfect the host.

Geographical distribution

Primarily distributed among New world monkeys in South America.

Zoonotic potential

These parasites appear to be highly host specific (Stuart et al., 1998), so there is little chance of transmission between people and monkeys.

Disease

Trypanoxyuris minutus has been implicated in the death of southern brown howler monkeys, *Alouatta guariba* (Amato et al., 2002; Souza et al., 2010). The presence *T. minutus* and other *Trypanoxyuris* species have been described in howler monkeys species and New world monkeys (Stuart et al., 1998; Felt and White, 2005; Vitazkova and Wade, 2006; Valdespino et al., 2010; Pinto et al., 2013; Maldonado-Lopez et al., 2014).

Controrchis sp.

Classification

Phylum: Platyhelminthes

Class: Trematoda

Order: Plagiochiida

Family: Dicrocoeliidae

Genera: *Controrchis*

Identification

Trematode eggs measure 20-51 μm in length and 13-31 μm in width (Cristobal-Azkarate, 2010).

A mean size of 41 x 21 μm was reported by Vitazkova and Wade (2006).

Biology

This trematode likely requires the two intermediate hosts, gastropod and an ant species (Kowalzik et al., 2010). Field evidence from Belize suggests that *Azteca* spp. (ants) found living in trumpet trees (*Cecropia peltata*) harbor metacercariae. Howler monkeys presumably eat the ants – and the parasite – incidentally.

Geographical distribution

Neotropical.

Zoonotic potential

This parasite species is primarily described in howler monkeys, though eggs have been recovered from other neotropical primates (Vitazkova and Wade, 2006; Trejo-Macias et al., 2007; Kowalzik et al., 2010).

Disease

Related trematodes are known to cause morbidity and mortality in other mammals, including humans (Kowalzik et al., 2010).

LITERATURE CITED

Adam, R. D., Y. R. Ortega, R. H. Gilman, and C. R. Sterling. 2000. Intervening transcribed spacer region 1 variability in *Cyclospora cayetanensis*. *Journal of Clinical Microbiology* 38: 2339-2343.

Amato, J. F., S. B. Amato, C. Calegario-Marques, and J. C. Bicca-Marques. 2002. *Trypanoxyuris minutus* associated with the death of a wild southern brown howler monkey, *Alouatta guariba clamitans*. Rio Grande do Sul, Brazil. *Arquivos de Instituto Biológico* 69: 99-102.

Arisue, N., T. Hashimoto, H. Yoshiwaka, Y. Nakamura, G. Nakamura, F. Nakamura, T.- A. Yano, and M. Hawegawa. 2002. Phylogenetic position of *Blastocystis hominis* and of stramenopiles inferred from multiple molecular sequence data. *Journal of Eukaryotic Microbiology* 49: 42-53.

Barta, J. R., M. D. Schrenzel, R. Carreno, and B. A. Rideout. 2005. The genus *Atoxoplasma* (Garnham 1950) as a junior objective synonym of the genus *Isospora* (Schneider 1881) species

- infecting birds and resurrection of *Cystoisospora* (Frenkel 1977) as the correct genus for *Isoospora* species infecting mammals. *Journal of Parasitology* 91: 726-727.
- Boorom, K. F., H. Smith, L. Nimri, E. Viscogliosi, G. Spanakos, U. Parkar, L.-H. Li., X.-N. Zhou, U. Z. Ok, S. Leelayoova, and M.S. Jones. 2008. Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. *Parasites and Vectors* 1: 1-16.
- Brooks, D. R., and D. R. Glen. 1982. Pinworms and primates: a case study in coevolution. *Proceedings of the Helminthological Society of Washington* 49: 76-85.
- CDC, Center for Disease Control and Prevention. 2014. DPDx: Laboratory identification of parasitic diseases of public health concern. <<http://www.cdc.gov/dpdx/balantidiasis/index.html>>. Accessed 12 August 2014.
- Chacín-Bonilla, L. 2010. Epidemiology of *Cyclospora cayetanensis*: A review focusing in endemic areas. *Acta tropica* 115: 181-193.
- Chapman, C. A., D. D. Bowman, R. R. Ghai, J. F. Gogarten, T. L. Goldberg, J. M. Rothman, D. Twinomugisha, and C. Walsh. 2012. Protozoan parasites in group-living primates: testing the biological island hypothesis. *American Journal of Primatology* 74: 510-517.
- Chu, D. M., J. B. Sherchand, J. H. Cross, and P. A. Orlandi. 2004. Detection of *Cyclospora cayetanensis* in animal fecal isolates from Nepal using an FTA filter-base polymerase chain reaction method. *The American Journal of Tropical Medicine and Hygiene* 71: 373-379.
- Coyle, C. M., J. Varughese, L. M. Weiss, and H. B. Tanowitz. 2011. *Blastocystis*: To treat or not to treat... *Clinical Infectious Diseases* 1-6.
- Cristóbal-Azkarate, J., B. Hervier, S. Vegas-Carrillo, D. Osorio-Sarabia, E. Rodríguez-Luna, and J. J. Veà. 2010. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. *Primates* 51: 231-239.
- Eberhard, M. L., M. N. Njenga, A. J. DaSilva, D. Owino, E. K. Nace, K. Y. Won, and J. M. Mwenda. 2001. A survey for *Cyclospora* spp. in Kenyan primates, with some notes on its biology. *Journal of Parasitology* 87: 1394-1397.
- Felt, S. A., and C. E. White. 2005. Evaluation of a timed and repeated perianal tape test for the detection of pinworms (*Trypanoxyuris microon*) in owl monkeys (*Aotus nancymae*). *Journal of Medical Primatology* 34: 209-214.
- Frenkel, J. K. 1977. *Besnoitia wallacei* of cats and rodents: With a reclassification of other cyst-forming isosporoid coccidia. *Journal of Parasitology* 63: 611-628.
- Gillespie, T. R., C. A. Chapman, and E. C. Greiner. 2005. Effects of logging on gastrointestinal parasite infections and infection risk in African primates. *Journal of Applied Ecology* 42: 699-707.

- Hendricks, L. D. 1977. Host range characteristics of the primate coccidian, *Isospora arctopitheci* Rodhain 1933 (Protozoa: Eimeriidae). *The Journal of Parasitology* 63: 32-35.
- Holsback, L., M. J. Cardoso, R. Fagnani, and T. H. Patelli. 2013. Natural infection by endoparasites among free-living wild animals. *Revista Brasileira de Parasitologia Veterinária* 22: 302-306.
- Hugot, J. P. 1999. Primates and their pinworm parasites: the Cameron hypothesis revisited. *Systematic Biology* 48: 523-546.
- Johnson, E. H., J. J. Windsor, and C. G. Clark, 2004. Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clinical Microbiology Reviews* 17: 553-570.
- Kulda, J., and E. Nohynkova. 1978. Flagellates of the human intestine and of intestines of other species. *Parasitic Protozoa* 2: 1-138.
- Lindsay, D. S., J. P. Dubey, and B. L. Blagburn. 1997. Biology of *Isospora* spp. from humans, nonhuman primates, and domestic animals. *Clinical Microbiology Reviews* 10: 19-34.
- Maldonado-López, S., Y. Maldonado-López, A. G. T. Ch, P. Cuevas-Reyes, and K. E. Stoner. 2014. Patterns of infection by intestinal parasites in sympatric howler monkey (*Alouatta palliata*) and spider monkey (*Ateles geoffroyi*) populations in a tropical dry forest in Costa Rica. *Primates* 1-10.
- McCarthy, J., and T. A. Moore. 2000. Emerging helminth zoonoses. *International Journal for Parasitology* 30: 1351-1359.
- Nakauchi, K. 1999. The prevalence of *Balantidium coli* infection in fifty-six mammalian species. *The Journal of Veterinary Medical Science and the Japanese Society of Veterinary Science* 61: 63-65.
- Olivier, C., S. Van de Pas, P. W. Lepp, K. Yoder, and D. A. Relman. 2001. Sequence variability in the first internal transcribed spacer region within and among *Cyclospora* species is consistent with polyparasitism. *International Journal for Parasitology* 31: 1475-1487.
- Peek, R., F. R. Reedeker, and T. van Gool. 2004. Direct amplification and genotyping of *Dientamoeba fragilis* from human stool specimens. *Journal of Clinical Microbiology* 42: 631-635.
- Pinto, H. A., F. C. Ferreira Junior, V. L. T. Mati, and A. L. D. Melo. 2013. *Trypanoxyuris (Paraoxyuronema) lagothricis* (Nematoda: Oxyuridae) in *Lagothrix cana* (Primates: Atelidae) from Brazil. *Revista Brasileira de Parasitologia Veterinária* 22: 307-311.
- Schuster, F. L., and L. Ramirez-Avila. 2008. Current world status of *Balantidium coli*. *Clinical Microbiology Reviews* 21: 626-638.

- Soave, R., and C.S. Weikel. 1990. *Cryptosporidium* and other protozoa including *Isospora*, *Sarcosystis*, *Balantidium coli*, and *Blastocystis*. Principles and Practice of Infectious Diseases 2122-2130.
- Souza, D. D., C. M. Magalhães, F. M. Vieira, and S. D. Souzalima. 2010. Occurrence of *Trypanoxyuris* (*Trypanoxyuris*) *minutus* (Schneider, 1866) (Nematoda, Oxyuridae) in *Alouatta guariba clamitans* Cabrera, 1940 (Primates, Atelidae) in Minas Gerais, Brazil. Revista Brasileira de Parasitologia Veterinária 19: 124-126.
- Stark, D., O. Phillips, D. Peckett, U. Munro, D. Marriott, J. Harkness, and J. Ellis. 2008. Gorillas are a host for *Dientamoeba fragilis*: An update on the life cycle and host distribution. Veterinary Parasitology 151: 21-26.
- Strausbaugh, L. J., and B. L. Herwaldt. 2000. *Cyclospora cayetanensis*: a review, focusing on the outbreaks of cyclosporiasis in the 1990s. Clinical Infectious Diseases 31: 1040-1057.
- Stuart, M., V. Pendergast, S. Rumfelt, S. Pierberg, L. Greenspan, K. Glander, and M. Clarke. 1998. Parasites of wild howlers (*Alouatta* spp.). International Journal of Primatology 19: 493-512.
- Traversa, D., A. Di Cesare, R. P. Lia, G. Castagna, S. Meloni, J. Heine, K. Strube, P. Milillo, D. Otranto, O. Meckes, and R. Schaper. 2011. New insights into morphological and biological features of *Capillaria aerophila* (Trichocephalida, Trichuridae). Parasitology Research 109: 97-104.
- Trejo-Macías, G., A. Estrada, and M. A. Cabrera. 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in southern Mexico. International Journal of Primatology 28: 931-945.
- Valdespino, C., G. Rico-Hernández, and S. Mandujano. 2010. Gastrointestinal parasites of howler monkeys (*Alouatta palliata*) inhabiting the fragmented landscape of the Santa Marta mountain range, Veracruz, Mexico. American Journal of Primatology 72: 539-548.
- Wobeser, G. A. 2013. Essentials of Disease in Wild Animals. John Wiley & Sons. Oxford, UK.
- Yang, J., and T. H. Scholten. 1977. *Dientamoeba fragilis*: a review with notes on its epidemiology, pathogenicity, mode of transmission, and diagnosis. The American Journal of Tropical Medicine and Hygiene 26: 16-22.
- Zhao, G. H., M. M. Cong, Q. Q. Bian, W. Y. Cheng, R. J. Wang, M. Qi, L. X. Zhang, Z. Lin, X. Q. Zhu. 2013. Molecular Characterization of Cyclospora-like Organisms from Golden Snub-nosed Monkeys in Qinling Mountain in Shaanxi Province, Northwestern China. PloS One 8: 1-5.

APPENDIX 2: LABORATORY PROTOCOLS

This appendix contains information on three fecal extraction methods: smear, flotation, and sedimentation.

Fecal smear

Fecal smears are ideal for helminth eggs, protozoan oocysts and cysts. Samples should be preserved in formalin or polyvinyl alcohol (PVA). The following protocol was modified and adapted from Gillespie (2006), and Hendrix and Robinson (2006).

Protocol

Pour some of the well-mixed PVA stool mixture onto a paper towel, and allow it to stand for 3 min to absorb out the PVA. Do not eliminate this step. With tongue depressor, apply some of the stool material from the paper towel to two places on a slide and allow to dry for several hours in a 37 degree incubator. Note, the mixture should be spread to the edges of the slide. The dry slides is then placed into an ethanol-iodine-solution (1-2 g iodine crystals/100 ml 70% ethanol) for approximately 5 min. Place the slides in 70% ethanol for 5 min. Place the slides into Trichrome stain for 10 min. Quickly dunk slides into 90% ethanol plus acetic acid. Dip the slides 3 times in 100% ethanol. Place the slides in two changes of 100% ethanol for 3 min each. Place the slides in xylene for 10 min. Place the slides in a second container of xylene for 10 min. Place cover slip on top using mounting medium Permount (or other mounting medium like Canada balsam). Allow the slide to dry overnight or 1 hour at 37 degrees.

Fecal flotation protocol

Weigh 1g of fecal sample on a small, clean, disposable weighing boat. Add the sample to a 15mL falcon/centrifuge tube. Fill the tube 2/3 with dH₂O (Add 10mL dH₂O) and screw cap onto the tube. Homogenize sample with dH₂O by gently inverting the tube until large particles are broken apart. Centrifuge sample at 400g (1800rpm) for 10 minutes. Pour off supernatant into a

clean, labeled beaker. Re-suspend fecal material in NaNO₃ solution (9.5g NaNO₃:30mL dH₂O) by adding 5mL NaNO₃ solution, cap tube, and gently invert tube once. Remove cap and place tube back in the centrifuge. Fill tube to just below rim with NaNO₃ solution (this should be done while tube is already in the centrifuge). Centrifuge sample at 1500rpm/400g for 10 minutes. Remove tube and place in holder. Fill to meniscus using glass pipette. Gently place a clean cover slip straight down over the lip of the tube, make sure it is centered. Leave for 10 minutes. Remove the cover slip from tube by lifting directly up without sliding/smearing. Place the cover slip on a clean, labeled slide. Examine slide under microscope. Record number of each organism, photograph representatives, and record flotation image number. Continue with the fecal sedimentation with same sample tube

Fecal sedimentation protocol

Good for the isolation and identification of trematodes and other heavy parasites. Pour off most of the supernatant. Using a clean Pasteur pipette, remove remaining supernatant. Transfer fecal pellet to a 50mL falcon containing 40mL sedimentation solution (small drop of dial:dH₂O). May need to use a pipette to transfer some solution back to the 15mL centrifuge tube to move material to 50mL falcon, or tap pellet out. Replace cap and gently invert 5 times or until material is loose/suspended. Using a glass funnel evenly lined with 2 layers of cheesecloth (or one piece folded in half), filter suspension into a clean, dry 100mL beaker. Using the same funnel with cheesecloth, filter suspension into a clean, dry, labeled 50mL falcon and screw on cap. Using a 5mL serological pipette and bulb, rinse cheesecloth in funnel with 5mL dH₂O into the falcon with suspension. Dispose of the cheesecloth with remaining pellet in waste bag to be autoclaved. Centrifuge suspension at 1500rpm/400g for 5 minutes. Gently pour off the supernatant without disturbing the sediment at the bottom. Using a clean Pasteur pipette, transfer 2 drops of the top

layer of sediment onto a clean, labeled slide. Apply cover slip and examine slide under microscope.

LITERATURE CITED

Gillespie, T. R. 2006. Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. *International Journal of Primatology* 27: 1129-1143.

Hendrix, C. M., and E. Robinson. 2006. *Diagnostic Parasitology for Veterinary Technicians*, 3rd ed. Mosby Inc., St. Louis, Missouri, 416 p.

APPENDIX 3: GLOSSARY

abundance: number of parasite individuals in a single host.

autoinfection: re-infection by a parasite without ever leaving a host. The entire parasite life cycle happens in a single organism.

definitive host: the host in which a parasite reaches sexual maturity.

direct transmission: simplest form of transmission in which parasite exchange is a direct result of skin-to-skin contact or in excretions or secretions (Wobeser, 2006).

excystation: the breakdown of the cyst once swallowed by the host. The act of excystation allows the parasite to easily disperse throughout the host when favorable conditions are present.

heteroxenous: refers to parasites that use either multiple sequential host species (complex life cycles) or concurrent host species.

intermediate host: in the case of indirectly transmitted parasite, the host in which sexual replication does not occur (Wobeser, 2006).

intensity: number of parasites specific to one species in a single host.

mean abundance: average number of individuals of a parasite species found in each host.

monoxenous: a parasite restricted to a single host during a parasite's life cycle.

oioxenous: a parasite that is highly host specific.

oocysts: encysted form of zygote that is shed from the host.

paratenic host: a host in which a parasite does not develop; rather, may help enhance transmission or

pathogenicity: capability of a parasite to cause disease.

prevalence: proportion of individuals infected with a particular parasite species at any given time.

schizogony: form of asexual reproduction with multiple rounds of mitosis

sporogony: multiple divisions of a zygote which produce sporozoites.

sporozoites: motile, infective parasite stage resulting from sporogony.

trophozoites: the active, motile feeding stage of a protozoan.

virulence: degree of pathogenicity of a parasitic organism.

zoonosis: disease in animals that is capable of transmitting to people.

APPENDIX 4: HOWLER MONKEY RAW DATA

Parasite presence/absence data from sampled ninety-six mantled howler monkeys, *Alouatta palliata aequatorialis*. Calculated distances are from first fecal sample collected in a group to nearest road, human settlement, agricultural plot, or the Bilsa research station. Basal area and percentage of trees greater than 40cm are based on a 10m circle plot.

Sample	Dist. to Road (m)	Dist. to Humans (m)	Distance to Agriculture Plot (m)	Distance from Bilsa (m)	Basal Area (m ² /ha)	Trees with DBH >40cm
AP2A	453	1597	1356	988	5	0.25
AP2B	453	1597	1356	988	5	0.25
AP2C	453	1597	1356	988	5	0.25
AP2D	453	1597	1356	988	5	0.25
AP2E	453	1597	1356	988	5	0.25
AP3A	2052	3186	1851	2562	1	0.00
AP3B	2052	3186	1851	2562	1	0.00
AP3C	2052	3186	1851	2562	1	0.00
AP3D	2052	3186	1851	2562	1	0.00
AP4A	1526	2837	1968	3002	14	0.12
AP4B	1526	2837	1968	3002	14	0.12
AP4C	1526	2837	1968	2930	14	0.12
AP4D	1526	2837	1968	2930	14	0.12
AP5A	677	1901	1693	1449	11	0.08
AP5B	677	1901	1693	1449	11	0.08
AP5C	677	1901	1693	1449	11	0.08
AP5D	677	1901	1693	1449	11	0.08
AP5E	677	1901	1693	1449	11	0.08
AP5F	677	1901	1693	1449	11	0.08
AP5G	677	1901	1693	1449	11	0.08
AP6A	103	17	0	31	3	0.06
AP6B	103	17	0	31	3	0.06
AP7A	207	988	750	966	4	0.04
AP8A	1424	2692	1967	2764	-	-
AP8B	1424	2692	1967	2764	-	-
AP8C	1424	2692	1967	2764	-	-
AP8D	1424	2692	1967	2764	-	-
AP9A	2079	3353	1439	3103	10	0.14
AP9B	2079	3353	1439	3103	10	0.14
AP10A	691	682	578	155	15	0.07
AP10B	708	683	559	131	15	0.07

AP10C	556	793	668	434	15	0.07
AP10D	718	663	582	175	15	0.07
AP10E	668	620	487	188	15	0.07
AP10F	693	637	555	167	15	0.07
AP11A	884	733	658	540	8	0.05
AP11B	873	749	644	534	8	0.05
AP11C	884	749	658	534	8	0.05
AP11D	884	749	658	534	8	0.05
AP11E	884	749	658	534	8	0.05
AP12A	2560	3801	910	3698	8	0.10
AP12B	2557	3798	696	3698	8	0.10
AP12C	2542	3811	734	3700	8	0.10
AP13A	87	57	0	98	4	0.00
AP13B	96	49	0	93	4	0.00
AP13C	115	45	0	98	4	0.00
AP13D	115	45	0	98	4	0.00
AP13E	115	40	0	93	4	0.00
AP13F	115	40	0	93	4	0.00
AP13G	125	41	0	93	4	0.00
AP14A	252	128	113	225	6	0.11
AP14B	261	126	122	225	6	0.11
AP14C	263	126	125	207	6	0.11
AP14D	251	132	109	225	6	0.11
AP14E	259	126	119	225	6	0.11
AP14F	259	126	119	225	6	0.11
AP14G	253	127	112	225	6	0.11
AP14H	252	127	113	225	6	0.11
AP14I	252	127	113	225	6	0.11
AP14J	244	120	105	196	6	0.11
AP15A	116	192	69	346	6	0.11
AP16A	2490	3737	859	2271	11	0.07
AP16B	2490	3737	859	2271	11	0.07
AP16C	2490	3737	859	2271	11	0.07
AP16D	2490	3719	859	2240	11	0.07
AP17A	44	1091	931	1017	6	0.08
AP17B	44	1081	931	1007	6	0.08
AP17C	44	1081	931	988	6	0.08
AP17D	44	1081	931	1017	6	0.08
AP17E	44	1081	931	1017	6	0.08
AP17G	44	1081	931	1017	6	0.08

AP18A	0	1147	1031	180	5	0.03
AP18B	0	1147	1031	180	5	0.03
AP18C	0	1211	1031	285	5	0.03
AP19A	238	1415	1145	1124	12	0.14
AP19B	238	1405	1145	1124	12	0.14
AP19C	238	1411	1145	1124	12	0.14
AP19D	238	1390	1145	1089	12	0.14
AP19E	238	1388	1145	1089	12	0.14
AP19F	238	1389	1145	1089	12	0.14
AP19G	238	1399	1145	1120	12	0.14
AP20A	285	2305	1953	2609	33	0.39
AP20B	285	2298	1953	2612	33	0.39
AP20D	285	2298	1953	2643	33	0.39
AP20E	285	2316	1953	2643	33	0.39
AP20G	285	2307	1953	2616	33	0.39
AP21A	332	300	173	5546	17	0.20
AP21B	332	300	173	5546	17	0.20
AP22A	1236	1075	270	5546	47	0.72
AP23A	685	3229	487	2755	16	0.15
AP23B	685	3219	487	2754	16	0.15
AP23C	685	3225	487	2755	16	0.15
AP23E	685	3241	487	2756	16	0.15
AP23F	685	3239	487	2756	16	0.15
AP23G	685	3216	487	2724	16	0.15
AP23H	685	3225	487	2755	16	0.15
AP24D	-	-	-	-	-	-

**Blastocystis* sp. presence was assessed using partial sequence from the PCR-based amplification of 18S ribosomal subunit.

APPENDIX 4: HOWLER MONKEY RAW DATA (CONTINUED)

Sample	<i>Cyclospora</i> sp.	<i>Isospora</i> sp.	<i>Strongyloides</i> sp.	<i>Capillaria</i> sp.	<i>Enterobius</i> sp.	<i>Trypanoxyuris</i> sp.	<i>Controrchis</i> sp.	<i>Blastocystis</i> sp.*	<i>Dientamoeba</i> sp.	<i>Chilomastix</i> sp.	<i>Balantidium</i> sp.	<i>Entamoeba</i> sp.	<i>Iodamoeba</i> sp.
AP2A	1	0	1	0	0	0	0	0	0	0	0	1	0
AP2B	0	0	1	1	0	0	0	1	0	0	0	0	0
AP2C	1	0	1	1	0	0	0	1	1	0	0	1	0
AP2D	0	0	0	0	0	0	0	1	0	0	0	0	0
AP2E	1	0	1	1	0	0	1	1	0	0	0	1	0
AP3A	1	0	0	1	1	0	0	1	1	0	0	1	0
AP3B	0	0	1	1	0	0	0	1	0	0	0	0	0
AP3C	1	0	1	0	0	0	0	1	0	0	0	0	0
AP3D	1	0	1	1	0	0	0	0	0	0	0	1	0
AP4A	1	0	0	1	0	0	1	1	0	0	0	0	0
AP4B	1	0	1	1	0	0	0	0	0	0	0	0	0
AP4C	0	0	1	1	0	0	0	1	0	0	0	1	0
AP4D	0	0	1	1	0	0	0	1	0	0	0	0	0
AP5A	0	0	0	0	0	0	0	0	0	0	1	1	0
AP5B	0	1	1	1	0	0	0	1	0	0	1	0	0
AP5C	0	0	1	1	0	0	0	0	0	0	0	0	0
AP5D	0	0	0	1	0	0	0	1	0	0	0	0	0
AP5E	0	1	1	1	0	1	0	0	0	0	1	0	0
AP5F	0	0	1	0	0	0	0	0	0	0	0	1	0
AP5G	0	0	1	1	0	0	0	0	0	0	0	0	0
AP6A	1	0	1	1	0	0	0	0	0	1	0	0	0
AP6B	0	0	1	1	0	0	1	0	0	0	0	1	0
AP7A	0	0	1	1	0	0	0	0	0	0	0	0	0
AP8A	0	0	1	1	0	0	0	1	0	0	0	0	1
AP8B	0	0	1	0	0	0	0	1	0	0	1	1	0
AP8C	0	0	1	0	0	0	1	1	0	0	0	1	0
AP8D	0	0	1	1	0	0	0	0	0	0	0	0	0
AP9A	0	0	1	1	0	0	0	1	0	0	0	0	0
AP9B	0	0	1	1	0	0	0	1	0	0	0	0	0
AP10A	0	0	1	1	0	0	0	0	0	0	1	1	0
AP10B	1	0	1	1	0	1	0	0	0	0	0	1	0
AP10C	0	0	1	1	0	0	0	1	0	0	1	1	0
AP10D	0	0	1	1	0	0	0	0	0	0	0	1	0
AP10E	0	0	1	1	0	1	0	1	0	0	0	1	0
AP10F	0	0	1	1	0	0	0	1	0	0	0	1	0
AP11A	0	0	1	0	0	0	0	1	1	1	0	1	0

AP11B	0	0	1	1	0	0	0	1	0	0	0	1	0
AP11C	0	0	1	1	0	0	0	1	0	0	0	1	1
AP11D	0	0	1	1	0	0	0	1	0	0	0	0	0
AP11E	1	0	1	0	0	0	0	0	0	0	0	1	0
AP12A	0	0	1	1	0	0	0	0	0	0	0	1	0
AP12B	0	0	1	1	0	1	0	1	0	0	0	1	0
AP12C	0	0	1	1	0	0	0	0	0	0	0	1	0
AP13A	0	0	1	1	0	1	0	0	0	0	0	0	0
AP13B	0	0	1	1	0	0	0	1	0	0	0	0	0
AP13C	0	0	1	1	0	0	0	1	0	0	0	0	0
AP13D	0	0	1	1	0	0	0	1	0	0	0	1	0
AP13E	-	-	-	-	-	-	-	1	-	-	-	-	-
AP13F	0	0	1	0	0	0	0	0	0	0	0	1	0
AP13G	0	0	1	1	0	0	0	1	0	0	0	1	0
AP14A	0	0	1	0	0	0	0	0	0	0	0	1	0
AP14B	0	0	1	0	0	0	0	0	0	0	0	0	0
AP14C	0	0	1	1	1	0	0	1	0	0	1	1	1
AP14D	1	1	1	1	0	0	0	1	0	0	0	1	0
AP14E	0	0	1	1	1	0	0	1	0	0	0	1	0
AP14F	0	0	1	0	0	0	0	1	0	1	0	0	0
AP14G	0	0	1	1	0	1	0	1	0	0	0	1	0
AP14H	1	0	1	1	0	0	0	0	0	0	0	0	0
AP14I	0	0	1	0	0	0	0	0	0	0	0	0	0
AP14J	0	0	1	0	0	0	0	0	0	0	0	1	0
AP15A	0	0	1	1	0	1	0	1	0	0	0	1	0
AP16A	0	0	1	1	0	0	1	0	0	0	0	0	0
AP16B	0	0	1	1	0	0	1	1	0	0	0	0	0
AP16C	0	0	1	1	0	0	0	0	0	0	0	1	0
AP16D	0	0	1	1	0	0	0	1	0	0	0	1	1
AP17A	1	0	1	1	0	0	1	0	0	0	0	0	0
AP17B	0	0	1	1	0	0	0	0	0	0	1	1	0
AP17C	0	0	1	1	0	0	0	0	0	0	0	0	0
AP17D	0	0	1	1	0	0	0	1	0	0	0	0	0
AP17E	1	0	1	1	0	1	0	0	0	0	0	1	0
AP17G	0	0	1	1	0	1	0	0	0	0	0	0	0
AP18A	0	0	1	1	0	0	0	0	0	0	0	1	0
AP18B	0	0	1	1	0	0	0	1	0	0	0	1	0
AP18C	0	0	0	1	0	0	0	0	0	0	0	1	0
AP19A	0	0	0	0	0	0	0	0	0	0	0	0	0
AP19B	0	0	1	1	0	0	0	1	0	0	0	1	0

AP19C	0	0	1	1	0	0	0	1	0	0	0	1	0
AP19D	1	0	1	1	0	0	0	0	0	0	0	1	0
AP19E	0	0	1	1	0	0	1	1	0	0	0	0	0
AP19F	0	0	1	1	0	0	1	1	0	0	0	1	0
AP19G	0	0	1	1	0	0	0	0	0	0	0	1	0
AP20A	0	0	1	1	0	0	1	1	0	0	0	0	1
AP20B	0	0	0	1	0	0	0	1	0	0	0	1	0
AP20D	0	0	1	1	0	0	1	-	0	0	0	1	0
AP20E	0	0	1	1	0	0	1	1	0	0	0	1	0
AP20G	1	0	0	0	0	1	0	1	0	1	0	1	0
AP21A	0	0	0	0	0	0	0	0	0	0	0	0	0
AP21B	0	0	1	1	0	0	0	1	0	0	0	1	0
AP22A	0	0	1	1	0	0	0	1	0	0	0	0	0
AP23A	0	0	1	1	0	1	0	1	0	0	0	0	0
AP23B	0	0	0	1	0	0	0	1	0	0	0	0	0
AP23C	0	0	1	0	0	0	0	1	0	0	0	0	0
AP23E	0	0	1	0	0	0	0	1	0	0	0	0	0
AP23F	0	0	0	0	0	0	1	1	0	0	0	0	0
AP23G	0	0	1	1	0	0	1	1	0	0	1	1	0
AP23H	0	0	1	1	0	0	0	1	0	0	0	1	0
AP24D	0	0	1	1	0	0	0	1	0	0	0	1	0

APPENDIX 4: HUMAN RAW DATA

Questionnaire data and gastrointestinal parasite presence/absence data from fifty-five sampled humans.

Sample ID	Family number	Monkeys near farm*	Age (years)	Boil water*	Monkeys near home*	Gender†	Treated last year*	Hunt wildlife*	Dist home to forest (m)	Hunt monkey*	How often hunt‡
HS1	10	3	46	1	1	0	1	0	0	0	0
HS2	10	3	47	1	1	1	1	0	0	0	0
HS3	10	2	-	2	2	1	2	2	-	2	5
HS4	4	1	45	1	1	1	0	0	40	0	0
HS5	4	1	42	1	1	0	1	0	40	0	0
HS6	4	1	5	1	1	1	1	0	40	0	0
HS7	10	1	22	1	1	1	0	1	200	0	2
HS8	5	0	70	2	1	1	1	0	200	2	0
HS9	5	0	40	2	1	1	0	0	-	0	0
HS10	5	0	7	2	1	1	1	0	200	0	0
HS11	5	0	38	2	1	0	1	0	200	0	0
HS12	5	0	11	2	1	1	1	0	200	0	0
HS13	3	1	52	0	1	0	1	0	100	0	0
HS14	3	1	33	0	1	1	0	1	200	0	4
HS15	3	1	57	1	1	1	1	0	100	0	0
HS16	13	1	24	0	1	1	0	1	100	0	2
HS17	13	1	6	0	1	0	0	0	100	0	0
HS18	13	1	11	0	1	0	0	0	100	0	0
HS19	13	1	10	0	1	0	0	0	100	0	0
HS20	13	1	9	0	1	0	0	0	100	0	0
HS21	13	1	12	0	1	1	0	0	100	0	0
HS22	13	1	14	0	1	0	0	0	100	0	0
HS23	13	1	16	0	1	0	0	0	100	0	0
HS24	13	1	47	0	1	0	0	0	100	0	0
HS25	13	1	46	0	1	1	0	1	100	0	2
HS26	1	2	-	2	1	1	2	2	-	2	5
HS27	4	2	24	0	1	0	1	0	50	0	0
HS28	10	1	32	1	1	1	0	0	0	0	0
HS29	3	1	26	0	1	1	0	0	80	0	0
HS30	3	1	57	0	1	1	0	0	100	0	0
HS31	3	1	28	0	1	0	0	0	80	0	0
HS32	13	1	8	0	1	0	0	0	100	0	0

HS34	13	1	17	0	1	0	0	0	100	0	0
HS36	-	-	-	-	-	-	-	-	-	-	-
HS38	2	0	27	0	1	0	0	0	5000	0	0
HS39	3	1	8	0	1	1	0	0	5000	0	0
HS40	3	0	9	0	1	0	0	0	5000	0	0
HS42	3	1	6	0	1	0	0	0	300	0	0
HS44	2	1	39	0	1	0	2	0	300	0	0
HS45	9	2	-	0	2	0	1	2	-	2	5
HS47	9	2	7	0	2	0	1	2	-	2	5
HS48	9	2	5	0	2	0	1	2	-	2	5
HS50	9	1	17	0	1	0	1	0	200	0	0
HS51	9	1	16	0	1	1	2	0	200	0	0
HS52	9	1	45	0	1	0	2	0	200	0	0
HS53	9	1	21	0	0	1	2	0	500	0	0
HS54	9	1	10	2	0	0	2	0	1000	0	0
HS55	9	1	39	2	0	0	2	0	1000	0	0
HS56	10	0	52	2	0	0	0	0	-	0	0
HS57	10	1	43	0	1	1	1	1	10	0	5
HS58	10	1	22	1	1	0	0	0	5	2	0
HS59	10	1	45	2	1	1	1	0	300	0	0
HS60	10	2	15	2	2	1	1	2	200	2	5
HS61	1	1	49	2	1	1	1	2	200	2	5

*No:0, Yes:1, or No Response:2. †Female:0, Male:1. ‡Never hunt wildlife:0, 2-3 times per week:2, Once per month:4, No response:5

APPENDIX 4: HUMAN RAW DATA (CONTINUED)

Sample ID	Ascarididae gen. sp.	<i>Entamoeba</i> spp.	<i>Strongyloides</i> spp.	<i>Capillaria</i> sp.	<i>Balantidium</i> sp.	<i>Blastocystis</i> sp.	<i>Blastocystis</i> sp. subtype
HS1	0	0	0	0	0	1	ST1-A
HS2	0	0	0	0	0	0	0
HS3	0	0	0	0	0	0	0
HS4	0	0	0	0	0	0	0
HS5	0	0	0	0	0	0	0
HS6	0	0	0	0	0	1	ST3-A
HS7	0	0	0	0	0	1	ST3-A
HS8	0	0	0	0	0	1	ST1-A
HS9	0	1	0	0	0	1	ST2-B
HS10	0	0	0	0	0	0	0
HS11	0	0	0	0	0	1	ST2-B
HS12	0	1	0	0	1	1	ST2-B; ST5-A
HS13	0	0	0	0	0	1	ST2-C
HS14	0	0	0	0	0	1	ST3-B
HS15	0	0	0	0	0	0	0
HS16	0	0	0	1	0	1	ST2-B
HS17	0	0	0	1	0	1	ST1-A
HS18	0	0	0	1	0	1	ST1-A
HS19	1	0	0	1	0	1	+
HS20	1	0	0	1	0	1	+
HS21	0	0	0	0	0	1	ST1-A
HS22	0	0	0	1	0	1	ST3-A
HS23	0	0	0	0	0	1	ST1-A
HS24	1	0	0	0	0	1	ST3-A
HS25	0	0	0	0	0	1	ST1-A
HS26	0	0	0	0	0	1	ST3-A
HS27	0	0	0	0	0	1	ST3-A
HS28	0	0	0	0	0	0	0
HS29	0	0	0	0	0	1	ST2-D
HS30	0	0	0	0	0	1	ST3-A
HS31	0	0	0	1	0	1	ST1-A
HS32	0	0	0	0	0	1	ST1-B; ST3-A
HS33	0	0	0	0	0	1	ST3-A

HS34	0	0	0	1	0	1	ST1-C
HS36	-	-	-	-	-	1	ST1-A
HS38	0	0	1	0	0	1	ST1-A
HS39	0	0	0	1	0	0	0
HS40	0	1	0	0	0	1	+
HS42	0	0	0	0	0	1	ST1-A
HS44	0	1	0	0	0	1	ST3-A
HS45	0	0	0	0	0	1	ST2-C
HS47	0	0	0	1	0	1	ST2-A
HS48	0	0	0	0	0	0	0
HS50	0	0	0	0	0	1	ST2-A
HS51	0	0	0	0	0	1	ST1-A
HS52	0	0	0	0	0	1	ST1-A
HS53	1	0	0	0	0	1	ST1-D; ST2-E
HS54	0	0	0	0	0	1	ST1-A
HS55	1	0	0	0	0	0	0
HS56	1	1	0	0	0	1	ST1-A
HS57	0	1	0	0	0	1	ST3-A
HS58	0	1	0	0	0	1	ST1-A
HS59	0	1	0	0	0	1	ST1-A
HS60	1	0	0	1	0	1	ST1-A
HS61	0	0	0	0	0	0	0

QUESTIONNAIRE

1) **Would you prefer to remain anonymous? Yes/No. If yes, skip to question 5. If you answer "yes," we will not be able to provide any results to you.**

2) Name: _____

3) Address: _____

4) Would you like to receive the results from our tests on the samples you provide? Yes/No

5) Age: _____

6) Gender: _____

7) Occupation: _____

8) How many people are in your household? _____

9) Have you been treated for any parasites or disease within the last 1 year? Yes/No

10) If yes, which parasites or diseases have been treated? _____

11) Is the water that you drink: boiled/filtered/straight from river/other?

12) What types of animals do you have near your home? _____

13) Do monkeys live within 1km of your home? Yes/No/Don't know

14) Do monkeys live within 1km of your farm? Yes/No/Don't know

15) Do you hunt wildlife? Yes/No/No answer

16) If so, how often do you hunt? _____

17) Do you hunt and/or eat monkeys? Yes/No/ No answer

18) If so, what type of monkeys do you generally hunt? _____

19) In meters, please estimate the distance from your home to the forest. _____

For Project Coordinator:

1) Location: _____

2) Code: _____

APPENDIX 5: CURRICULUM VITAE

WILLIAM DANIEL HELENBROOK

Work Address: 129 Illick Hall, Department of Environmental and Forest Biology
State University of New York – College of Environmental Science and Forestry (SUNY ESF)
Syracuse, NY 13210
Phone Number: 239.470.1200
Email Address: wdhelenb@syr.edu

EDUCATION

Doctoral Candidate, Conservation Biology (August 2007-December 2014)

- State University of New York (SUNY) - College of Environmental Science and Forestry, Syracuse, NY (Co-advisors: C.M. Whipps and W.M. Shields)
- Dissertation: Effects of ecological disturbance on parasite communities in both people and mantled howler monkeys (*Alouatta palliata aequatorialis*) living in Ecuador

Master of Arts, Biological Sciences (2006)

- SUNY Buffalo State College, Buffalo, NY (Advisor: A.M. McMillan)
- Thesis: Non-invasive Sampling of Mandrill and Drill Monkeys for Use in Genetic Analyses

Bachelor of Science, Environmental Studies (2001)

- SUNY University at Buffalo, Amherst, NY

Bachelor of Arts, Psychology (2001)

- SUNY University at Buffalo, Amherst, NY

TEACHING AND PROFESSIONAL EXPERIENCE

SUNY ESF

- Adjunct Instructor, EFB307: Principles of Genetics Lecture (Summer 2014)
- Adjunct Instructor, EFB496: Species and Ecosystem Conservation Seminar (Spring 2014)
- Adjunct Instructor, EFB496/EFB796: Conservation Genetics Seminar (Fall 2013)
- Teaching Assistant, EFB462/EFB662: Animal Physiology (Fall 2013)
- Teaching Assistant, EFB200: Physics of Life (Fall 2013)
- Adjunct Instructor, EFB307: Principles of Genetics Lecture (Summer 2013)

- Adjunct Instructor, EFB413: Conservation Biology Lecture (Spring 2013)
- Co-Instructor, EFB307/EFB309: Principles of Genetics Lecture and Laboratory (Fall 2012)
- Co-Instructor, EFB497: Emerging Diseases of Wildlife and Humans Seminar (Spring 2012)
- Co-Instructor, EFB307: Principals of Genetics Lecture (Fall 2011)
- Adjunct Instructor, EFB307: Principals of Genetics Lecture and Laboratory. (Summer 2011)
- Faculty Search Committee Member: Environmental Forestry and Biology (Spring 2010)
- Teaching Fellow, Graduate Assistant Colloquium on Teaching and Learning (Spring 2009)
- Teaching Assistant, EFB480: Animal Behavior Recitation (2008-2012)
- Teaching Assistant and Lab Instructor, EFB307: Principles of Genetics (2007-2010)
- Graduate Student Travel Grant Committee Member (Fall 2008)

Cardiff University, Wales

- Demonstrator, Molecular Ecology and Evolution. Cardiff University (Fall 2006)

SUNY Buffalo State College

- Teaching Assistant, BIO211: Cell and Genetics (Spring 2006)
- Teaching Assistant, BIO213: Ecology, Evolution, and Behavior (Fall 2005)
- Laboratory Instructor, BIO626: Botany (2004-2005)
- English as Second Language Tutor, Portland International Community School (Fall 2001)

RESEARCH EXPERIENCE AND FIELD EXPERIENCE

Laboratory Supervisor. Principals of Genetics. SUNY ESF (Fall 2011)

Principal Investigator, Estacion Biologia Bilsa de la Fundacion Jatun Sacha. June 2009 - August 2013

- Recruited and organized a field team of 6-8 volunteers to sample people and mantled howler monkeys living in an Ecuadorian tropical rainforest.
- Collected information relating to forest structure, location (GPS data points), and demographics of mantled howler monkeys.
- Administered questionnaires to individuals throughout local communities.
- Trained and organized four undergraduates in laboratory methods in the Fish and Wildlife Disease Laboratory in Syracuse, NY.

Principal Investigator, Ometepe, Nicaragua. June-August 2008

- Tested field research methods and conducted preliminary field research on host-parasite interactions in mantled howler monkeys

Senior Research Aid, SUNY College at Buffalo. September 2005-August 2006

- Conducted molecular sexing project of avian populations, and trained and supervised undergraduate students.

Principal Investigator, SUNY College at Buffalo and Buffalo Zoological Gardens. August 2004-May 2006

- Research experience with gel and capillary electrophoresis, microsatellite and mtDNA PCR development, and standard use of molecular biology equipment and protocols.

Volunteer Educator and Supervisor, Pandrillus, Drill Rehabilitation and Breeding Center. October 2002-August 2003

- Project management responsibilities of multi-million dollar primate facilities, including accounts, planning and reporting.
- Supervising and training of 40 staff in primate rehabilitation.
- Education of local communities, including onsite tours, school visits, university lectures and television appearances related to conservation issues.
- Veterinarian assistant performing surgeries, administering anesthesia, medications and maintain breeding records.

Principal Investigator, La Suerte Biological Field Station, Costa Rica. August-September 2002

- Research measuring tropical forest structure and its relationship to mantled howler monkey demographics. Plot surveys were utilized to determine forest succession comparisons throughout various primate groups.

Research Assistant, SUNY Buffalo, Anthropology Department. August 2000-January 2001

- Monitored alloparenting aspects of primate juvenility in Japanese macaques at the Toronto Zoo.

PRESENTATIONS

April 15, 2014. Spotlight on Student Research. Syracuse, NY. *Impact of changing landscapes on parasite communities in people and howler monkeys of Ecuador.* Helenbrook, W.D., S.V. Stehman, S.E. Wade, W.M. Shields, and C.M. Whipps.

February 6 and 11, 2014. Parasitology EFB 453/653, SUNY Environmental Science and Forestry. *The Kinetoplastids: Trypanosoma and Leishmania.*

February 4, 2014. Conservation Biology EFB 413, SUNY Environmental Science and Forestry.

Speciation and Phylogenetics

January 24, 2014. From Lab to Landscape: Integrated Infectious Disease Research. SUNY Environmental Science and Forestry, Syracuse. *Impact of changing landscapes on parasite communities in people and howler monkeys of Ecuador.* Helenbrook, W.D., S.V. Stehman, S.E. Wade, W.M. Shields, and C.M. Whipps

June 26-29, 2013. The 88th Annual Meeting of the American Society of Parasitologists. Quebec City, Quebec. *Influence of forest structure and human encroachment on parasite communities of mantled howler monkeys, Alouatta palliata.* Helenbrook, W.D., Shields, W.M., and Whipps, C.M.

October 15-18, 2012. The International Association for Ecology and Health. Kunming, China. *'Til death do they part': host-parasite relationships of New World monkeys and the anthropogenic disturbances that influence them, Alouatta palliata.* Helenbrook, W.D., Shields, W.M., and Whipps, C.M.

July 13, 2012. American Society of Parasitologists. *A tale of anthropogenic disturbance: association of forest degradation and human proximity on parasitism in mantled howler monkeys.*

March 11, 2012. International Conference on Emerging Infectious Diseases, Atlanta, Georgia. *A tale of anthropogenic disturbance: how forest degradation and human proximity to mantled howler monkey populations influence parasitism.* Helenbrook, W.D., Shields, W.M., and Whipps, C.M.

May 1, 2012. Life Science Symposium, Syracuse University. *A tale of anthropogenic disturbance: how forest degradation and human proximity to mantled howler monkey populations influence parasitism.*

April 13, 2012. Graduate Student Association, SUNY ESF. *A tale of anthropogenic disturbance: how forest degradation and human proximity to mantled howler monkey populations influence parasitism.*

April 12, 2012. Spotlight on Student Research, Syracuse, NY. *Expanded Morphological Characterization of Endoparasites in Mantled Howler Monkeys.* Helenbrook, W.D., and C.M. Whipps.

April 12, 2012. Spotlight on Student Research, Syracuse, NY. *Identification and preliminary genetic sequencing of the endoparasitic protozoan Blastocystis species infecting Ecuadorian humans and howler monkeys using PCR-based techniques.* Sanford J.M., Helenbrook W.D., and Whipps C.M.

April 3, 2012. C-STEP Research and Multicultural Office. *A tale of anthropogenic disturbance: how forest degradation and human proximity to mantled howler monkey populations influence parasitism.*

April 12, 2011. Spotlight on Student Research, Syracuse, NY. *Expanded Morphological Characterization of Endoparasites in Mantled Howler Monkeys.* Helenbrook, W.D., and C.M. Whipps.

March 1, 2011. SUNY College of Environmental Science and Forestry. General Biology II: Cell Biology and Genetics. *Meiosis and Sexual Reproduction.*

February 25, 2009. Syracuse University, Program on Latin America and the Caribbean. *Host Parasite Relationships of New World Monkeys and the Anthropogenic Effects That Influence Them.*

November 29, 2007. Principles of Genetics lecture, SUNY Environmental Science and Forestry. *Molecular Markers in Conservation Genetics: When, Why and How We Use Them.*

December 2006. Primate Society of Great Britain. *Assessment of Drill Monkey Population Structure and Genetic Diversity.*

September 2006. American Society of Primatologists. *Non-Invasive Sampling of Mandrill and Drill Monkeys for Use in Genetic Analyses.*

April 14, 2005. Buffalo Museum of Science. *Population Genetics: Children's Forensic Chemistry Class.*

April 25, 2005. Student Research and Creativity, State University of New York College at Buffalo. *Non-Invasive Fecal Sampling of Mandrill, Mandrillus sphinx and Drill, Mandrillus leucophaeus, for Use in Genotyping Studies.*

May 4, 2003. Pandrillus, Drill Rehabilitation and Breeding Center, Afi Mountain, Cross River State, Nigeria. *Primate behavior and ecology in West Africa.*

GRANTS and AWARDS

Spotlight on Student Research. April 15, 2014. Syracuse, NY. *Impact of changing landscapes on parasite communities in people and howler monkeys of Ecuador.* Helenbrook, W.D., S.V. Stehman, S.E. Wade, W.M. Shields, and C.M. Whipps. 2nd place.

From Lab to Landscape: Integrated Infectious Disease Research. 2nd Place Poster Presentation. *Impact of changing landscapes on parasite communities in people and howler monkeys of Ecuador.* SUNY ESF, Syracuse, NY. January 24, 2014.

American Society of Parasitologists - Dresden Student Travel Grant, July 2013

National Science Foundation's Ecology and Evolution of Infectious Diseases Program: Next generation sequencing (NGS) at The International Association for Ecology and Health, Kunming, China. October 2012

American Society of Parasitologists - Dresden Student Travel Grant, July 2012

SUNY ESF Travel Grant 2012

SUNY ESF Graduate Student Association Travel Grant 2012

Sigma Xi Award. 2010

Leroy C. Stegeman Award. 2009

SUNY ESF Graduate Student Association Travel Grant. 2009

SUNY ESF Graduate Student Association Research in Need. 2009

Program in Latin America and the Caribbean, Summer Research Grant. 2008

SUNY ESF Teaching Assistantship. 2007-2013

SUNY College at Buffalo John Urban Memorial Biology Scholarship. 2006

American Society of Primatologists Conservation Small Grant Award. 2005

State University of New York College at Buffalo Teaching Assistantship. 2004-2006

PUBLICATIONS

Helenbrook, W., S.E. Wade, W. Shields, S.V. Stehman, and C. Whipps (*Accepted*). Gastrointestinal parasites of Ecuadorian mantled howler monkeys, *Alouatta palliata aequatorialis*, based on fecal analysis. *Journal of Parasitology*.

Helenbrook, W., W. Shields, S.V. Stehman, and C. Whipps (*In Review*). Influence of forest structure and human proximity on parasite diversity and prevalence in mantled howler monkey populations. *Conservation Biology*.

Helenbrook, W., and C. Whipps (*In Review*). Molecular phylogenetic analysis of *Blastocystis* in sympatric human and nonhuman primate populations. *Parasitology Research*

Helenbrook, W., W. Shields, and C. Whipps (*In Review*). An epidemiological approach – risks associated with gastrointestinal parasitism in an Ecuadorian community. *Parasitology*.

Schlaepfer, M., **W. Helenbrook,** K. Searing, and K. Shoemaker (2010). Assisted colonization: evaluating contrasting management strategies (and values) in the face of uncertainty. *Trends in Ecology and Evolution* 24(9):471-472.

Helenbrook, W. (2006). Non-invasive Sampling of Mandrill and Drill Monkeys for Use in Genetic Analyses. MA Thesis, SUNY College at Buffalo, Buffalo, New York.

Helenbrook, W., A. McMillan (2006). Non-invasive Sampling of Mandrill and Drill Monkeys for Use in Genetic Analyses. *American Journal of Primatology* 68(S1): 88.

ADDITIONAL TRAINING

- International Association for Ecology and Health. Next generation sequencing (NGS) Workshop. October 2012

- Panel speaker. Grant Writing Workshop. SUNY ESF 2012
- Graduate Student Association Representative, SUNY-ESF and Syracuse University Liaison. 2009-Present
- Graduate Student Association, Environmental and Forest Biology Representative. 2008-2009
- Graduate Student Union Representative, SUNY ESF. 2007-2008
- Graduate Research Assistant, Psychology Department, SUNY College at Buffalo. 2005-2006
- Graduate Student Union Representative, SUNY College at Buffalo. 2005-2006
- Volunteer Program Coordinator, Science and Education, Oregon Museum of Science and Industry, Oregon. 2000-2001
- Internship, Earth Spirit/University at Buffalo. 1999-2000