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Suraja Raj April 25, 2013

Intestinal Parasite Infections in the Human and Mountain Gorilla Populations of the Virunga Volcanoes Region

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Bachelor of Science
University of California, Los Angeles
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Thesis Committee Chair: Christine L. Moe, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

Intestinal Parasite Infections in the Human and Mountain Gorilla Populations of the Virunga Volcanoes Region

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Urbanization and migration can create stresses on population health and on the ecosystem. Habitat encroachment can cause increased contact and competition between animals and humans. In Rwanda, the endangered mountain gorillas (*Gorilla berengei berengei)* live in a habitat bordered by an extremely dense human population. Bisate is one community nearest the Volcanoes National Park and gorilla reserve. This population of about 20,000 rural agriculturists has limited access to education, healthcare, opportunities for economic development and basic services such as roads, electricity and potable water. Living in close proximity, humans and gorillas have frequent contact through humans entering the park to collect spring water and gorillas leaving the park to forage in agricultural fields. This contact poses a potential threat for transmission of zoonoses and also of human pathogens to the gorilla population. Five rounds of cross-sectional microbiological data on intestinal parasites (protozoa and helminthes) in almost 5,000 human stool samples were collected in Bisate between 2002 and 2011 through The Dian Fossey Gorilla Fund International's mass chemotherapy program. Microbiological data on 400 gorilla stool samples was collected through a 2010 census of mountain gorillas in the Virunga Volcanoes region. Overall, parasitic infections decreased in the human population from 91.8% to 52% during the study time frame. Soil-transmitted helminths were the most common parasites detected in the human samples. Age and administrative sector were associated with increased odds of parasitism and sex was associated with increased odds of infection by multiple parasites. Habituation and residence in DRC and Uganda were found to be associated with decreased odds of polyparasitism in gorillas. We qualitatively compared the number and types of parasite species detected in humans and gorillas in Rwanda and found that both populations were infected with *Ascaris, Trichuris, Trichostrongylus* and Hookworm. Monitoring of infectious disease in both human and gorilla populations and implementation of programs to improve health and limit human-gorilla contact, are critical for both wildlife conservation and public health in this area of Rwanda.

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Abbreviations

DRC	Democratic Republic of the Congo
DFGFI	Dian Fossey Gorilla Fund International
IRB	Institutional Review Board
MOH	Ministry of Health
STH	Soil-Transmitted Helminths
UNICEF	United Nations Children’s Fund
VIF	Variance Inflation Factor
WHO	World Health Organization

Chapter 1: Literature Review

The Global Burden from Gastrointestinal Parasites

Global Burden and Epidemiology

Gastrointestinal parasites (helminths and protozoa) affect billions of people worldwide each year, accounting for 25% of known infectious diseases (Fig 1). While these parasites have been largely controlled in more developed regions of the world, including Europe and the United States, parasites remain a disease of poverty. The majority of parasitic infections occur in the humid tropics, where substandard access to water, sanitation and housing dominate, and most of the human population lives below the World Health Organization (WHO) poverty line (less than US $2 per day). It is this population that disproportionately carries the burden of parasitic infections—70% of the worm burden carried by 15% of the global population (Peter J Hotez et al., 2008).

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Figure 1: Taxonomic distribution of etiological agents causing infectious disease in humans (Alum, Rubino, & Ijaz, 2010)

It is difficult to know the exact burden of gastrointestinal parasites in humans because of complexity associated with measuring morbidity. Parasitic infections by worms are often chronic in nature, affecting their hosts more by way of intensity (the number of parasites found in an infected host) and rate of reinfection, rather than infection itself. Individuals living in rural, low-income settings will often harbor multiple parasite species (polyparasitism). While polyparasitism in children can be associated with increased wasting, overall, the implications of polyparasitism on morbidity has not been well explored (Mupfasoni et al., 2009). In addition, while protozoal infections may not be considered chronic, the nature of their transmission through contaminated food and water makes it difficult to quantify the burden of the disease. Further, parasitic disease often act as opportunistic infections in those with HIV/AIDS or in malnourished immunosuppressed persons, causing repeated bouts of diarrheal disease and significant morbidity. These subtle morbidities on human development and unique methods of transmission, have led to controversy over the applicability of metrics such as the Disability-Adjusted Life Year (DALYs). Lastly, morbidity due to parasitism is affected by a variety of water, sanitation and environmental factors. Thus, incidence and prevalence can vary geographically (Harhay, Horton, & Olliaro, 2010). Considering all these factors, the global burden is likely underestimated and thus parasites are often perceived to be less of a health risk than they actually are (Alum et al., 2010).

The Parasites

Chronic infection with parasites can lead to malnutrition and stunting. Both are correlated with decreased cognitive performance, particularly in children. Parasitism is also correlated with low levels of hemoglobin, vitamin and mineral deficiencies and inefficient absorbance of nutrients. Furthermore, high intensity of parasitic disease can lead to severe immunosuppression and pave the way for other opportunistic infections (Alum et al., 2010). Gastrointestinal parasites are generally categorized into two groups, protozoa and helminths (Harhay et al., 2010). The major parasites in each category are described below.

Helminths

Soil-transmitted helminths (STH) are a group of parasitic nematodes that include the roundworm (*Ascaris lumbricoides)*, the whipworm *(Trichuris trichura)* and hookworm (*Ankylostoma duodenale* and *Necatur americanus)*. This class of parasites make up the majority of parasitic infections worldwide. Approximately 807 million people are affected annually with

Ascaris, 604 million are infected with *Trichuris* and 576 million are infected with hookworm (Peter J Hotez et al., 2008). Helminths are generally transmitted through the fecal-oral pathway, whereby eggs are ingested in food that has been contaminated with feces (Bogtish, 2005; Howard, 2003). Roundworm eggs must fully develop in the soil, and once ingested, they penetrate the intestinal mucosa. These can then enter the liver and lungs before re-entering the gastrointestinal tract, maturing into adults and laying eggs. The whipworm resides in the colon where it matures and lays eggs. Finally, the hookworm is unique in that the eggs hatch in the soil and the larvae burrow into the skin through the soles of feet. The larvae enter the circulatory system where they travel through capillaries, the lungs and epiglottis to reach the gastrointestinal tract and "hook" onto the host to lay eggs (Bethony et al., 2006; Bogtish, 2005). The eggs of helminths are passed through feces and the cycle perpetuates itself. Most STH eggs can remain viable in the soil for months, while hookworm larva can survive in the soil for several weeks. Adult worms can survive for several years and start producing eggs after 4-6 weeks (Brooker, Clements, & Bundy, 2006). Most cases of helminth infection go undetected, however high intensities are linked to chronic malnutrition, stunting, cognitive delay as well as immunosuppression.

Other parasitic nemotodes include trematodes and flukes. Schistosomiasis is caused by one common fluke, *Shistosma sp.*, which affects over 200 million people worldwide and like other gastrointestinal parasites is of greater threat to humans in low-

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(http://www.globaled.uconn.edu/teachers_water/images/image025.gif)

Figure 2: Fecal-oral route transmission of disease (Howard, 2003)

middle income countries. The *Schistosoma sp.* is contracted generally through swimming or other contact with freshwater that contains snails that carry the schistosome larvae. Upon infection, the parasite migrates through tissue and matures into adult worms in the blood vessels of the host. Schistosomiasis can be asymptomatic for years, however, chronic schistosomiasis as a result of reinfection can lead to complications such as bladder cancer, kidney failure, and liver or spleen damage ("Center for Disease Control and Prevention (CDC)," 2012, "This Wormy World," n.d.).

Strongyles include *Strongyloides stercoralis,* the threadworm. While the global burden of threadworm infection is unknown, the Centers for Disease Control and Prevention estimates that between 3-100 million people are infected worldwide. Like STHs, strongyles are transmitted through contact with contaminated soil, for example, by walking barefoot or through contact with sewage. Adult female worms live threaded in the wall of the small intestine where they hatch eggs. Unlike other helminths, strongyles are unique because of their ability to undergo autoinfection whereby the threadworm larvae can turn into the infectious form once more and reinfect the host. Another common strongyle is the pinworm (*Enterobius vermicularis*) which is particularly common in young children (Bogtish, 2005; "Center for Disease Control and Prevention (CDC)," 2012).

As opposed to nematodes, cestodes are characterized by proglottids, which contain sexually mature reproductive systems. These are shed along with eggs by cestodes. Common cestode parasitic infections include infections by *Taenia solium* and *Taenia saginata.* Detection of tapeworms in stool is not as common as other worms (Harhay et al., 2010). Most people with Taeniasis feel mild to no symptoms; however, it can cause digestive pains, loss of appetite and other gastrointestinal discomfort. Infection is often associated with contaminated meat, particularly beef or pork that has not been cooked properly ("Center for Disease Control and Prevention (CDC)," 2012). If *Taenia* (particularly *T. saginata*) eggs are consumed, a condition called cystercercosis can develop, where cysts form in the muscles or brain of the infected person. These cysts can last for years, and the inflammation of these cysts cause painful nodules in muscles or seizures when in the brain. *T.solium* is primarily an intestinal infection (Bogtish, 2005; Harhay et al., 2010).

Protozoa

Protozoal infections such as those caused by *Giardia sp., Cryptosporidium sp*. and *Entamoeba histolytica* are often associated with severe diarrheal disease. Outbreaks of *Giardia* associated with contaminated water are common, especially in areas of flooding. Infection is highly prevalent and asymptomatic in rural areas in developing countries. In addition, amoebic dysentery, caused by *Entamoeba histolytica,* is the most common cause of death from a parasitic disease worldwide, after malaria, and causes about 100,000 deaths annually. Most protozoa are transmitted through contaminated water and poor hygiene, through the fecal-oral transmission route (Harhay et al., 2010).

Environmental Considerations & Risk Factors

<u>Household Factors</u>

Socioeconomic status, poverty and degree of urbanization all play an important role in disease transmission (Clements, Deville, Ndayishimiye, Brooker, & Fenwick, 2010; World Bank, 2006). An environment with dirty water, poor sanitation and substandard housing will propagate transmission of parasites. A lack of adequate water as well as no sanitary means of disposing feces (poorly maintained toilets or shared sanitation facilities) also increases transmission rates and reinfection (Harhay et al., 2010; Lilley, Lammie, Dickerson, & Eberhard, 1997). An estimated 13% of the world's population live in households where water is collected from unprotected sources and thus contributes to the likelihood of endemic and epidemic waterborne and diarrheal disease (Bartram & Cairncross, 2010). Lack of an adequate water supply or a water source without any treatment (chlorination, boiling etc.) poses a risk of transmission of parasites such as *Giardia* and *Cryptosporidium.* A 2012 article by Salyer et al. examined *Cryptosporidium* in non-human primates, humans and livestock in Uganda. The study examined human demography, behaviors and interactions with animal and how these factors affect risk of *Cryptosporidium* infection. The study reported a strong association between fetched water from an open water source and infection (Salyer, Gillespie, Rwego, Chapman, & Goldberg, 2012). In addition, parasites can also be transmitted by contaminated fruit and vegetables. Produce can be contaminated in the fields, by the use of manure for fertilizer, watewater for irrigation, or during processing and handling (Alum et al., 2010). Without proper washing and cooking, it is possible for eggs and cysts to be transmitted to humans. Finally, a lack of handwashing with soap, due to inadequate access to water, soap or education, perpetuates transmission of gastrointestinal parasites through the fecal-oral route. As a result of these factors, it has been shown that intestinal nematode infections contribute to 3% of DALYs (Disability Adjusted Life Years) of the total burden of ill health that could be prevented by improved water, sanitation and hygiene (Bartram & Cairncross, 2010).

Population density and clustering of houses can also promote increased transmission within and between family and community members (Peter J Hotez et al., 2008). Gastrointestinal parasites can be readily transmitted between members of a family that live in the same household. Densely populated spaces in areas that lack in access to clean water and safe disposal of excreta make for an ideal breeding ground for parasites and subsequent disease transmission (World Bank, 2006).

Furthermore, certain subgroups of the population tend to be at higher risk for infection from parasites. For example, specific occupations can put people at risk for gastrointestinal parasites. Hookworm has been shown to have a higher prevalence in people who work in the agricultural sector because farming increases their contact with soil (World Bank, 2006). Tending livestock and working in forests can also increase risks of infection (Salyer et al., 2012). Studies have shown that infection with STHs has a convex relationship with age. Compared to any other age group, school-aged children between the ages of 5 and 15 years, are at greater risk for having a higher worm burden. As children age, the prevalence and intensity of infection goes down (Peter J Hotez et al., 2008).

Ecological Considerations

A variety of environmental factors affect the spatial and temporal distribution of gastrointestinal parasites. Both biotic and abiotic factors affect the distribution of parasites. Microorganisms, such as algae, may promote or limit the growth of helminths and protozoa (Alum et al., 2010). Increased land surface temperature, increased precipitation, a higher vegetation index, greater elevation, decreased distance to water bodies and lower altitudes have all been shown to increase the distribution of STHs as well (Appleton & Gouws, 1996; Brooker et al., 2003; Clements et al., 2010; Pullan et al., 2011). Temperature and humidity play a large role in the transmission of parasites that require incubation in the environment, such as STHs (Alum et al., 2010). Moist conditions favor the eggs of STHs, and thus flooding, altered water drainage patterns and soil saturation affect the prevalence and intensity of STH transmission (Lilley et al., 1997). Moisture can also affect the survival of eggs, cysts and oocysts in the environment. *E.histolytica* can survive as little as 18 hours or as long as 10 days depending on whether the environment is dry or moist, respectively (Alum et al., 2010). As a result, prevalence and intensity of STH infections can vary both spatially and temporally because of environmental factors.

Anthropogenic changes to the environment can also affect the ecological factors that influence disease transmission. In Haiti, shifts in land and water use as a consequence of economic development or environmental degradation, may have increased the soil moisture and expanded the distribution of hookworm. Consequently, contact between pathogens and the human population increased. These environmental changes may have increased human exposure to infectious agents or indirectly increased disease transmission by expanding vector habitat, as was the case with schistosomiasis in Egypt (Lilley et al., 1997). Habitat disturbance, deforestation, geographic isolation and forest fragments can affect parasite transmission dynamics (Gillespie, Chapman, & Greiner, 2005; Gillespie, Nunn, & Leendertz, 2008; Gillespie & Chapman, 2006; Goldberg et al., 2007).

Infections in Non-Human Primates

Research in Uganda has shown how various attributes of forest fragments can affect parasite infection dynamics in primates. The study found a greater risk of rhabitoid nematode infections were found in Red Colobus monkeys in areas with higher stump density. The density of tree stumps remaining after harvest by people, a proxy for increased human contact, reduced food availability for monkeys and increased ground time because of tree range restriction (Gillespie & Chapman, 2006).

Increased habitat overlap between humans and other animals as a result of anthropogenic changes can compound the effect of environmental factors on disease transmission and the potential for zoonotic transmission. Concern about zoonosis is greater for animals that have a low degree of evolutionary difference with humans, such as great apes (Gillespie, 2006). Non-human primates are more likely to carry pathogens that can be transmitted to humans as well as suffer from similar clinical manifestations. Increased exposure to bodily fluids, tissues and feces of non-human primates can increase risk of disease transmission to humans (Calvignac-Spencer, Leendertz, Gillespie, & Leendertz, 2012). Non-human primates can have infections such as yellow fever, tuberculosis, malaria, Chagas disease, as well as giardiasis and cryptosporidiosis (Chapman, Gillespie, & Goldberg, 2005). Perhaps most infamous, is the case of HIV/AIDS which reportedly evolved from chimpanzees (Gao et al., 1999; Keele et al., 2006). In addition, there have been cases of suspected transmission from humans to primates, or anthropozoonosis. An Ebola outbreak in the bordering regions of the DRC and Gabon caused an 80% reduction in the Western Lowland Gorilla population (Rwego, Isabirye-Basuta, Gillespie, & Goldberg, 2008).

Treatment and Prevention

Water Sanitation and Hygiene

Risk of intestinal parasite infections is strongly linked to poverty, poor hygiene and sanitation. Studies of the effectiveness of sanitation interventions and mass drug administration have shown that sanitation interventions can reduce risk of infection with intestinal parasites (Mupfasoni et al., 2009). Worldwide, about 2.6 billion people lack access to improved sanitation. The 2012 WHO and UNICEF Joint Monitoring Programme Report showed that in sub-Saharan Africa only 30% of the population has access to improved sanitation (*Progress on Drinking Water and Sanitation*, 2012). Without improvements in sanitation and behavior change related to open defecation, it is impossible to sustainably decrease the burden of parasitic disease (Tchuem Tchuenté, 2011). In addition, improving water quality and quantity by creating sustainable access to safe water supplies is crucial. Access to water also improves hygiene and can mitigate transmission due to contaminated hands, food, etc. (Moe & Rheingans, 2006). Esrey et. al. analyzed 144 studies to examine the relationship between improved water and sanitation interventions and helminth infections. This meta-analysis indicated water and sanitation interventions can substantially decrease disease burden due to parasitic infections. A 29% median reduction in ascariasis and a 26% median reduction diarrheal disease was found in rigorous studies (Esrey, Potash, Roberts, & Shiff, 1991).

Anti-helminthic Medication

While such interventions address the underlying problems, water and sanitation interventions are often costly and can take a long time to implement. Thus, chemotherapeutic interventions tend to be a much more common approach for controlling many parasitic diseases (Tchuem Tchuenté, 2011). Mass drug administration of chemotherapeutic drugs is often used to decrease morbidity due to parasites. Annual doses of albendazole and mebendazole can cure up to 95% of roundworm infection, but has shown to be less effective on whipworm and hookworm infections. These drugs are very inexpensive and have limited side effects, but administration must be repeated frequently because reinfection occurs within 3-6 months without proper sanitation (P J Hotez, 2009). The WHO recommends treating “high-risk communities” (greater than 50% prevalence of STH infections among school-aged children) at least two times a year. For “low-risk communitites” (between 20-50% prevalence of STH infection among school-aged children), once a year preventative chemotherapy is recommended. Preventative chemotherapy through mass drug administration is generally administered to high-risk groups, such as preschool children, women of child-bearing age and those in high-risk occupations (Albonico, De Silva, Engels, Gabrielli, & Savioli, 2006). However, issues of coverage and reinfection must be considered in chemotherapeutic interventions (Tchuem Tchuenté, 2011). Furthermore, veterinary models have suggested the development of parasite resistance to chemotherapy (Kaplan, 2004). As a result, researchers have been investigating combinations of drugs as well as vaccine development as options for parasitic disease control (Alum et al., 2010).

Rwanda as a Unique Case in Gastrointestinal Parasitism

Rwanda is a landlocked country in eastern Africa, with a population of 11,689,696 people, that is growing at a rate of approximately 2.75% per year (“CIA-World Factbook Rwanda,” n.d.). It is the most densely populated country in Africa with a population density of 403.4 people/km^2 (“Country Profile-Rwanda,” 2010). In rural areas this number is thought to be as high as 820 people/km^2 (Gray & Kalpers, 2005). The majority of the population lives in rural areas, with only 19% of the population living in urban areas. Following the Rwandan genocide and civil war of the early 1990s, the country was left unstable and in extreme poverty. Since then, Rwanda has rehabilitated its economy. Today, Rwanda’s economy includes tourism, minerals, coffee and tea. Sixty percent of Rwanda’s population continues to live below the poverty line, and most people make their living from subsistence agriculture, or mineral and agro-processing (“CIA-World Factbook Rwanda,” n.d.).

Amongst its diverse terrains, including plateaus, rolling hills and savannah, is the volcanic region in the Northern Province. Here, Rwanda is well known as home to one of the last remaining populations of the mountain gorilla (*Gorilla berengei beringei*). The endangered species’only remaining habitats are in the Virunga Volcanoes region (part of the Albertine Rift) that spans Rwanda, Uganda and the Democratic Republic of the Congo (DRC), and in the Bwindi Impenetrable Forest in Uganda. In Rwanda, the Volcanoes National Park (VNP) covers an area of around 120 km^2 and its gorilla population is estimated to be slightly above 480 individuals (Gray et al., 2013).

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Figure 3: Volcanoes National Park Zonation Map (Volcanoes National Park Management Plan, 2012)

Gorilla tourism is the primary source of income for Rwanda. In 2008, 17,000 people visited Rwanda to see the gorillas and this number has only increased since (Nielsen, 2010). In June of 2012, the price of gorilla tourism went from US$500 to US$750, and the number of tourists has not waned (F.Ndagijimana, personal communication, June 2012). However, the gorillas in the VNP are faced with a number of threats, including habitat loss, poaching and disease transmission from humans. This last point is of particular concern as the region has a very high human population density, exacerbated by intensive movement of military and other personnel into the area during the wars of the 1990s, and continued traffic from ecotourism today (Sleeman, Meader, Mudakikwa, Foster, & Patton, 2000).

A string of villages closely border Rwanda's VNP (*Volcanoes National Park Management Plan*, 2012). During the political and ethnic tension of the 1990s in Rwanda, fighting caused significant displacement of the human population from this area to the capital region. The area faced much deforestation, as well as agricultural and sanitation problems. Corpses were discarded in rivers, and are thought to have affected the water quality of the Nile basin and its biodiversity. Land mines were placed around the Virungas, killing both humans and, likely, gorillas (Kanyamibwa, 1998). Of 187 environmental impacts reported from 1990-2005 as a result of conflict in the DRC and Rwanda, 10.2% were accounted for by *Gorilla sp.* in Rwanda and the DRC (Glew & Hudson, 2007). While much information is still lacking on the extent of effect, the war left the area very fragile both environmentally, and socioeconomically (Kanyamibwa, 1998).

Today, this area has high rates of poverty and is very densely populated. Many of the people in this region have difficulty accessing health facilities and adequate care. In the Northern Province of Rwanda, 55.1% of women reported at least one problem in accessing a health facility, including distance, money or support. This problem disproportionately affects the poor, who make up 79.6% of those having trouble accessing health facilities. Furthermore, only 71.2% of rural households in Rwanda have access to an improved source of drinking water, and 56.7% of rural households must travel thirty minutes or longer round trip in order to obtain drinking water. An estimated 71.2% of rural households in Rwanda have access to an improved source of drinking water, and only 59% use improved (not shared) sanitation facilities (*Rwanda Demographic and Health Survey 2010*, 2012). The volcanic soil of the area prevents water from being stored in the water table. Alternating rainy and dry seasons complicate access to water, and affects disease transmission. As a result of poor water and sanitation, the population is especially vulnerable to communicable diseases (DFGFI, n.d.; *Rwanda Demographic and Health Survey 2010*, 2012). The area has one of the highest prevalence rates of intestinal parasites in humans in Rwanda. Almost 83.1% of the population is infected with STHs and 8.6% of the population suffers from schistosomiasis (Mupfasoni et al., 2009).

Throughout the years, there has been an increase in overlapping environment and interactions between gorillas and humans in the Virungas area. Anthropogenic changes to habitats have especially limited gorilla habitat to patchy areas and forced primates and humans into more frequent contact (Chapman et al., 2005). Furthermore, the area lacks any sort of buffer zone between the homes or agriculture of the local population and the national park that is home to the mountain gorillas except for a three foot stone wall designed to keep out wild buffalo (*Volcanoes National Park Management Plan*, 2012). Lastly, while ecotourism is aimed at increasing awareness of the endangered species, it has also led to increased habituation of gorillas (meaning that they are accustomed to human presence). Visits from tourists can increase transmission of diseases such as gastrointestinal parasites and respiratory diseases between humans and primates (Guerrera et al., 2003).

Apes and humans are 98.2% genetically similar, facilitating disease transmission between species. The introduction of new pathogens into immunologically naïve populations can result in widespread morbidity and mortality for both gorillas and humans. Respiratory disease such as measles and tuberculosis, as well as gastrointestinal parasites of human origin such as *Trichuris trichura* and *Strongyloides stercoralis,* have been found in mountain gorilla populations and can have deleterious effects on population dynamics, susceptibility and resistance (Ferber, 2000). In 1988, a measles outbreak spread through three of seven habituated gorilla groups in the Virungas of Rwanda putting incredible stress on the population and threatening gorilla progress (Guerrera et al., 2003).

Few studies have examined gastrointestinal parasites in primates. In nature, parasitic infections are common, and low-intensity infections are often asymptomatic. However, high burdens of strongyles and rhabitoid nematode infections have been shown to cause substantial morbidity, and even mortality, in primates (Anderson & May, 1979; DePaoli & Johnsen, 1978; Harper, Rice, London, Sly, & Middleton, 1982). The first descriptions of parasites in the mountain gorillas of the Virunga Mountains were from 1976 -1978. There were reported cases of strongyles, helminths, protozoa and nematodes (J. M. Rothman, Bowman, Eberhard, & Pell, 2002). Since then, more studies have explored parasitic disease in gorillas and the potential for parasitic disease transmission between humans and great apes. Great ape populations with increased interaction and habitat overlap with human populations have shown increased prevalence and diversity, as genetic similarities in the parasites, as well as similar patterns of antibiotic resistance with parasites that infect humans (Guerrera et al., 2003; Lilly, Mehlman, & Doran, 2002; Nizeyi, Mwebe, Nantezat, Cranfieldt, & Kalemall, 1999; Rwego et al., 2008). Though transmission between humans and great apes has been difficult to prove, the similarity between parasitic infections in the two populations suggests transmission. Parasites that have been recorded in both humans and mountain gorillas include *Ascaris lumbricoides, Trichuris trichura, Entamoeba histolytica, Entamoeba coli, Iodamoeba butschlii, Endolimax nana,* as well as *Cryptosporidium,* with data suggesting there may be more (J. Rothman & Bowman, 2003).

A study conducted in the Bwindi mountain gorilla population in Uganda indicated that the most common periods of interaction between mountain gorillas and humans are during entrance into the parks en route to villages and markets, harvesting, human work in the National Park, and other livelihood tasks. The same study indicated that people reported defecating while in the forest, and only 36.6% reported burying their waste (Guerrera et al., 2003). Contact with human feces poses a risk for parasitic exchange and disease transmission (Mupfasoni et al., 2009). In the Uganda study, 81.7% of those interviewed were unaware of the risk of disease transmission from humans to primates or vice versa (Guerrera et al., 2003).

Because of both environmental and health needs of animals and humans in Rwanda, there is a high concentration of government and non-governmental agencies working in the area to improve the livelihood and health of people as well as reduce human-great ape conflict. In 2007, the government of Rwanda worked with The Access Project to launch a nationwide Neglected Tropical Disease program. The program provided anti-heliminthic drugs through schools to children in Rwanda (MOH & Project, 2010). In the Northern Province of Rwanda, organizations such as The Dian Fossey Gorilla Fund (DFGFI) and the Mountain Gorilla Veterinary Project, long known in the region for their mountain gorilla research and conservation efforts, have been working to address the healthcare needs of the local community. These efforts are motivated by the belief in “one health”—the understanding that the health and well-being of humans, animals and the environment are intimately connected. Educational interventions that highlight the importance of hygiene, and explain how to prevent intestinal parasite transmission and prevention, as well as the economic significance of the endangered mountain gorillas, can augment efforts to improve healthcare. The adoption of improved water and sanitation facilities (such as latrines, and clean water supplies) has the potential to significantly improve the health and wellbeing of the people and ecosystem surrounding the VNP.

CHAPTER 2: RESEARCH OBJECTIVES AND RATIONALE

Study Objective

The objective of this study is to understand the prevalence of intestinal parasites in both human and mountain gorilla populations by conducting a secondary data analysis of data collected in the VNP region of northern Rwanda, and the Virunga Massif across the DRC, Rwanda and Uganda.

Specific Aims

1. Determine the prevalence of intestinal parasites in humans in the Bisate catchment area surrounding the VNP in five separate years and the relation between age, sex and administrative sector and parasite prevalence.
2. Determine the prevalence of intestinal parasites in mountain gorillas in the Virungas and the relation between age, habituation and country of residence and parasite prevalence.
3. Compare the species prevalence and overlap of intestinal parasites between mountain gorillas and humans in the same geographic region to inform development of hypotheses about disease transmission between the two populations.

Rationale

A better understanding about parasites in northern Rwanda can inform decisions on environmental, health and economic interventions in this region. By improving water and sanitation infrastructure as well as providing regular anti-helminthic medication, children will be able to better perform in school and absenteeism and drop-out rates could decrease. This will lead to a healthier and more productive population. In addition, addressing poor sanitation practices will not only affect the threat to human health, but can also mitigate the threat of biodiversity loss and disease on the endangered mountain gorilla populations (Sleeman et al., 2000). By preserving biodiversity, Rwanda will ensure sustained economic returns from gorilla tourism, allowing both the local population and country as a whole to benefit. Fostering synergy between animal and human well-being can more effectively address the needs of vulnerable populations.

Chapter 3: Manuscript

Contribution of Student

I traveled to Rwanda in May of 2012 and worked along with the Dian Fossey Gorilla Fund International (DFGFI) in an assessment of their Ecosystem Health Community Development program. During my time in Rwanda, I familiarized myself with the program as well as the data they had collected through deworming campaigns at the Bisate Health Center. I cleaned the data referring to the Ecosystem Health Community Development manager for questions on protocol and lab records. The data was provided to me by DFGFI. Dr. Thomas Gillespie provided the Virunga mountain gorilla data, which was collected through a 2010 census conducted by protected area authorities in the DRC, Rwanda and Uganda (L'Institut Congolais pour La Conservation de la Nature, The Rwanda Development Board, and The Uganda Wildlife Authority) in partnership with local non-governmental organizations.

Under the guidance of Dr.Christine Moe and Dr.Thomas Gillespie, I conceptualized the research questions for this project, conducted a review of the literature and wrote the entire manuscript. I also conducted all statistical analysis and created all the tables and figures for the results of my analysis.

INTRODUCTION

Global Burden and Risk Factors

Gastrointestinal parasites (helminths and protozoa) affect billions of people worldwide each year, accounting for 25% of known infectious diseases. While these parasites have been largely controlled in more developed regions of the world, they remain a disease of poverty. The majority of parasitic infections occur in the humid tropics, where poor access to water, sanitation and housing dominate and most of the population lives below the World Health Organization (WHO) poverty line (less than US $2 per day). It is this population that disproportionately bears the burden of parasitic infections—70% of the worm burden carried by 15% of the global population (Peter J Hotez et al., 2008).

Intestinal parasites are generally categorized into two groups, protozoa and helminths (Harhay et al., 2010). Protozoal infections such as those caused by *Giardia sp., Cryptosporidium sp.* and *Entamoeba histolytica* are often associated with severe diarrheal disease. Soil-transmitted helminths (STHs)--including the roundworm (*Ascaris lumbricoides)*, the whipworm *(Trichuris trichura)* and hookworm (*Ankylostoma duodenale* and *Necatur americanus*)—make up the majority of parasitic infections worldwide (Peter J Hotez et al., 2008). Helminths and protozoa are generally transmitted through the fecal-oral pathway, whereby eggs are ingested in food or water that has been contaminated with feces (Harhay et al., 2010; Howard, 2003). Chronic infection with parasites can lead to malnutrition and stunting. Both are associated with impaired cognitive development, particularly in children. Parasitism is also associated with to low levels of hemoglobin, vitamin and mineral deficiencies , as well as inefficient absorbance of nutrients. Furthermore, high numbers of infections can lead to severe immunosuppression and create a predisposition for other opportunistic infections (Alum et al., 2010).

There are a variety of risk factors associated with parasitic infections. Socioeconomic status, poverty and degree of urbanization all play an important role in the transmission of parasites (Clements et al., 2010; World Bank, 2006). An environment with dirty water, poor sanitation and substandard or crowded housing will promote transmission of parasites (Harhay et al., 2010; Lilley et al., 1997). Furthermore, certain subgroups of the population, such as school-aged children (5-15 years of age) and people who work in agriculture, tend to be at higher risk for infection from parasites (Peter J Hotez et al., 2008; World Bank, 2006). Environmental factors such as increased land surface temperature, increased precipitation, higher vegetation index, elevation, decreased distance to water bodies, and lower altitudes have all been shown to increase the distribution of STHs as well (Alum et al., 2010; Appleton & Gouws, 1996; Brooker et al., 2003; Clements et al., 2010; Pullan et al., 2011). Anthropogenic changes to the environment can also affect ecological factors that influence disease transmission. In Haiti, shifts in land and water use as a consequence of economic development or environmental degradation may have increased soil moisture and expanded the distribution of hookworm, resulting in greater contact between parasites and the human population (Lilley et al., 1997). Habitat disturbance, deforestation, isolated areas, and forest fragments can severely affect parasite transmission dynamics (Gillespie et al., 2005, 2008; Gillespie & Chapman, 2006; Goldberg et al., 2007). Increased habitat overlap between humans and other animals as a result of anthropogenic changes can compound the effects of environmental factors on disease transmission and increase the risk of zoonotic transmission.

Concern about zoonosis is greater for animals that have a low degree of evolutionary difference with humans, such as great apes (Gillespie, 2006). Non-human primates have been shown to carry such diseases such as yellow fever, tuberculosis, malaria, Chagas disease as well as giardiasis and cryptosporidiosis (Chapman et al., 2005).

Because great apes and humans share 98.2% of genetic material, disease transmission is facilitated between the species. The introduction of new pathogens into immunologically naïve populations can result in widespread morbidity, and mortality, for both gorillas and humans (Ferber, 2000). In nature, parasitic infections are common, and low-intensity infections are often asymptomatic. However, high burdens of strongyles and rhabitoid nematode infections have been shown to cause substantial morbidity and even mortality in primates (Anderson & May, 1979; DePaoli & Johnsen, 1978; Harper et al., 1982). An increase in habitat overlap between great ape and human populations can increase the risk of disease transmission. Great ape populations with increased interaction and habitat overlap with human populations have shown increased prevalence and diversity, genetic similarities in the parasites, as well as similar patterns of antibiotic resistance with parasites that infect humans (Guerrera et al., 2003; Lilly et al., 2002; Nizeyi et al., 1999; Rwego et al., 2008). Though transmission between humans and great apes has been difficult to prove, the similarity between the parasitic infections in the two populations suggests transmission.

Rwanda

Amongst Rwanda's diverse terrains, is the volcanic region of the Northern province. This is home to one of the last remaining populations of the mountain gorilla (*Gorilla berengei beringei*). The endangered species has only two remaining habitats. One is the Virunga Volcanoes region (part of the Albertine Rift) that covers Rwanda, Uganda and the Democratic Republic of the Congo (DRC) and the second is in the Bwindi Impenetrable Forest in Uganda. In Rwanda, the Volcanoes National Park (VNP) covers an area of around 120 km^2 and its gorilla population is estimated to be slightly over 480 individuals (Gray et al., 2013). However, the gorillas in the VNP are faced with a number of threats including habitat loss, poaching and disease transmission from humans, also known as anthropozoonosis.

This last point is of particular concern as Rwanda has a very high human population density, exacerbated by intensive movement of military and other personnel into the area during the wars of the 1990s and continued traffic due to ecotourism today (Sleeman et al., 2000). The area is characterized by high rates of poverty and is densely populated—as high as 820 people/km^2 in some rural areas (Gray & Kalpers, 2005). The majority of the population lives in rural areas, with only 19% of the population living in urban areas. Many of the people in this region have difficulty accessing health facilities and adequate care. The volcanic soil of the area prevents water from being stored in the water table. As a result of poor water and sanitation, the population is especially vulnerable to communicable diseases (DFGFI, n.d.; *Rwanda Demographic and Health Survey 2010*, 2012). The area has one of the highest prevalence of intestinal parasites in humans. Almost 83.1% of the population is infected with STHs (Mupfasoni et al., 2009).

Throughout the years, there has been an increase in overlapping environment and interactions between gorillas and humans in the Virungas area. Anthropogenic changes to habitats have especially limited gorilla habitat to patchy areas and forced primates and humans into more frequent contact (Chapman et al., 2005). Furthermore, the area lacks any sort of buffer zone between the homes and agriculture of the local population and the national park except for a three foot stone wall designed to keep out wild buffalo (*Volcanoes National Park Management Plan*, 2012). While ecotourism is aimed at increasing awareness about the endangered species, it has also led to increased contact between gorillas and human tourists. All the mountain gorillas in Rwanda are habituated, meaning that they are regularly monitored by humans, and are consequently accustomed to their presence.

Currently, there is limited understanding of the causal pathway of disease transmission between humans and gorillas in Rwanda. A better understanding about parasites in northern Rwanda can inform decisions on environmental, health and economic interventions in this region. By improving water and sanitation infrastructure as well as providing regular anti-helminthic medication, children will be able to better perform in school and absenteeism and drop-out rates could decrease. This will lead to a healthier and more productive population. In addition, addressing poor sanitation practices will not only affect the threat to human health, but can also mitigate the threat of biodiversity loss and disease to the endangered mountain gorilla populations (Sleeman et al., 2000). By preserving biodiversity, Rwanda will ensure sustained economic returns from gorilla tourism, allowing both the local population and the country as a whole to benefit. This belief in "one health"—the understanding that the health and well-being of humans, animals and the environment are intimately connected—fosters synergy between animal, environmental and human well-being to more effectively address the needs of vulnerable populations.

The objective of this study is to understand the prevalence of intestinal parasites in both human and mountain gorilla populations. The study conducted a secondary data analysis of data collected in the VNP region in Northwestern Rwanda and the Virunga Massif across the DRC, Rwanda and Uganda to address the following aims: (1) Determine the prevalence of intestinal parasites in humans in the Bisate catchment area surrounding the VNP in five separate years and the relation between age, sex and administrative sector and parasitic prevalence. (2) Determine the prevalence of intestinal parasites in Virunga mountain gorillas and the relation between age, habituation and country of residence and parasitic prevalence. (3) To compare the species prevalence and overlap of intestinal parasites in mountain gorillas and humans to inform development of hypotheses about disease transmission between the two populations.

METHODS

Ethics Statement

Ethical approval was sought by DFGFI for Bisate Health Center data from The Government of Rwanda's Ministry of Health in Kigali, Rwanda, the Ruhengeri Health District as well as the Kinigi Administrative District. Stool samples were only collected from patients who provided verbal consent after fully understanding the study. This analysis was submitted for ethical review by the Emory University Institutional Review Board (IRB), and a determination letter was issued stating that IRB review was not required. The analysis is quite specific to a particular area or Rwanda, and therefore does not meet IRB criteria for generalizable "research." There were no risks in executing this analysis.

Data Source

DFGFI gathered cross-sectional data on intestinal parasites in humans from five separate years between 2002 and 2011 as part of their Ecosystem Health Community Development Program. Data from the mountain gorilla population were collected as part of a complete census of the mountain gorillas in the Virunga Massif in 2010. The census was conducted by protected area authorities in the DRC, Rwanda and Uganda (L'Institut Congolais pour La Conservation de la Nature, The Rwanda Development Board, and The Uganda Wildlife Authority) in partnership with local non-governmental organizations (Gray et al., 2013).

Study Site

Human Study Site

The Bisate catchment encompasses a population of approximately 20,000 located in Musanze District, the western most district in the Northern province of Rwanda (I. Munyarugero, personal communication, June 2012). The catchment area is part of a series of communities that borders Rwanda's VNP.

Gorilla Study Site

The Virunga Massif consists of 450 km^2 that spans the border of northwestern Rwanda, southwestern Uganda and the eastern DRC (Gray et al., 2013; Robbins et al., 2011). The massif boasts seven volcanoes, two of which are active. The forest in the Virungas is characterized as afro-montane and has several habitat types spanning an altitude of 1500-4500m (Robbins et al., 2011). In addition, it is home to one of the last remaining populations of mountain gorillas, the other population being located in the Bwindi Impenetrable National Park in Uganda. The Virunga Massif has been home to intensive conservation efforts over the last forty years, but in recent years, has also been subject to war and instability (Gray et al., 2013). Furthermore, the region is characterized by its proximity to some of the highest rural human population density in the world (up to 820 people/km^2) contributing to its ecological vulnerability (Robbins et al., 2011).

Study Population

Human Study Population

Intestinal parasite data collection in 2002 and 2005 was part of a larger household survey and was administered to a convenience sample of residents who took part in the survey, lived within the catchment area and consented to providing a sample. The Ecosystem Health Community Development program manager collected fecal specimens from four sectors in the Bisate catchment area in 2002 and six sectors (two previous sectors and four new sectors) in 2005. In 2008, 2010 and 2011, eligible participants consisted of anyone who attended the mass drug administration at the Bisate Health Center—regardless of age or sex.

Gorilla Study Population

In 2010, a census of both habituated and unhabituated mountain gorillas was conducted in the Virunga Massif. Fecal specimens were collected and genotyped to confirm that gorillas were not double counted. The 2010 census showed a total of 480 mountain gorillas in the Virunga Massif, including 36 social groups (12 of which were unhabituated and 14 solitary males). Previous analysis of this data has shown this was a 26.3% increase in the mountain gorilla population since the last census in 2003 (Gray et al., 2013; Robbins et al., 2011).

Data Collection

Bisate Health Center Data

Setting

The local health center in the catchment area was the recruitment base for the human data collection. Once a year, DFGFI helps to organize a mass drug administration through the health center. Community health workers educated the public prior to the mass drug administrations about parasitic infections as well as water, sanitation and hygiene practices. The health center was open to whoever chose to attend. The health center was chosen as the point of testing because of its centralized location and because of DFGFI's long-standing presence in the community working on conservation efforts, as well as providing employment for many residents for gorillas tracking.

This image has been redacted due to copyright restrictions

(http://41.222.244.11/adminrwanda/)

Figure 4: The Administrative Sectors Bordering the Volcanoes National Park (Center for GIS, National University of Rwanda)

Sample Size

The sample size for each of the years in which data were collected is as follows: 2002-2003 (n=1002), 2005 (n=936), 2008 (n=616), 2010 (n=988), 2011 (n=966). Justification for the sample size was that about 10% of those who came to the deworming campaign were sampled.

Participant Selection

Community health workers promoted the deworming efforts to the public prior to the mass drug administrations and the health center was open to whoever chose to attend. Data were collected from both minors and adults. In 2002 and 2005, local leadership identified "high-risk" people in the catchment area and tried to focus the sampling population. In 2008, 2010 and 2011, testing for parasites was done on a convenience sample of health center attendees.

Timeline

Data were collected during the following times: November 6, 2002-February 26, 2003, July 15, 2005- October 25, 2005, June 2008-August 2008, June 21, 2010- August 23, 2010, and June 20, 2011- July 2011. The majority of data collection occurred during the dry season.

Data Collection

Villagers were asked to bring stool specimens to the Bisate Health Center. Direct fecal smears were prepared and analyzed via microscopy by trained lab technicians at the health center and the data were recorded.

The following variables and identifiers were included in the datasets: name, sex, age, address (village, cell, sector) and results from fecal smears (type of parasite recorded as presence/absence of parasite). Only data from 2005 included lab results on egg counts in the fecal smears and it was recorded as number of eggs per gram of feces. Data from 2005 are also de-identified and do not include any demographic information (sex, age and administrative sector).

The direct fecal smears procedure used at the Bisate Health Center for parasitic surveillance was a follows: a slide was prepared with one drop of physiologic saline solution and one drop of Lugol's Iodine (10% Iodine solution) on the left and right side of the slide, respectively. A sample of human fecal matter was mixed with each of the solutions on the slide, making sure to keep the two solutions separate. Each slide was then covered with a cover slip and examined microscopically. The Lugol-sample mix was used to identify cysts, while the physiologic saline sample mix was used to identify vegetative forms and parasite shapes.

Mountain Gorilla Census Data

Stool samples were collected from every mountain gorilla in the Virungas for genotyping as well as for examination of intestinal parasites. Further details on the methodology of the census can be found in the 2013 article by Gray et. al (Gray et al., 2013).

Non-invasive assessment of gastrointestinal parasite infection of gorillas was conducted. All samples were examined macroscopically for consistency, presence of blood, mucus, tapeworm proglottids, and adult or larval nematodes. An aliquot of 2g of feces was then placed in a polypropylene tube containing 15 ml of 10% neutral buffered formalin and stored at ambient temperature (20–25°C) until analysis. From each fecal sample, helminth eggs and larvae were recovered via sodium nitrate floatation and fecal sedimentation. Both techniques utilized standardized methodologies previously described by Gillespie (Gillespie 2006). Slides prepared by each method were examined with compound microscope, and parasites were identified on the basis of egg or larvae coloration, shape, contents, and size. Each parasite species per sample was quantified and representatives measured to the nearest 0.1 mm with an ocular micrometer. If needed, one drop of Lugol's iodine solution was added to aid in species identification.

Data Management

Human Parasite Data

The DFGFI data on human parasites from the Bisate Health Center were extensively cleaned. New dichotomous variables (presence/absence) were created for each parasite. Results for each subject were consolidated into one record for ease of analysis. All parasite names were standardized. In the case where multiple parasite tests were done on the same subject during the same year but with inconsistent results, all replicate records were deleted. Information about egg count (2005 only) and trophozite or cyst forms found in fecal samples were not included in the newly created dichotomous variables. A categorical age variable was created to reflect differences between young children (0-5), school-aged children (6-12 and 13-18), adults (19-50) and the elderly (51 and over). School-aged children were defined according to the Ministry of Education age ranges for primary and secondary school children (*Rwanda Education Statistics*, 2012).

New variables were also created to represent polyparasitism (infection with two or more, three or more, or four or more species of parasites), and various taxa of intestinal parasites (cestoda, terematoda, nematoda and protozoa). Also, parasites found in the human population were categorized according to the environmental classification for excreta-related diseases published by Faechem (Table 1) (Faechem, Bradley, Garelick, & Mara, 1983).

Table 1: *Environmental classification of excreta-related diseases found in humans of the Bisate catchment area, Rwanda from 2002-2011 (Modified from Feachem R.G. et. al.)*

Category and epidemiological Features	Infection	Environmental Transmission Focus	Major Control Measures
I. Non-latent; Low Infective Dose	Amoebiasis *(E.histolytica/ E.dispar* *Entamoeba coli* *Iodamoeba butschlii)* Balantidiasis Enterobiasis Giardiasis Hymenolepiasis Trichomoniasis	Person-to-person	Increased water access Improved personal hygiene Health education Improved housing Provision of toilets
II. Non-Latent; Medium or High Infective Dose; Moderately Persistent; Able to multiply	N/A	N/A	N/A
III. Latent and Persistent; No Intermediate Host	Ascariasis Hookworm Infection Strongyloidiasis Trichuriasis Trichostrongyliasis	Fecal contamination of soil, fields and crops	Provision of toilets Treatment of excreta prior to land application
IV. Latent and Persistent; Animal Intermediate Host	Taeniasis	Animal access to human feces Humans eating improperly cooked meet	Provision of toilets Treatment of excreta prior to land application Better cooking and meat inspection
V. Latent and Persistent; Aquatic Intermediate Hosts	Paragonomiasis	Contact with water Foodborne via raw or smoked fish or water plants	Provision of toilets Treatment of excreta prior to discharge Control of animal reservoirs Control of intermediate hosts Cooking of water plants and fish Reducing water contact
VI. Spread by Excreta Related Insects	N/A	N/A	N/A

A combined dataset was created with data from 2008, 2010 and 2011. The combined dataset did not include 2005 data because of the lack of identifiers, or 2002 data because administrative sector, cell and village boundaries were different at that time (the Government of Rwanda redrew administrative boundaries in 2002) (I. Munyarugero, personal communication, June 2012).

Gorilla Parasite Data

Minimal cleaning was needed for the Virunga mountain gorilla data, however minor spelling mistakes were corrected. New dichotomous variables (presence/absence) were created for each parasite. A new dichotomous variable was also created for the presence of any parasites. Similar to the human data, new variables were also created to represent polyparasitism and for various taxa of intestinal parasites. Parasites that were labeled as “larvated strongyles” are unknown and were combined with the group “unknown strongyles.” Since gorilla age/sex was determined by fecal specimen diameter, any age/sex groups that were unidentifiable because the stool sample was smashed, or other reasons, were grouped together as “unknown.”

Statistical Analysis

Descriptive statistics were analyzed separately for all parasites and taxa of parasitic infections for both the Bisate Health Center and the Virunga mountain gorilla datasets. Frequencies and proportions were recorded for all categorical variables. Mean egg count and standard deviation was recorded for Virunga mountain gorilla data. Descriptive statistics were calculated for each of the five years of data from the Health Center.

In order to examine the relationship between variables of interest and parasitism, a chi-square test for association was conducted to compare frequencies of parasitic disease across each of the variables of interest, including age group, administrative sector and sex in the Bisate

Health Center data, and age group, country and habituation status in the Virunga mountain gorillas data.

The combined 2008, 2010 and 2011 Bisate Health Center dataset was used to further examine the relation between age group, sex and administrative sector and parasitic infection. Logistic models were built to evaluate the association between sex, age group and administrative sector and parasitism, a dichotomous outcome. First, crude models relating each of the variables to parasitism were created and the odds ratio and confidence interval were recorded. Next, interactions of year and age, as well as year and sector, were evaluated by inclusion of interaction terms. The significance of the interaction term was evaluated in order to determine the inclusion of the term in the model. Confounders were also assessed for significance in the associative model. The odds ratios from the resulting final model were compared to the crude models and the associations of sex, age group and administrative sector, given the presence multiple factors, were noted. The final model was assessed for collinearity through linear regression and examining the variance inflation factor.

As infection with multiple species of parasites is associated with greater morbidity, polyparasitism in this sample was explored. The logistic modeling process was replicated with the outcome of polyparasitism.

A sub-analysis was conducted with this data to examine the association between the same three demographic variables (age group, sex, sector) and specific environmental classifications of parasites. The majority of parasite species are non-latent and have a low infective dose (Category I), or are latent and persistent with no animal intermediate host (Category III). Category I parasites are primarily transmitted person-to-person, while Category III parasites are transmitted via soil, crops and fields contaminated with fecal matter.

Due to the high prevalence of parasitic infections transmitted through fecal-oral pathways and person-to-person contact, the analysis was expanded to examine how associations with demographic variables changed for these two specific classifications of parasites. Two logistic models were used to explore this relationship, one where Category I parasites were the outcome, and the other with Category III parasites as the outcome. Once more, potential confounders were examined.

Because parasitism is the norm in nature, polyparasitism was the only outcome modeled for the Virunga mountain gorilla population, rather than the presence of any parasitism in general (>90% of the population). Logistic models were built to evaluate the association between habituation status, age/sex group, country of residence and parasitism, a dichotomous outcome. Crude and final models were then compared. The same methodology used to model parasitism in humans was used for the mountain gorilla data. An interaction term between country of residence and habituation status was evaluate for significance for inclusion in the final model.

Comparison between human and gorilla population

Differences in parasite prevalences between the human and mountain gorilla populations were compared qualitatively due to methodological constraints.

Assumptions

A number of assumptions were made in the analysis of the data. First, missing values were ignored in the statistical analysis. However, there were very few missing values, so the analysis was minimally affected. Next, in conducting logistic regression, it is assumed that the logit is linearly associated with x (the risk factors of interest). Furthermore, chi-square tests assumed (1) independent random samples and (2) expected cell-count greater than or equal to 5. Lastly, a Variance Inflation Factor (VIF) of greater than ten was considered an indicator of collinearity. All statistical tests were evaluated at a 5% significance level ($\alpha=0.05$) unless otherwise stated. Data management was conducted in Microsoft Excel (2008) software, and all statistical analysis was conducted in SAS Version 9.3 software (SAS Institute, Cary, N.C). Confounders were identified by checking if the odds ratios of risk factors of interest were within 10% of each other in the crude and adjusted models. The effect of clustering within gorillas and humans in families was ignored.

RESULTS

Table 2: *Human population demographics from the Bisate catchment, Rwanda (2002, 2008, 2010, 2011)*

Demographic Characteristics	**n (%)** 2002† (n=988)	**2008 (n=616)**	**2010 (n=982)**	**2011 (n=996)**
Sex				
Female	505 (51.1)	411 (66.7)	700 (71.2)	664 (66.7)
Age*				
0-5	304 (30.8)	113 (18.3)	88 (9.0)	127 (12.7)
6-12	30 (3.0)	144 (23.4)	96 (9.8)	135 (13.6)
13-18	10 (1.0)	46 (7.5)	40 (4.1)	48 (4.8)
19-50	598 (60.6)	209 (33.9)	514 (52.3)	512 (51.4)
51+	45 (4.6)	104 (16.9)	244 (24.9)	174 (17.5)
Sector**				
Bisate	250 (25.4)	--	--	--
Gitaraga	261 (26.5)	--	--	--
Kareba	240 (24.3)	--	--	--
Kinigi	--	615 (99.8)	742 (75.6)	820 (82.3)
Musanze	--	--	206 (21.0)	145 (14.6)
Nyabitsinde	235 (23.8)	--	--	31 (3.1)
Shingiro	--	1 (0.2)	34 (3.5)	--

*n=2 missing in 2002; n=1 missing in 2008
**n=2 missing in 2002
†Sector boundaries were changed in 2002

Descriptive statistics for sex, age and sector of residence for the human populations sampled at the Bisate Health Center in 2002, 2008, 2010 and 2011 illustrated that the majority of those sampled were female (over 50%) and were between 19-50 years of age (Table 2). Fewer than 10% of those sampled were between 13-18 years. In 2008, 2010 and 2011, over 75% of those sampled were overwhelmingly from the Kinigi sector. As sector boundaries were redrawn in 2002, it was difficult to compare sector distribution in 2002 to that of subsequent years. However, the sample from 2002 was fairly evenly distributed between the geographic sectors.

Table 3: *Virunga mountain gorilla population demographics in 2010 (n=400)*

Demographic Characteristics	**n (%)**
Status	
Habituated	270 (67.5)
Age/Sex Group*	
Adult Female	46 (11.5)
Infant	47 (11.8)
Juvenile	53 (13.3)
Medium	148 (37.0)
Silverback	60 (15.0)
Solitary Silverback	11 (2.75)
Unknown	35 (8.8)
Country	
Rwanda	207 (51.8)
DRC	179 (44.8)
Uganda	14 (3.5)
*Age/Sex group was based on stool specimen diameter. Where age could not be identified from the specimen, age was categorized as unknown.	

Demographic data from the Virunga mountain gorilla population indicated that the majority of gorillas resided in Rwanda or the DRC, with 51.8% and 44.8% respectively (Table 3). In more recent years, national park services have increased the surveillance of gorillas, and so 67.5% of the gorillas in the Virunga Massif were habituated.

Table 4: *Intestinal parasites examined in stool specimens from humans in 2002, 2005, 2008, 2010 and 2011 and the Virunga mountain gorillas in 2010*

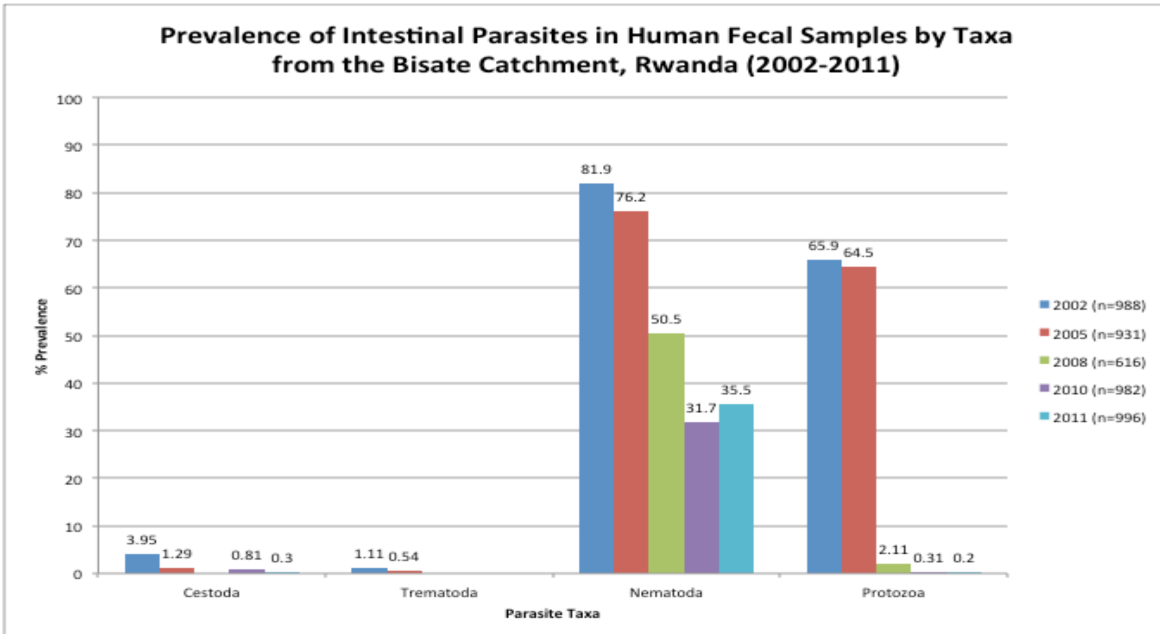
	Humans					Gorillas
Taxa of Parasite	**2002**	**2005**	**2008**	**2010**	**2011**	**2010**
Cestoda						
Anoplocephalidae						+
Hymenolepis*	+				+	
Taenia	+	+		+	+	
Trematoda						
Paragonimus	+	+				
Schistosoma						+
Nematoda						
Ascaris	+	+	+	+	+	+
Capillaria						+
Enterobius	+	+				+
Hookworm	+	+	+	+	+	+
Mammomonogamus						+
Oesophagostomum						+
S.stercoralis		+	+	+	+	
S.fuelleborni						+
Trichuris	+	+	+	+	+	+
Trichostrongylus				+	+	+
Protozoa						
Balantidium	+	+				
Entamoeba coli	+	+	+	+	+	
E.histolytica/E.dispar* **	+	+	+	+	+	
Giardia			+	+	+	
Iodamoeba					+	
Trichomonas				+	+	
Fungal						
Candida			+	+	+	
Unknown						+

Genera in **bold** are known for zoonoses between humans and non-human primates
Genera highlighted in gray are were found across all years or both humans and gorillas.
*Includes both *Hymenolepis nana* and *Hymenolepis diminuta*
** *E.histolytica* cannot be differentiated from *E.dispar* without PCR

Considerable temporal variation was observed in the parasites detected in the human population. The only parasites identified in all five years were the STHs, as well as *Entamoeba coli* and *E. histolytica/E.dispar* (Table 4). Still fewer parasites were identified in both the human and gorilla populations (*Ascaris,* hookworm, *Trichuris* and *Trichostrongylus*).

Table 5: *Prevalence of intestinal parasites in human stool specimens in the Bisate catchment, Rwanda (2002, 2005, 2008, 2010 and 2011)*

Taxa of Parasite	**n (%)**				
	2002 (n=988)	**2005 (n=931)**	**2008 (n=616)**	**2010 (n=982)**	**2011 (n=996)**
Any Parasite	907 (91.8)	838 (90.0)	356 (57.8)	510 (51.9)	518 (52.0)
Cestoda					
Hymenolepis	6 (0.6)	--	--	--	1 (0.1)
Taenia	33 (3.3)	12 (1.3)	--	8 (0.8)	2 (0.2)
Trematoda					
Paragonimus	11 (1.1)	5 (0.5)	--	--	--
Nematoda					
Ascaris	691 (69.9)	662 (71.1)	274 (44.5)	299 (30.5)	326 (32.7)
Enterobius	2 (0.2)	1 (0.1)	--	--	--
Hookworm	61 (6.2)	21 (2.3)	4 (0.7)	5 (0.5)	0 (0.0)
S.stercoralis	--	0 (0.0)	3 (0.5)	1 (0.1)	3 (0.3)
Trichostrongylus	--	--	--	1 (0.1)	3 (0.3)
Trichuris	442 (44.7)	263 (28.3)	53 (8.6)	11 (1.1)	26 (2.6)
Protozoa					
Balantidium	63 (6.8)*	4 (0.4)	--	--	--
Entamoeba coli	710 (71.9)	627 (67.4)	54 (8.8)	206 (21.0)	222 (22.3)
E.histolyltica/ E.dispar	679 (68.7)	641 (68.9)	37 (6.0)	6 (0.6)	8 (0.8)
Giardia	--	--	7 (1.1)	7 (0.7)	7 (0.7)
Iodamoeba	--	--	--	--	2 (0.2)
Trichomonas	--	--	--	2 (0.2)	0 (0.0)
Fungal					
Candida	--	--	11 (1.8)	18 (1.8)	29 (2.9)
Polyparasitic Infections					
≥2	800 (81.0)	711 (76.4)	69 (11.2)	32 (3.3)	79 (7.9)
≥3	646 (65.4)	511 (54.9)	4 (0.7)	1 (0.1)	0 (0.0)
≥4	331 (33.5)	171 (18.4)	1 (0.2)	0 (0.0)	0 (0.0)
*n=1 missing					



Overall prevalence of parasitic infection in humans decreased from 91.8% in 2002 to

Figure 5: Prevalence of intestinal parasites in human fecal samples by taxa from the Bisate catchment, Rwanda (2002, 2005, 2008, and 2011)

52.0% in 2011 (Table 5). However, levels of parasitic infection seem to plateau around 50% starting in 2008. Infections of nematodes were the most common in all five years, followed by protozoan infections (Figure 5). Very few trematodes and cestodes were observed. At the species level, *Ascaris* was the most commonly detected infection, with over half of the sample population infected in 2002 and 2005, and 32.7% of the sample population infected in 2011. *Trichuris* was also highly prevalent in 2002 with almost 45% of the sample population infected, but there was a dramatic decrease to 2.6% by 2011. *Entamoeba coli* infections were also prevalent over the years and ranged from 71.9% in 2002 to 22.3% in 2011. While *Entamoeba coli* and *Iodamoeba butschlli* are non-pathogenic intestinal protozoa, they may serve as an indicator of environmental conditions that may expose the population to other pathogens associated with fecal contamination. The *Candida* fungal infections are similar in this way in that they are generally co-infections of parasitic infections. *Entamoeba histolytica/Entamoeba dispar* infections decreased drastically from 2002-2011. The prevalence of polyparasitism was high in 2002 (81.0%), but decreased to less than 10% by 2011.

The overwhelming majority of infections in the human study population fell into Category I and Category III environmental classifications, with smaller representation from Category IV and V (Appendix: Figure 9). Overall prevalence within each category decreased over the five years and was consistent with the overall trends observed in parasite infection in this sample population.

Table 6: *Prevalence of intestinal parasites in mountain gorilla stool specimens from the Virunga Massif in 2010 (n=400)*

Taxa of Parasite	**n (%)**	**Avg Egg Burden* (SD)**
Any Parasite	364 (91.0)	49.4 (62.5)
Cestoda		
Anoplocephalidae	302 (75.0)	27.8 (41.2)
Trematoda		
Schistosoma	3 (0.8)	0.01 (0.12)
Nematoda		
Ascaris	2 (0.5)	0.01 (0.16)
Capillaria	1 (0.3)	0.002 (0.05)
Enterobius	6 (1.5)	0.02 (0.17)
Hookworm	12 (3.0)	0.1 (1.13)
Mammomonogamus	13 (3.3)	0.1 (1.73)
Oesophagostomum	205 (51.3)	7.6 (21.0)
S.fuelleborni	32 (8.0)	0.6 (3.93)
Trichostrongylus	259 (64.8)	9.3 (21.3)
Trichuris	2 (0.5)	0.005 (0.07)
Unknown Strongyles	212 (53.0)	4.16 (8.75)
Unknown	3 (0.8)	0.008 (0.09)
Polyparasitic Infections		
≥2	326 (81.5)	--
≥3	268 (67.0)	--
≥4	185 (46.3)	--

*Average egg burden is defined as the average number of eggs per gram in a stool specimen of a gorilla
**n=1 missing

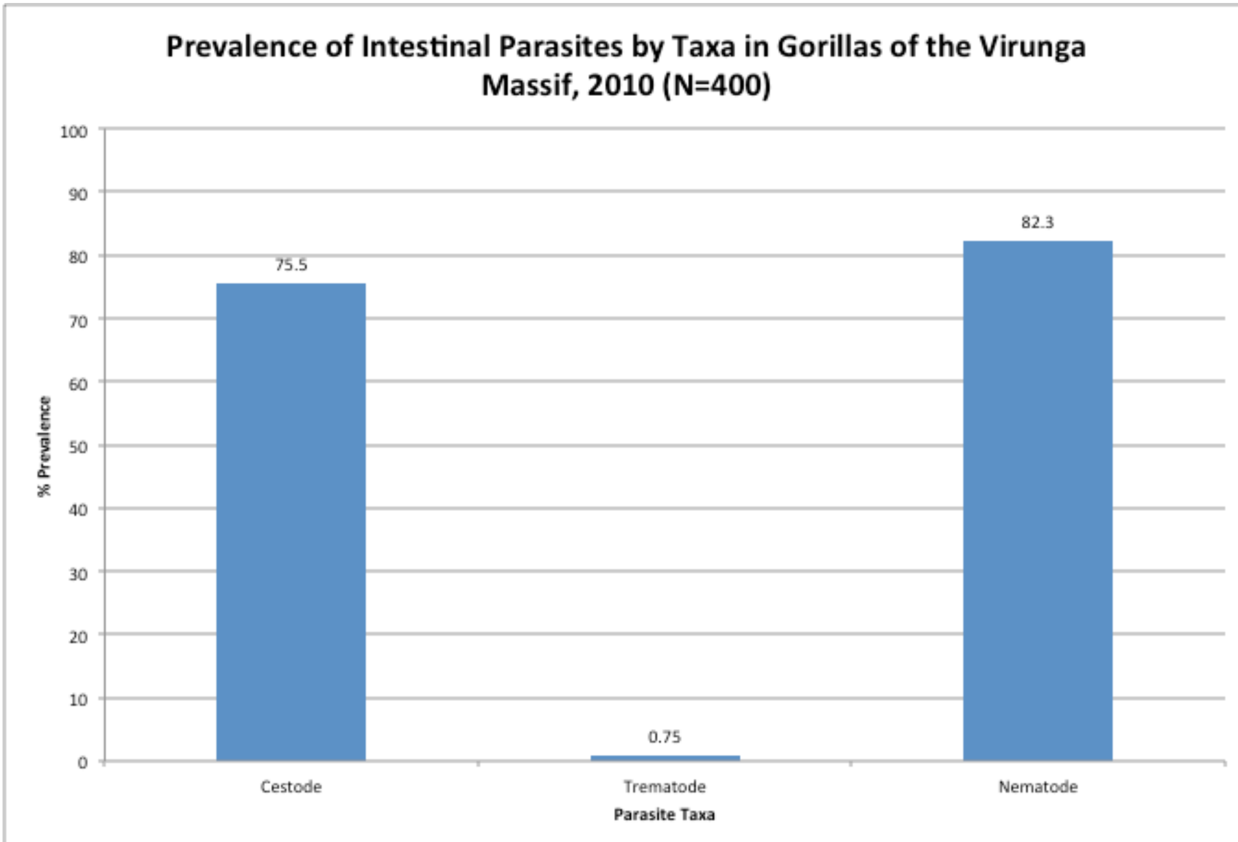


Figure 6: Prevalence of intestinal parasites by taxa in gorillas of the Virunga Massif, 2010 (N=400)

Examination of the Virunga mountain gorilla data showed that 91% of the gorillas were infected with intestinal parasites (Table 6). The majority of these infections were from nematodes or cestodes (Figure 6). No data were available on the presence of protozoan infections as laboratory methodologies were limited. Most of the gorillas were infected with *Anaplocephalidae* family (75.0%) as well as *Oesophagostomum* (51.0%). Furthermore, polyparasitism was very common in the mountain gorilla population with 81.0% infected with two or more species of parasites, and 46.3% infected with four or more species of parasites.

Table 7: *Prevalence of types of intestinal parasites stratified by sex, age category and sector in human stool specimens from combined 2008, 2010, 2011 data, Bisate catchment, Rwanda*

	n (%)				
Demographic Characteristic	**Cestodes (n=11)**	**Nematodes (n=976)**	**Protozoa (n=18)**	**Any Parasite (n=1384)**	**Polyparasitism (n=180)**
Sex					
Female	6 (54.6)	681 (69.8)	15 (83.3)	956 (69.1)	145 (80.6)
P-Value*	--	0.3	0.2	0.4	<0.001
Age					
0-5	1 (9.1)	112 (11.5)	3 (16.7)	154 (11.1)	15 (8.3)
6-12	1 (9.1)	155 (15.9)	2 (11.1)	210 (15.2)	26 (14.4)
13-18	0 (0.0)	55 (5.6)	1 (5.6)	74 (5.4)	8 (4.4)
19-50	6 (54.6)	448 (45.9)	10 (55.6)	655 (47.3)	92 (51.1)
51+	3 (27.3)	206 (21.1)	2 (11.1)	291 (21.0)	39 (21.7)
P-value*	--	0.2	--	0.1	0.4
Sector					
Kinigi	10 (90.9)	815 (83.5)	17 (94.4)	1144 (82.7)	167 (92.7)
Musanze	1 (9.1)	137 (14.0)	1 (5.6)	206 (14.9)	11 (6.1)
Shingiro	0 (0.00)	24 (2.5)	0 (0.00)	34 (2.5)	2 (1.1)
P-value*	--	0.9	--	0.1	<0.001
* P-values are reported only where expected cell count was adequate for a chi-square test					

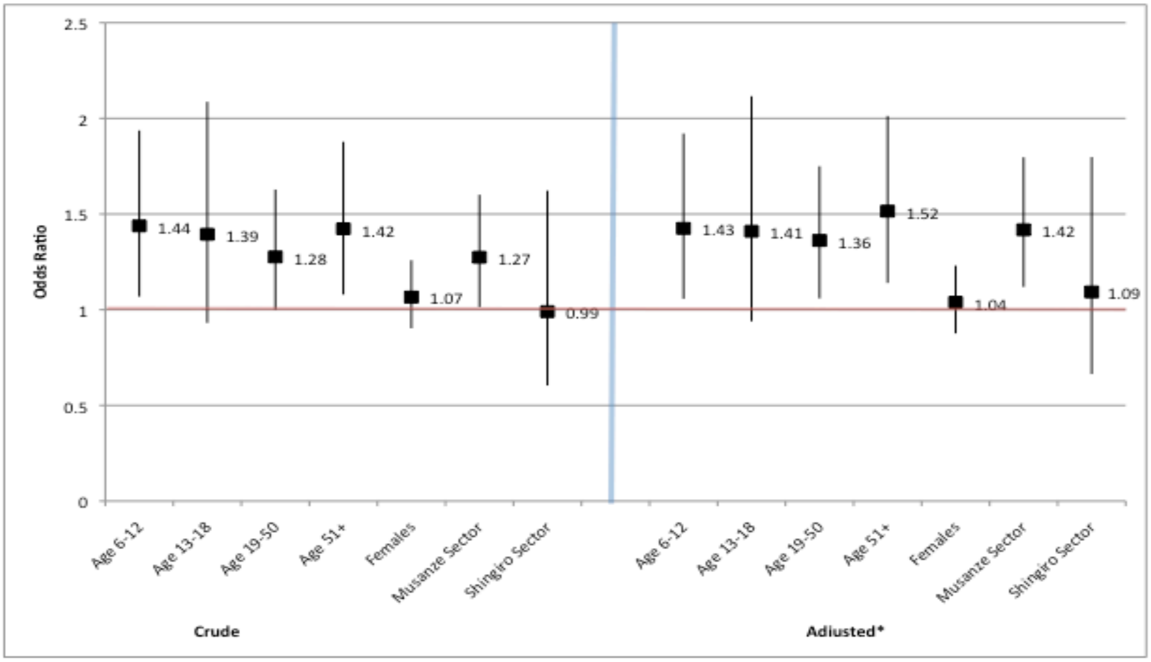
Using the combined dataset of the cross-sectional data from 2008, 2010 and 2011, a bivariate analyses of the taxa and environmental classification categories of various parasites with sex, age and administrative sector, identified trends in parasitism. Analysis of intestinal parasites was stratified by sex, age and sector. The prevalence of polyparasitism was the only variable that was significantly different across sectors and sexes (p-value<0.001) (Table 7). No significant differences were found between environmental classification category and any of the demographic variables in the combined dataset (Appendix: Table 11). In contrast, specific taxa and environmental classification categories were significantly different across sex, age and sector when 2002 was examined separately (Table 10 & Appendix: Table 12). This suggested that there were significant changes in both the population distribution as well as levels of parasitism from 2002 to later years.

Table 8: *Prevalence of types of intestinal parasites in Virunga mountain gorillas, stratified by habituation status, age group in 2010*

Demographic Characteristic	n (%) Cestoda (n=302)	Trematoda (n=3)	Nematoda (n=330)	Any Parasite (n=364)	Polyparasitism (n=326)
Status					
Habituated	196 (64.8)	3 (100.0)	217 (65.8)	242 (66.5)	214 (65.6)
P-value*	0.05	--	0.11	0.2	0.1
Age					
Adult Female	38 (12.6)	0 (0.0)	39 (11.8)	43 (11.8)	41 (12.6)
Infant	23 (7.62)	2 (66.7)	31 (9.4)	36 (9.9)	26 (8.0)
Juvenile	38 (12.6)	0 (0.0)	41 (12.4)	47 (13.0)	43 (13.2)
Medium	117 (38.7)	1 (33.3)	128 (38.8)	140 (38.5)	128 (39.3)
Silverback	53 (88.3)	0 (0.0)	52 (15.8)	56 (15.4)	54 (16.6)
Solitary Silverback	9 (3.0)	0 (0.0)	8 (2.4)	9 (2.5)	9 (2.8)
Unknown	24 (8.0)	0 (0.0)	31 (9.4)	33 (9.0)	25 (7.7)
P-value*	<0.001	--	0.03	--	<0.001
Country					
Rwanda	159 (52.7)	2 (66.7)	187 (56.7)	197 (54.1)	184 (56.4)
DRC	130 (43.1)	1 (33.3)	133 (40.3)	154 (42.3)	130 (39.9)
Uganda	13 (4.0)	0 (0.0)	10 (3.0)	13 (3.6)	12 (3.7)
P-Value*	0.19	--	<0.001	0.007	<0.001

* P-values are reported only where expected cell count was adequate for a chi-square test

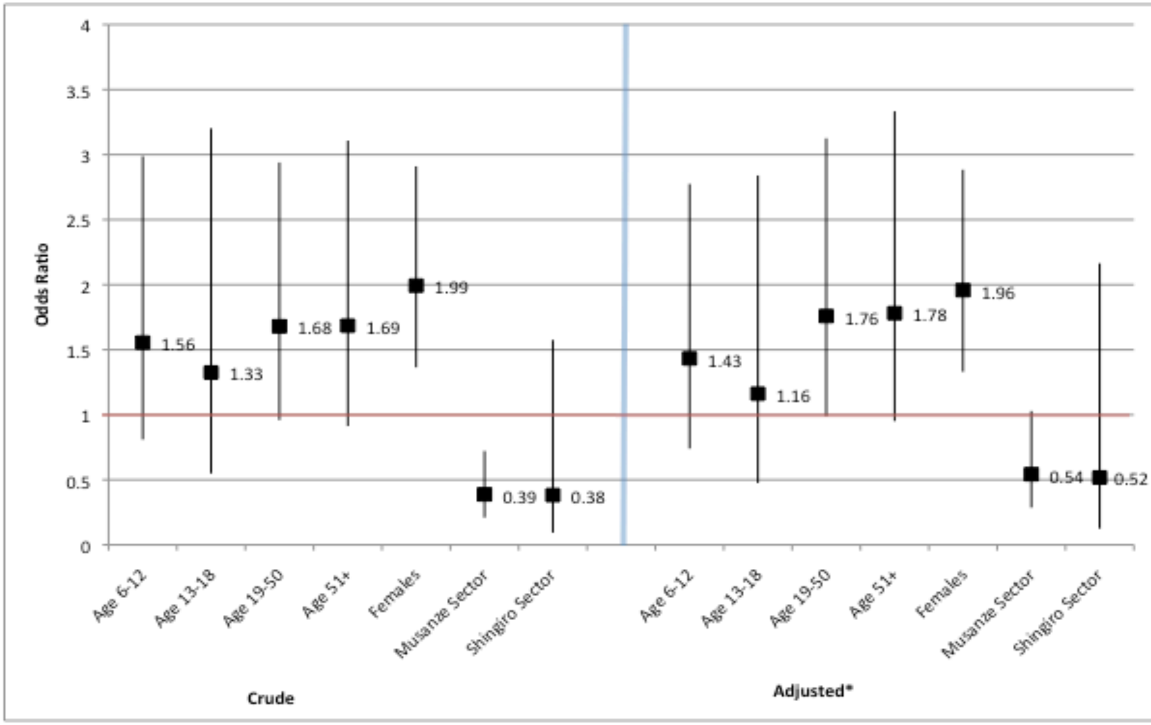
Stratified analysis suggested that prevalence of polyparasitism changed significantly in the gorilla population across age group and country of residence (p-value<0.001) (Table 8). General parasitic infection varied significantly across countries with higher prevalence in Rwanda compared to the DRC and Uganda (p-value=0.007). However, few significant differences were found between specific parasitic taxa and demographic characteristics. Only the prevalence of nematode infections was significantly different between countries (p-value<0.001), and the prevalence of cestode infections was significantly different across age groups (p-value<0.007) with the highest prevalence in silverbacks.



Reference Categories: Age 0-5, Males, Kinigi Sector; *Adjusted model includes Age, Sex, Sector and Year

Figure 6: Logistic regression crude and adjusted associations of age, sex and sector with parasitism in the human population, Bisate catchment, Rwanda

Four final models were constructed from the combined Bisate Health Center data for each of four outcomes: any parasitism, polyparasitism, Category I parasites and Category III parasites. Figure 6 illustrates the crude and adjusted odds ratios and confidence intervals of the primary variables of interest (sex, age group and sector) with the outcome of parasitism. Both interaction terms (year and age, as well as year and sector) were discarded from the model. Year was included in the model as a confounder because prevalence of parasitism and polyparasitsm varied significantly between years. In contrast to the stratified analysis which showed no significant difference of age as a whole with parasitism, mulitivariate analysis shows significant associations of individual age groups with parasitism.When controlling for the effects of other factors, age greater than five years was significantly associated with parasitism. Specifically, school-aged children ages 6-12 years had a 44% greater crude odds of infection than children ages 0-5 years [OR: 1.44 [1.07, 1.94]]. In addition, those aged 51 years and over had 42% greater odds of infection compared to under-five year olds [OR: 1.42 [1.08, 1.89]]. Both age categories maintained a greater association with any parasitic infection after adjusting for sex and sector in the final model. Children ages 6-12 years had a 43% greater odds [OR: 1.43 [1.06, 1.92]], and the elderly had a 52% greater odds of parasitic infection than young children [OR: 1.52 [1.14, 2.01]]. In addition, the final model indicated that the 19-50 year-old age group had a 36% greater odds of parasitic infection than 0-5 year-olds [OR: 1.36 [1.06, 1.75]]. Additionally, people living in the Musanze sector had a 42% greater odds of any parasitic infection than those living in the Kinigi sector [OR: 1.42 [1.12,1.80]].



Reference Categories: Age 0-5, Males, Kinigi Sector; *Adjusted model includes Age, Sex, Sector and Year

Figure 7: Logistic regression crude and adjusted associations of age, sex and sector with polyparasitism in the human population, Bisate catchment, Rwanda

Logistic regression for crude and adjusted associations of demographic variables with polyparasitism showed several significant associations. Logistic regression showed no significant associations between age and polyparasitism, and this was consistent with results from the stratified analysis. The crude model showed that females had twice the odds of polyparasitic infection compared to males [OR: 2.00 [1.36, 2.91]]. In the final model, the association between being female and polyparasitism remained significant with a 96% greater odds of infection than males [OR: 1.96 [1.33, 2.88]] (Figure 7). The crude model indicated that living in Musanze sector was protective and associated with a 61% lower odds of infection than living in Kinigi sector [OR: 0.39 [0.21, 0.72]]. However, this association was no longer significant in the adjusted model.

Two supplemental models were created that examined the outcome of the presence or absence of Category I and Category III parasites. The final adjusted model for the outcome of Cateogry I parasitism included year and Cateogry III parasites as confounders, and the final adjusted model for the outcome of Category III parasitism included year and Category I parasites as confounders. The year variable was included as a confounder because parasitism varies significantly across years. Category I and III variables were included as confounders because their transmission pathways are related to similar conditions and an individual can have multiple infections that fall under different environmental classification cateogries.

These supplemental models showed few significant associations of age groups or sectors with Category I or Category III parasites (Appendix: Figure 10 & Figure 11). In the crude model, only 19-50 year-olds had a 60% greater odds [OR: 1.6 [1.15, 2.25]] of infection with Category I parasites and a 49% greater odds of infection in the adjusted model [OR: 1.49 [1.06, 2.08]] compared to 0-5 year-olds. For Category III parasites, none of the variables were significant in the crude models, but in the multivariate model there were some significant associations. In the final Category III model, the elderly (over 51 years) had a 51% greater odds of Category III parasitism than 0-5 year-olds [OR: 1.51 [1.14, 2.03]] and living in Musanze sector was associated with a 39% greater odds of Category III parasitism than living in Kinigi sector [OR: 1.39 [1.09, 1.78]].

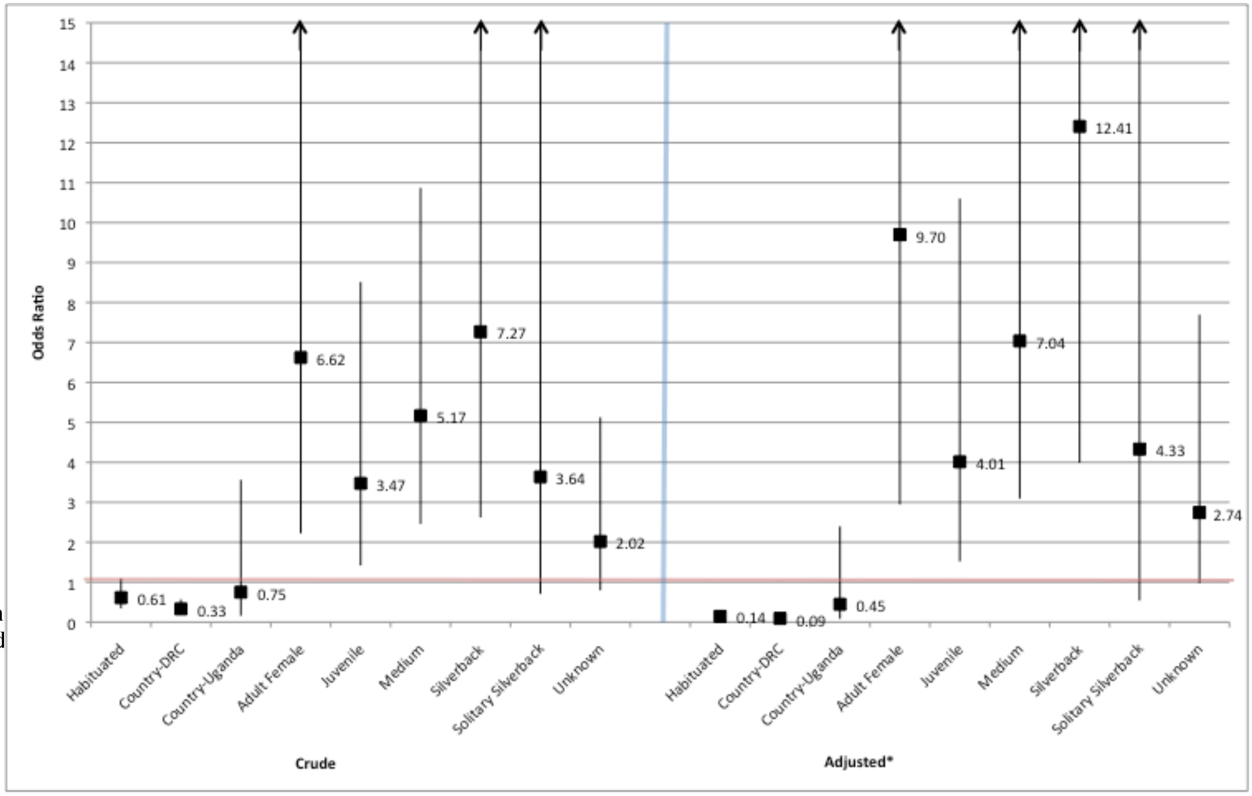


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Figure 8: Logistic regression crude and adjusted associations of habituation status, age/sex group and country of residence with polyparasitism in Virunga mountain gorillas

The final logistic regression model for Virunga mountain gorilla demographic data and the outcome of polyparasitism included only the risk factors of interest—age, habituation status and country of residence. An interaction term of habituation status and country of residence was tested to account for a greater number of habituated gorillas in Rwanda, but this term was not significant at the 5% level in the model and was consequently removed. In contrast to the analyses of parasitic infections in humans, all the risk factors (habituation status, age/sex group and country) were significantly associated with polyparasitism in gorillas. Interestingly, habituation seemed to be associated with lower odds of polyparasitism (Figure 8). Habituated gorillas was associated with a 37% lower odds of infection with multiple parasites compared to unhabituated gorillas in the crude model [OR: 0.61 [0.35, 1.10]]. In the final model adjusted for country and age group, habituation was still independently associated with a 86% lower odds of infection with multiple parasite species [OR: 0.14 [0.07, 0.31]]. In addition, gorillas in the DRC had a 67% lower odds of polyparasitism in the crude model [OR: 0.33 [0.19, 0.57]], and a 91% lower odds of polyparasitism in the adjusted model [OR: 0.09 [0.05, 0.20]] compared to gorillas in Rwanda. All the age/sex groups had greater odds of polyparasitism compared to infant gorillas. However, these odds ratios had large confidence intervals, and thus, precision was low for the estimate of the degree of association. The results from logistic regression in the Virunga mountain gorilla population are consistent with the significant difference found in stratified analysis conducted for age/sex group and country of residence. However, with regard to habituation status, stratified analysis showed no significant difference in polyparasitic infections between habituated versus unhabituated mountain gorillas.

Table 9: C*omparison of intestinal parasite prevalence in humans and mountain gorillas of Rwanda (2010)*

	Prevalence in Population (%)				
Species	**Hookworm**	***Ascaris***	***Trichuris***	***Trichostrongylus***	**Polyparasitism**
Humans (n=982)	0.5	30.5	1.1	0.1	3.3
Gorillas (Rwanda)	3	0.5	0.5	64.8	81.5

The prevalence of parasites found both in the mountain gorillas of Rwanda as well as the human population of Bisate could only be compared qualitatively for 2010 because this was the only year in which data was gathered on both humans and gorillas. While the overall population prevalence of polyparasitism was not similar in mountain gorillas (81.5%) and humans (3.3%), it is noteworthy that there is species overlap in infections (Table 9). The four parasites compared were *Ascaris,* hookworm, *Trichuris* and *Trichostrongylus*. The results show different patterns of infection in the humans and mountain gorillas. In particular, *Ascaris* infection was highest in humans (30.5%), yet lowest in the mountain gorillas (0.5%). Meanwhile, *Trichostrongylus* was highest in mountain gorillas (64.8%), but lowest in humans (0.1%). However, because the data and methodology were limited, further comparison of the prevalence of parasites common to both populations could not further examined.

DISCUSSION

The results of this analysis provide a series of snapshots of parasitic prevalence in humans and mountain gorillas around the Virungas.

Parasitic Infections in the Bisate Area

Soil-transmitted Helminth Infections

General trends in prevalence of parasite infection in the Bisate sample of the local population agree with previously published patterns. Data from the 2008 National Health Report in Rwanda, suggest that STH prevalence was highest in the Northern Province at 83.1% (*National prevalence survey on soil-transmitted helminths & schistosomiasis in school-aged children*, 2008). The prevalence in our study area for the same year is lower—50.5% of parasitic infections were due to nematodes (STH and *Strongyloides*). The decreased parasite prevalence may be due, in part, to yearly mass drug administration programs run through the Bisate Health Center.

In addition, this study found that *Ascaris* and *Trichuris* were the most prevalent helminth infections. Previous studies in Rwanda and elsewhere in East Africa have found similarly high levels of these low-intensity infections in study populations (Brooker et al., 2009; Mupfasoni et al., 2009; *National prevalence survey on soil-transmitted helminths & schistosomiasis in school-aged children*, 2008). Prevalence of hookworm infection seem to be lower in the Bisate catchment compared to those reported by other studies in East Africa. Our analysis found that fewer than 5% of the sample population were infected with hookworm in each of the five years, yet other studies in East Africa report hookworm prevalence more comparable to that of *Ascaris* or *Trichuris* (Brooker et al., 2009; Kabatereine et al., 2005). Hookworms prefer moist, tropical soil, and so this difference could be due in part to the differing environmental conditions and volcanic soil unique to northwestern Rwanda.

Protozoan Infections

Few studies have examined the prevalence of intestinal protozoa in Sub-Saharan Africa. In 2013, an estimated 74.7% of children on Pemba Island, Tanzania harbored at least one intestinal protozoa species at baseline measurement (Speich et al., 2013). The same Pemba Island study reported an 18% prevalence of *E.histolytica/E.dispar* in school children and a 16.4% prevalence of *Giardia sp. Giardia* infections have an estimated prevalence of 20-30% in developing countries (Speich et al., 2013). Infection with *Giardia sp.* was less than 5% in all years from 2002-2011 in the Bisate sample population. It may be that these drastic differences are due to increased access to water through building of water tanks, improved housing or infrastructure and increased access to healthcare since 2002, facilitated by local NGOs. However, further research is needed to confirm or deny whether this is the case. Furthermore, it is likely that the low sensitivity of microscopy in this study led to an underestimation of protozoan infections (Gillespie, 2006; Spencer, 1982).

Risk Factor: Age

Our findings that parasitic prevalence was lower in adults and infants than school-aged children are consistent with previous literature. While stratified bivariate analysis did not show any significant difference between age groups and parasitism, our logistic regression models showed greater associations of children aged 6-12 years and 13-18 years with parasitism than of children under-five and adults 19-50 years old. Fewer than 20% of children aged 6-18 years in 2002-2011 were infected with multiple parasite species. This is a lower prevalence than data from a 2008 study conducted in Rwanda that found that 23% of school aged children (10-16 years) had dual infections and 4.8% had triple infections (*National prevalence survey on soil-transmitted helminths & schistosomiasis in school-aged children*, 2008). This difference could possibly be due to differences in categorization of age groups, definitions of multiple parasite infections, geographic location (the entirety of Rwanda, as opposed to solely the Bisate catchment), as well as methods of data collection.

Risk Factor: Administrative Sector

The multivariate analysis from this study suggest that administrative sector is significantly associated with parasitism, as well as polyparasitism. While data is not available on distance to the park boundary and distance to the health center, these factors may contribute to the significant association between sector and parasitism. Musanze sector is further from the park boundary than Kinigi sector, but also further from the Bisate Health Center (*Volcanoes National Park Management Plan*, 2012). People in this area may not have the same access to medical care and chemotherapy.

Environmental Classification of Parasites

Supplementary analysis showed very few significant associations between demographic factors and environmental classification of the parasites. However, environmental classification is an imprecise way to account for differences in transmission. Future studies should incorporate data on environmental sampling, socioeconomic status, education and behaviors to better capture the true association between demographic characteristics and parasitic prevalence.

Parasitic Infections in Virunga Mountain Gorillas

Previous studies have examined parasitic prevalence in mountain gorillas in Bwindi Impenetrable Forest and the Virungas. The data from this analysis show that the vast majority of mountain gorillas (91%) were infected by gastrointestinal parasites. This is consistent with findings from the Central African Republic on western lowland gorillas (*Gorilla gorilla gorilla)*, 90% of which were infected with parasites (Lilly et al., 2002). The majority of infections were from *Anoplocephalidae*, *Oesophagostomum* and Strongyles. This is again consistent with previous studies of gorillas from the Virungas that reported Strongyles in 97% of the gorilla population and *Anoplocephalidae* in 85% of the population (Kalema-Zikusoka, Rothman, & Fox, 2005; Sleeman et al., 2000).

The species richness of parasites in the gorillas from this Virunga census seemed to be less than that observed in previous studies of mountain gorillas. Particularly, previous studies have found several more species including, *C.wehri, Hyostrongylus sp., Impala sp. Loa loa sp. M.devias, and P.gorillae* (Kalema-Zikusoka et al., 2005). It is not clear why these parasites were not recorded in this study; however, it may relate to differing diagnostic methodology. Furthermore, the Virunga mountain gorillas were specifically screened to examine only helminths and thus no data exists to compare prevalence of protozoans . Studies have found *Cryptosporidium sp.*and *Giardia sp.* in the gorillas of Bwindi (Lilly et al., 2002; Nizeyi et al., 1999). In addition, previous studies report non-pathogenic protozoa such as *E. coli* and *Iodoamoeba butschlii*, as well as pathogenic strains such as *Balantidium coli* and *E. histolytica* (Kalema-Zikusoka et al., 2005; Lilly et al., 2002).

Among studies that have looked at single species infections and demographic factors, significant differences have been found between whipworms, tapeworms, ascaroids, hookworm and gorilla age/sex classes; however, these studies did not report any significant differences in strongyles and infection age/sex class (Lilly et al., 2002). While our study stratified analysis at the taxa level, our study did find significant differences across age groups for cestode and nematode infection, and differences across countries for nematode infection and any parasitic infection.

To our knowledge, no previous studies have examined the relation between polyparasitism and demographic factors in mountain gorillas. Our study observed significant differences in overall polyparasitism by age/sex as well as country of residence. Of particular interest is the negative association between habituation of gorillas and polyparasitism. Stratified analysis showed that 66% of habituated gorillas were infected with multiple species of parasites. However, the results of the logistic model, indicate that habituation was associated with an 86% lower odds of polyparasitism [OR: 0.14 [0.07, 0.31]]. We expected that increased contact with humans would be associated with greater odds of infection with multiple parasites. It may be that although the species richness of parasites is not greater, habituation may affect worm burden due to more frequent exposure to human fecal contamination. Unfortunately, there is no non-invasive method to reliably determine worm burden (Gillespie 2006). Furthermore, although the vast majority of habituated mountain gorillas are in Rwanda, some of the Virunga mountain gorillas of DRC and Uganda are also habituated. Interaction between habituation and country of residence was not significant. Still, differences between countries in terrain, soil or water conditions and disease transmission routes may explain this unexpected outcome. Logistic regression analysis also showed that mountain gorillas in the DRC and Uganda have a lower odds of polyparasitism compared to gorillas in Rwanda. The results from the Ugandan gorillas are limited due to a small sample size, and subsequently large confidence interval. However, in the DRC, there was a 91% decrease in odds of polyparasitism [OR: 0.09 [0.05, 0.20]]. DRC represents a mix of habituated and unhabituated gorillas, less population density and far less tourism. It may be that the differences in parasitic prevalence in DRC and Rwanda are due to these other factors.

Parasite Infection Comparison Between Humans and Mountain Gorillas

This study found that infections with *Ascaris*, *Trichuris*, hookworm and *Trichostrongylus* were common between the human and gorilla populations. Few studies have documented human to gorilla transmission of parasitic diseases. However, one study was conducted in the Central African Republic and also showed overlap in infection by strongylates, ascarids and hookworm in humans and western lowland gorillas (Lilly et al., 2002). Due to the methodological constraints of this analysis in working with two independent datasets collected for different reasons and comprised of different sets of information, this study was unable to thoroughly examine species overlap in parasite infections of humans and mountain gorillas. In addition, our diagnositic methodology limited diagnosis to helminth infections, and so comparisons cannot be made in relation to protozoal infections were not possible. Still, a previous molecular study has documented similarities in *Cryptosporidium* infection in variants in humans, livestock and primates in western Uganda. While the effects of *Cryptosporidium* on primates could not be determined from fecal consistency, negative effects could not be discounted (Salyer et al., 2012). Future studies of Virunga mountain gorilla and human populations may consider employing genetic analysis to examine the extent of similarities between protozoa in humans and mountain gorillas.

Transmission between humans and gorillas has been documented for other infectious agents in the Virungas. A 1988 outbreak of respiratory disease in VNP gorillas resulted in at least six deaths and twenty-seven other cases. Analysis has suggested, that measles was the primary infection, which is not present in gorillas in their natural environment but can be easily transmitted from humans. This resulted in a subsequent successful measles vaccination campaign by the Mountain Gorilla Veterinary Project (Wallis & Lee, 1999).

Outside the Virungas, there are documented cases of infections in non-human primates such as yellow fever, tuberculosis, malaria, and Chagas disease (Chapman, Gillespie, & Goldberg, 2005). Ebola outbreaks in the DRC and Gabon have caused reductions in the western lowland gorilla population as well (Rwego, Isabirye-Basuta, Gillespie, & Goldberg, 2008). Learning from these past incidents and increasing efforts to understand human-gorilla pathogen transmission can help to improve conservation efforts.

Strengths & Limitations

Few studies have explored the intersection of human health and primate conservation with respect to the endangered mountain gorilla populations. Our analysis was able to use preexisting data in order to begin to address this area. To our knowledge, this is the first analysis of human and gorilla parasitic prevalence in the Bisate catchment area. The increasing overlap between the human and great ape populations in this area make it critical to explore the health consequence of more frequent human-ape contact and this is of primary interest to both a development and conservation audience. As no other research has examined parasitic prevalence in both populations side by side, the information from this analysis is important in preparing for future studies.

However, results from the study should be interpreted cautiously and there are several limitations to this study that should be acknowledged. First, sampling methodology varied slightly from year-to-year for the Bisate Health Center data. In early years (2002, 2005) the data were collected as part of a household survey, and in the later years, the data were collected at the health center itself. Furthermore, the sample was not randomized. This raises questions as to how representative the sampled population is of the Bisate catchment area. Future studies should employ a random sampling technique in order to ensure that samples are obtained from a representative population. Furthermore, because the data in this analysis are cross sectional for five separate years, temporality cannot be established and no causal inferences can be made. Ideally, a case-control or longitudinal cohort design would be more suitable for studying changes in parasitic prevalence and the associations with specific risk factors.

Both the Bisate Health Center and Virunga mountain gorilla data were limited by laboratory methodology. Parasitic diagnoses were done via microscopy by direct fecal smears or following parasite isolation by fecal floatation or sedimentation. Definitive diagnosis without more advanced laboratory equipment and procedures is difficult and thus the sensitivity and specifictiy of the diagnostic tests in this study were low. As a result, for the purposes of this study, pathogen data were aggregated into categories by taxa to mitigate the limitations of microscopy. In addition, no data were collected regarding intensity of infection in the Bisate Health Center data. Data on mountain gorilla egg burden was collected for the purpose of ensuring full coverage of the slide and cannot provide insight into the intensity of infection in gorillas. Thus, in the gorilla data, non-invasive methodological constraints limit the extent of meaningful analysis. In both sets of data, inter-observer variability is also of concern. No data is available on the training of the technicians who examined the fecal specimens. Particularly with microscopy, it is possible that certain parasites were missed. Future studies should employ multiple diagnostic methods for parasites, and if possible, molecular methods to provide reliable infection estimates. Furthermore, validating diagnosis of parasites between and within observers would provide some quality control/quality assurance measures to improve the reliability of the data.

Data management and cleaning was a concern in this study, and future standardization of data recording would help to ensure that missing values and improperly recorded information are kept to a minimum.

In our statistical modeling, we ignored the effect of clustering of gorillas and humans as part of families. Because this information was not available for the human datasets, we ignored this during logistic regression. It is likely that the infection status of the study subjects may not be entirely independent (i.e. the infection status of one subject may affect the infection status of another subject). In addition, the available data on risk factors was sparse. Coupling future clinical data with good survey data on demographics, geospatial distributions of the populations, behavior, environment, water and sanitation would strengthen model building and our understanding of risk factors in the area. Thus, while our analysis can point in the direction of further research, it is limited in its ability to draw conclusions about significant risk factors for parasite infection in this context.

Our available data to compare gorilla and human infections in the same geographic region and year were limited. The data were not matched and varied temporally, and thus we were unable to statistically compare levels of parasitism and species overlap. Further research is needed to better establish correlation and causation and a definite relationship between parasitism in humans and primates. Future studies should be designed to collect data from humans and gorillas concurrently, and better evaluate the effect of household and geographic risk factors.

Finally, this study focuses on a very specific population of humans and mountain gorillas. The Bisate Health Center data was collected for the purpose of monitoring the DFGFI deworming program. While it is likely that there are similarities between this community and others surrounding the VNP, we do not know that the Bisate catchment is representative of all the communities that surround mountain gorilla habitats. This may limit the external validity of this study. While this is certainly a limitation, the results of these analyses are still useful and important for generating hypotheses about parasitic infection risks in Rwanda's VNP region, particularly given the dearth of information in this area.

Because the human and mountain gorilla habitat, livelihood and culture are different in Rwanda compared to other areas in Africa with an interface between human and primate habitats, the external validity of the study is low. However, the lessons learned from this study and methods used can be applied in other settings to examine the nexus of human, animal and ecosystem health in different contexts around the world.

There are many challenges in working on "one health" as it requires the close collaboration and pooling of resources from different groups that work with humans, animals and the environment. Despite the limitations of this study, utilizing available information is an important start to making connections between conservation and human health and development. The concept of "one health" recognizes that the fate and well-being of people, animals and the environment are inter-dependent. This includes the dynamic nature of the environments that generate health problems, the ever-changing populations within them and the inevitability of increasing contact between humans and animals. In East Africa, and particularly Rwanda, the mountain gorilla population of the Virungas is a vital source of economic revenue through tourism, in addition to the benefits they provide associated with preserving biodiversity. A better understanding of the Bisate catchment and the communities surrounding the mountain gorilla habitat will help to inform efforts to ensure that the appropriate resources are available to meet the needs of human and animal populations in the area. This examination of parasitic infection prevalence in humans and mountain gorillas will encourage greater synergy between human and veterinarian diagnostics, as well as encourage pooling of resources, infrastructure and skill sets to support better conservation, development and health.

CONCLUSIONS

This study found that parasite prevalence decreased between 2002-2011 in the human population in the Bisate catchment surrounding the VNP, and that the majority of infections were caused by STH. In the Bisate catchment, certain administrative sectors and age groups are associated with greater odds of and higher prevalence of parasitism in humans. Analyses of the data from Virunga mountain gorillas suggest that the mountain gorilla groups of Rwanda have a greater odds of infection by more species of parasites than the mountain gorillas of other countries. The presence of specific parasites in both the human and gorilla populations suggest potential transmission of infection between these two populations when they live side-by-side. The most commonly detected human parasites in this study are transmitted by fecal contamination of soil and crops as well as poor sanitation and hygiene. Further in-depth research should be done to examine the risk of transmission between these populations. It is critical to understand the different factors (demographics, geospatial distributions of the populations, behavior, environment, water and sanitation) that affect zoonoses and anthropozoonoses in this setting in order to fully address health and ecological concerns. Further research is needed to understand the health connections between humans and great ape populations in order to develop more effective and sustainable solutions for these fragile environnments.

CHAPTER 4: LESSONS LEARNED AND RECOMMENDATIONS

This project has been an invaluable learning experience, allowing me to learn and grow both as a researcher as well as a public health practitioner. While I was not involved in the initial program design or data collection process, the challenges and limitations I encountered in the process of analyses has taught me important lessons on data management, study design and aligning program goals with the greater mission of the implementing body or NGO. Particularly, my experience in planning the analysis and cleaning the data has reinforced the importance of clear protocols for both sampling and lab methodologies. In addition, I have learned the importance of a standardized database for all data to be entered for ease of analysis as well as data management.

I find that a clear vision of the purpose of data collection and how it ties into a larger research goal is important in order to guide analysis. It is also a challenge learning how not to overinterpret data, and knowing the limits of the data that you work with. However, given the limitations, I am proud of the analyses that I was able to provide and that I was able to help translate collected data into knowledge that can be used to guide future research and programmatic improvements.

Retrospectively, there are a few things that could have been done differently in the analysis. First, I think that in my research question and specific aims could have been narrowed, and consequently I could have narrowed the scope of the analyses in order to allow for greater depth in analysis. In addition, I think using existing open-source spatial data to look at population distributions, such as distance to the park and, if available, locations of water sources, would have added a lot to the descriptive analysis and may have allowed better hypothesis generation with respect to trends in parasite prevalence and spatial patterns. Future studies should certainly look into capitalizing on, or if possible, gathering geospatial data. In addition, it would have been more meaningful to use cluster analysis with the mountain gorilla families. Learning to account for clustering in logistic regression could have strengthened our ability to make inferences from the data. Given fewer time constraints and more resources, I would have also liked to explore more of the data collected during the mountain gorilla census (for example, location and home range patterns), strengthen the level of analysis conducted on gorilla data and subsequently the understanding of parasitism in the mountain gorillas.

Recommendations

My recommendations for The Dian Fossey Gorilla Fund International are three fold: (1) increasing capacity for study design, (2) improving data management and (3) increased collaboration and integration between conservation and health projects. Future study designs should focus on the information needed to answer important questions for understanding parastic prevalence and should include demographics, geospatial distributions of the populations, behavior, environment, water and sanitation. As alluded to earlier, data management could be improved through standardization of data collection and entry procedures. Future studies should attempt to match data from humans and gorillas by ensuring similar temporality and sampling procedures such that more comparisons can be made between the populations. Furthermore, this would be a step forward in better integrating environmental conservation, community health and development, to promote the “one health” model. Based on the results of this project, further efforts to compare mountain gorilla and human data should be made. Perhaps in the future, data could be collected from samples all across the Rwandan side of the Virungas so as to better understand the prevalence of parasites in different villages and the difference along the mountain gorilla habitat. DFGFI is well positioned to be a leader in “one health” given their long-standing presence in Rwanda as well as their resources. Improving the understanding of parasitic prevalence in humans and gorillas as well as associated risk factors, can have a great impact on primate conservation and human health and development in fragile areas.

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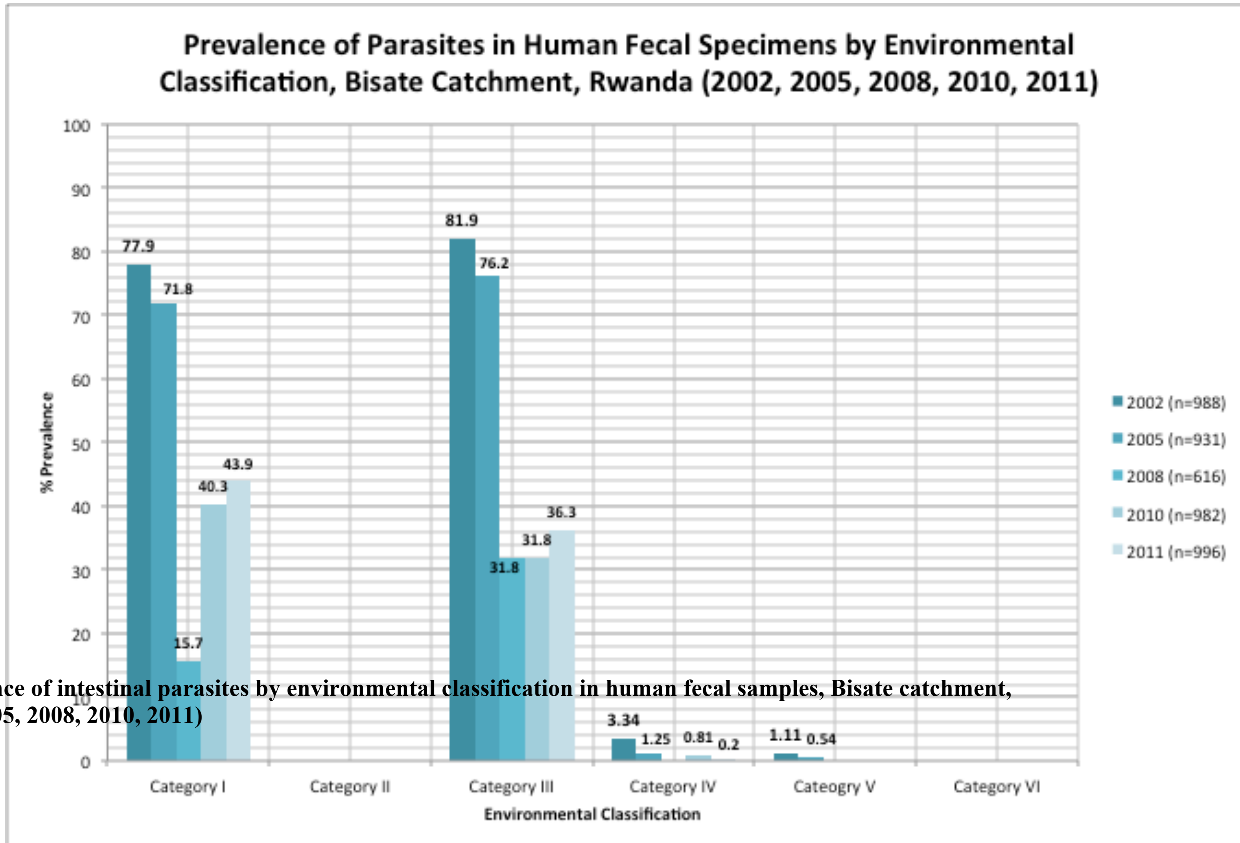
APPENDIX: SUPPLEMENTAL TABLES

Table 10: *Prevalence of types of intestinal parasites stratified by sex, age category and sector in human stool specimens in 2002, Bisate catchment, Rwanda**

Covariate	n (%) Cestoda (n=39)	Trematoda (n=11)	Nematodes (n=809)	Protozoa (n=651)	Any Parasite (n=907)	Polyparasitism (n=800)
Sex						
Female n (%)	17 (43.6)	9 (81.8)	419 (51.8)	355 (54.5)	471 (51.9)	430 (53.8)
P-value	0.3	0.04	0.36	0.002	0.09	<0.001
Age**						
0-4 n(%)	6 (15.4)	3 (27.3)	173 (21.4)	128 (19.7)	259 (28.6)	170 (21.3)
5-20 n(%)	3 (7.7)	3 (27.3)	88 (10.9)	124 (19.1)	26 (2.9)	87 (10.9)
21-30 n(%)	8 (20.5)	2 (18.2)	142 (17.6)	220 (33.8)	8 (0.9)	148 (18.5)
31-40 n(%)	13 (33.3)	1 (9.1)	264 (32.7)	113 (17.4)	569 (62.8)	257 (32.2)
41+ n(%)	9 (23.1)	2 (18.2)	140 (17.4)	66 (10.1)	44 (4.9)	137 (7.15)
P-value†	0.54	--	<0.001	<0.001	--	<0.001
Sector**						
Bisate	18 (27.7)	4 (36.4)	203 (25.5)	18 (27.7)	230 (25.4)	207 (25.9)
Gitaraga	160 (24.7)	5 (45.5)	225 (27.9)	160 (24.7)	241 (26.6)	212 (26.6)
Kareba	148 (22.8)	0 (0.0)	191 (23.7)	148 (22.8)	217 (23.9)	187 (23.4)
Nyabitsinde	161 (24.8)	2 (18.2)	188 (23.3)	161 (24.8)	217 (23.9)	192 (24.1)
P-value†	0.03	--	0.19	0.03	0.8	0.55

*Data from 2002 were examined separately because administrative boundaries of sectors changed and data on locations of households is not available
** n=1 missing for age; n=2 missing for sector
† P-values are reported only where expected cell count was adequate for a chi-square test



ure 9: Prevalence of intestinal parasites by environmental classification in human fecal samples, Bisate catchment, anda (2002,2005, 2008, 2010, 2011)

Table 11: *Prevalence of types of intestinal parasites stratified by sex, age category and sector in human fecal specimens from combined 2008,2010 and 2011 data, Bisate catchment, Rwanda**

	n (%)			
Covariates	**Category I (n=541)**	**Category III (n=976)**	**Category IV (n=10)**	**Category V (n=0)**
Sex				
Female	383 (70.8)	681 (69.8)	5 (50.0)	
P-Value**	0.2	0.3	--	
Age				
0-5	50 (9.2)	112 (11.5)	1 (10.0)	
6-12	75 (13.9)	155 (15.9)	1 (10.0)	
13-18	27 (5.0)	55 (5.6)	0 (0.0)	
19-50	276 (51.0)	448 (45.9)	5 (50.0)	
51+	113 (20.9)	206 (21.1)	3 (30.0)	
P-value**	0.08	0.2	--	
Sector				
Kinigi	450 (83.2)	815 (83.5)	9 (90.0)	
Musanze	79 (14.6)	137 (14.0)	1 (10.0)	
Shingiro	12 (2.2)	24 (2.5)	0 (0.0)	
P-value**	0.66	0.9	--	

*Data from 2005 were not included because no data were collected on sex, age or sector; Data from 2002 were not included because administrative boundaries of sectors changed and data on locations of households is not available

Table 12: *Prevalence of intestinal parasites by environmental classification stratified by sex, age category and sector in human fecal specimens in 2002, Bisate catchment, Rwanda**

Covariates	n (%) Category I (n=541)	Category III (n=976)	Category IV (n=10)	Category V (n=0)
Sex				
Female	410 (53.3)	419 (51.8)	16 (48.5)	9 (81.9)
P-Value	0.01	0.4	0.8	0.04
Age**				
0-5	210 (27.3)	231 (28.6)	4 (12.1)	5 (44.5)
6-12	20 (2.6)	20 (2.5)	2 (6.1)	1 (9.1)
13-18	8 (1.0)	5 (0.6)	0 (0.0)	0 (0.0)
19-50	495 (64.4)	514 (63.6)	25 (75.8)	5 (45.5)
51+	36 (4.7)	38 (4.7)	2 (6.1)	0 (0.0)
P-value†	<0.001	<0.001	--	--
Sector**				
Kinigi	206 (26.8)	203 (25.2)	10 (30.3)	4 (36.4)
Musanze	198 (25.7)	225 (27.9)	3 (9.1)	5 (45.5)
Kareba	175 (22.8)	191 (23.7)	8 (24.2)	0 (0.0)
Nyabitsinde	189 (24.6)	188 (23.3)	12 (36.3)	2 (18.2)
P-value†	0.04	0.2	0.09	--

*Data from 2002 were examined separately because administrative boundaries of sectors changed and data on locations of households is not available

** n=1 missing for age; n=2 missing for sector

† P-values are reported only where expected cell count was adequate for a chi-square test

'ategory III parasites

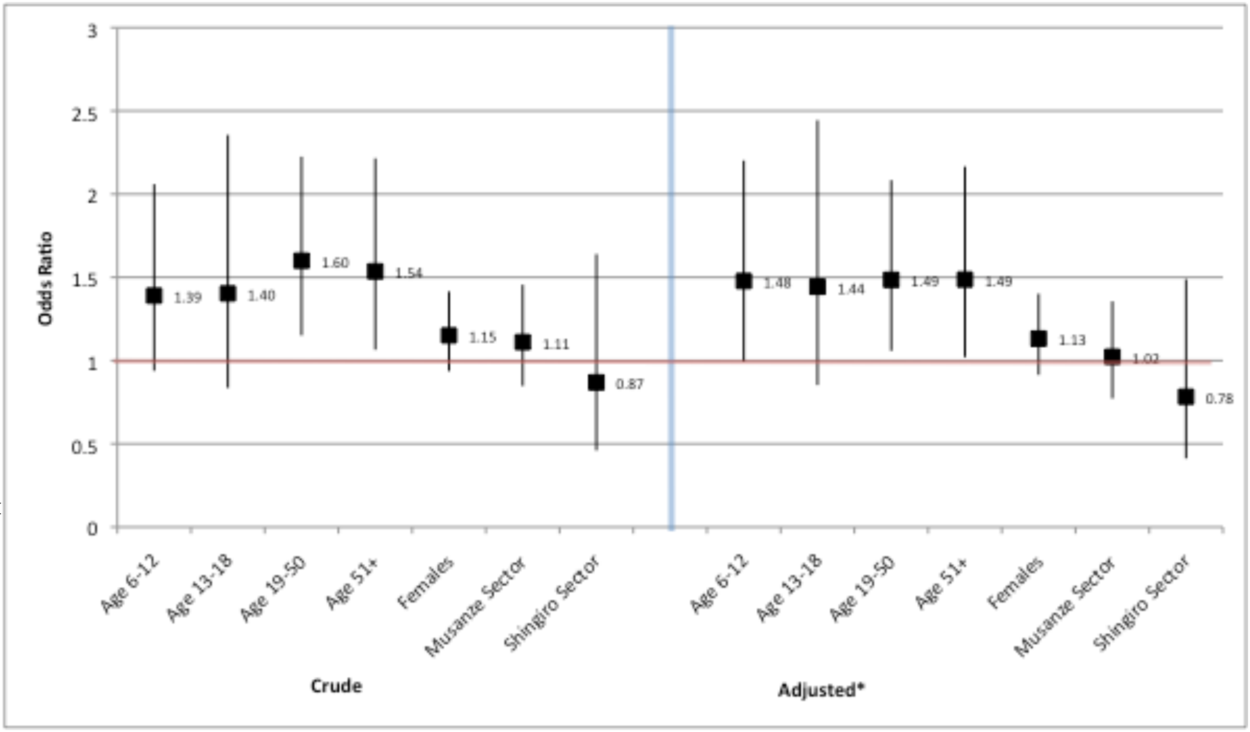


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Figure 10: Logistic regression crude and adjusted associations of age, sex and sector with Category I parasites

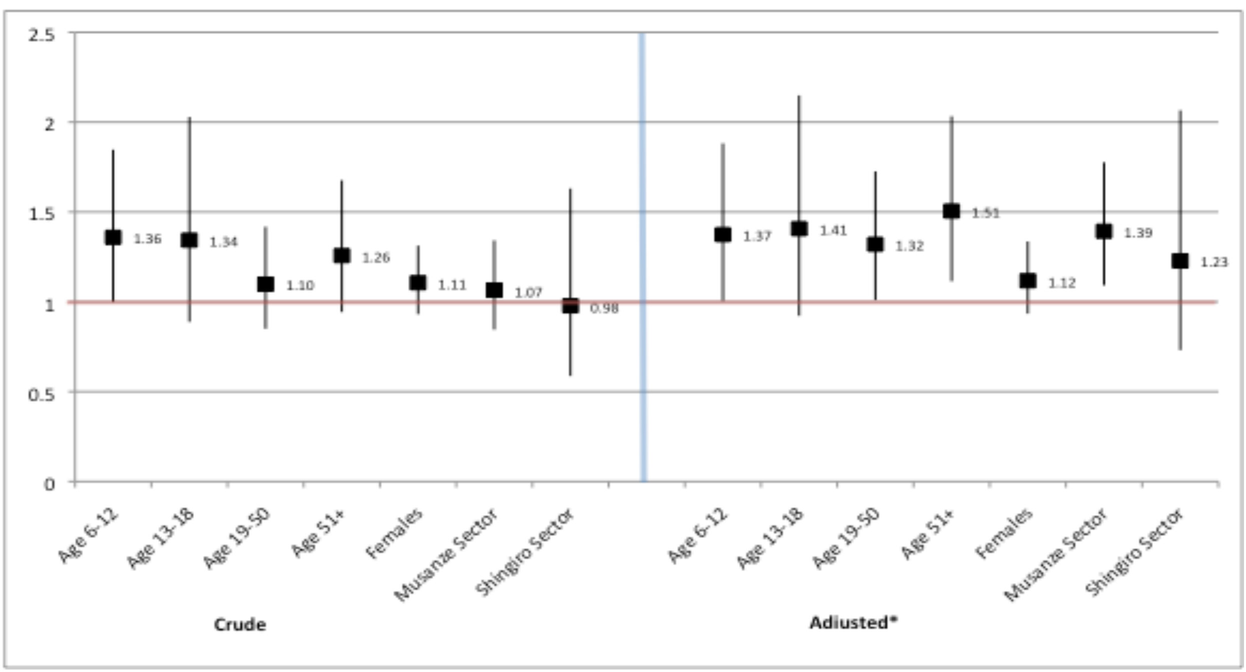

Figure 11: Logistic regression crude and adjusted associations of age, sex and sector with Category III parasites