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Effects of Anthropogenic Disturbance on Zoonotic Pathogen Transmission in People, Wild Primates and Domesticated Animals in the Greater Gombe Ecosystem, Tanzania

By

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Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences

Population Biology, Ecology, and Evolution

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Abstract

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By

Michele M. Parsons

Disease ecology involves understanding the mechanisms driving the complex interactions occurring between pathogens, their hosts, and the shared environment. The primary aim of this dissertation was to test theoretical principles of ecology and epidemiology that underlie zoonotic disease emergence in a complex natural system. Broadly, this research focused on examining how land use changes and multi-host interactions affect pathogen exchange from humans to non-human primates (NHPs), specifically chimpanzees (*Pan troglodytes schweinfurthii*) and baboons (*Papio anubis*) in habitats experiencing varying degree of anthropogenic disturbance from humans and domesticated animals in and around Gombe National Park, Tanzania. Using low-cost GPS data-loggers to establish the extent of domesticated animal overlap with NHP habitat, I found little evidence of domesticated animal mobility into the park, but rather identified potential hotspots for pathogen spillover where wild chimpanzees are known to raid agricultural crops that have high visitation rates by domesticated animals and village residents. My research uncovered a complex cycle of *Cryptosporidium* occurring in Gombe with humans, baboons and a subset of chimpanzees infected with *C. hominis* subtype IfA12G2; another subset of chimpanzees infected with *C. suis*; and all positive domesticated animals infected with *C. xiaoi*. The dominance of *C. hominis* subtype IfA12G2 among humans and NHPs suggests regular cross-species transmission. The finding of *C. suis*, a pig subtype, in chimpanzees is novel. Bacterial studies determined that humans are the likely source for antimicrobial resistance genes that spread to NHPs, regardless of human density. I found that NHPs have resistant pathogenic strains of *Salmonella* and *Shigella* that are similar in genotype and resistance pattern to strains locally circulating in humans. As NHPs are not routinely administered antimicrobials, this suggests spillover of resistance genes and associated pathogens from humans to NHPs. *Salmonella* from domesticated animals represented a different genotype cluster and were not as drug resistant. My research deepens our understanding of the ecology and epidemiology of zoonotic enteric pathogen transmission at the human-animal-wildlife interface in western Tanzania. Our results highlight the importance of considering the spread of infectious diseases in wildlife conservation.

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CHAPTER 1

The application of disease ecology theory and field research to further our understanding of the risk for emerging zoonoses among wild primates at the human-animal interface

An understanding of zoonotic disease emergence requires us to consider various ecological and epidemiological processes operating at different times and scale (Levin 1992; Wilcox & Gubler 2005). In its most simple form, we are interested in the interactions between pathogen, host(s) and the environment (Anderson & May 1991). However, biological systems are complex. Disease emergence will be influenced by pathogen evolution, virulence and transmission both within and between host species based on host density, structure and demography (Altizer et al. 2003; Antia et al. 2003; Dobson 2004; Keesing et al. 2010; Levin 1996). Host-pathogen interactions operate in a stochastic environment altered by biotic (e.g. vector biology, biodiversity), and abiotic (e.g climate, temperature) factors (Altizer et al. 2006; Bradley & Altizer 2007; Grenfell & Dobson 1995; Kurtenbach et al. 2006). A theoretical consideration of these complex and interconnected relationships allow us to better understand how pathogens are capable of infecting new host species, and whether the disease will perpetuate or die out in the new species. In addition, many of these factors are influenced by anthropogenic disturbance which is a leading contributor to the emergence of infectious diseases in humans, domesticated animals and wildlife (Daszak et al. 2000; Deem et al. 2001). Understanding

these relationships can provide valuable information for developing intervention strategies for improving public health, veterinary practices and wildlife conservation.

1.1 PRINCIPLES of DISEASE ECOLOGY and ITS ROLE in ZOONOTIC DISEASE EMERGENCE

The primary aim of this dissertation tests theoretical principles of ecology and epidemiology that underline zoonotic disease emergence in a complex natural system. The studies within assess how multi-host interactions and anthropogenic disturbance affect pathogen exchange from humans to wildlife, specifically non-human primates (NHPs). The following sections outline the history, concepts, theories, and ideas that form the basis for this work.

Yellow fever – historical case study in zoonotic disease and One Health

Yellow fever was historically a major public health challenge, causing severe disease and widespread epidemics in the Americas and Africa in the 18th and 19th centuries (Monath 1989; Robert. E. Shope 1997). Its global dissemination was linked to the concurrent spread of the *Aedes aegypti* mosquito, from Africa on ships to other tropical ports in the Americas and subsequent urban centers, due to increased commerce (Benavides et al. 2012; Monath 1989). In 1901, with the assistance of Carlos Finlay, The U.S. Army Yellow Fever Commission led by Walter Reed, determined that yellow-fever was blood borne and transmitted by mosquito with a 12-day extrinsic incubation period (Reed et al. 1901). Subsequent vector control measures coincided with significant reductions in yellow fever cases, particularly in urban settings. Based on these findings, there was general consensus that humans were the only susceptible vertebrate host and

areas where the domesticated *A. aegypti* mosquito was controlled or didn't naturally exist would be free of the disease (Bryan et al. 2004).

In 1932 a small outbreak in the Brazilian jungle led to the discovery of an independent rural transmission cycle of yellow fever not transmitted by *A. aegypti* (Soper et al. 1933) that in subsequent studies, was shown to cause disease in forest vertebrates, notably primates (Soper 1967). Similarly in Africa, scientists identified a sylvatic jungle cycle of yellow fever principally maintained by monkeys and *A. africanus* mosquitos (Mahaffy 1949; Smithburn et al. 1949) that breed in tree holes (Mutebi & Barrett 2002). While differences between ecological setting and vector exist in South America and Africa, both endemic sylvatic cycles allow for the maintenance of yellow fever in the absence of human hosts. This creates opportunity for focal human outbreaks when exposed while working or living in the jungle (Soper 1935).

Since the 1800s, the medical area has published on the similarities of disease processes among humans and animals (Pasteur et al. 1878; Pasteur 1881). However, human and animal medicine were practiced in relative silos until the turn of the 20th century (Rüegg 2004) when it became clear that infectious diseases and the pathogens that cause them do not operate in silos, but rather are complex interactions between humans, animals and the environment in which they live. The establishment of a One Health approach to understanding complex disease dynamics has important outcomes in how we shape our public health approaches and conservation strategies in fighting disease. The discovery of sylvatic yellow meant that yellow fever eradication was not possible, that spillover from sylvatic settings would remain a possibility where ever humans and tropical rainforest intersected and prevention strategies would focus on

vaccination and mosquito control campaigns (Barnett 2007). Another outcome is that we can recognize that ecological links exist between animal and human health, with potential for zoonotic diseases to serve as biomarkers for assessing ecological connections (Thompson 2013).

Origins and Emergence of Zoonotic Diseases

Zoonotic diseases are those transmitted naturally between vertebrate animals and humans, with or without establishment of a new life-cycle in humans (Daszak 2000). Wildlife and domesticated animals can serve as reservoirs from which previously unknown pathogens may emerge in humans. Infectious diseases that were previously rare or unknown of humans have increased in incidence during the last two decades, and this is not solely attributable to an increase in reporting (Jones et al. 2008). Many (~60%) of these diseases are zoonotic in origin with the majority (71%) caused by pathogens that originated in wildlife (Jones et al. 2008; Taylor et al. 2001). Examples of emerging diseases include influenza, plague, and HIV (Gao et al. 1999; Krause 1992; Subbarao et al. 1998; Walsh et al. 2003; Walsh et al. 2005).

Researchers looking at the ecological and epidemiological characteristics of twenty-five major human infectious diseases, have found biological distinctions between temperate and tropical diseases (Table 1) that have implications for their origin (domesticated animal versus wildlife), establishment, and persistence in humans (Wolfe et al. 2007). The majority of temperate diseases but none of the tropical diseases were classified as crowd diseases, which trigger brief local epidemics that can only be sustained regionally in large populations (Greenwood 1935). Many temperate diseases

(i.e. measles, smallpox, influenza A) appear to have relatively recent origins (~11,000 years ago). Humans acquired these diseases from domesticated animals during the rise in agriculture when human civilization created an environment for increased numbers of domesticated animals and humans to live in close proximity to one another (Diamond & Ordunio 2005; Furuse et al. 2010; McNeill 2010). Temperate diseases were also more likely to have shorter infectious periods and provide longer immunity, which would explain local epidemic dynamics that would stutter out in the absence of naïve immune hosts.

Tropical diseases (i.e. yellow fever, dengue fever, AIDS) exhibit quite different epidemiological and ecological characteristics. The tropical disease infectious state is often chronic (weeks-decades) with limited immunity. Phylogenetic analyses of strains from many of these pathogens have origins in non-human primates (Gao et al. 1999; Mackenzie et al. 2004; Soper 1944). The host shift of tropical diseases from wild primate to humans could be influenced by the close genetic relationship between these species, and because most non-human primates reside in tropical areas (Davies & Pedersen 2008). Tropical pathogens are more likely to maintain a sylvatic cycle in non-human reservoirs and are frequently associated with vector transmission, when compared to temperate pathogens (Wolfe et al. 2007). Selective evolution of mosquito vectors may occur more readily in tropical settings where there is a higher diversity of species that may be viable vectors for a given pathogen and in warmer temperatures the vectors may be active year round compared to temperate regions where vector activity would stop in the colder season (Pascual et al. 2006; Pongsiri et al. 2009).

Multi-host Pathogen Population Biology

In population biology, most studies have favored experimentation using single-host, single pathogen interactions with pathogens adapted to the single host, rather than using a generalist pathogen that can infect and be transmittable by multiple host species (Woolhouse et al. 2001). The selective advantage of generalism is not well understood in evolutionary terms. One might expect that ecological specialists would be favored over multi-host ecological generalists. Single-host pathogens would be well-adapted to a narrower niche where evolutionary processes could occur at a faster rate, resulting in fewer fixed deleterious alleles and a lighter mutation load (Whitlock 1996). This is particularly relevant to parasites that are under continual selective pressure to coevolve with their hosts and may modulate virulence intensity relative to transmissibility (Lipsitch 1997; Woolhouse et al. 2002).

Although this theoretical approach is elegant in its simplicity, in reality many pathogens have the capacity to infect multiple host species, and zoonotic pathogens regularly make the ecological jump from a primary animal reservoir species to humans. The evolution of a generalist requires the pathogen be able to make contact with the new host species, infect the new species and subsequently transmit disease (Woolhouse et al. 2005). Modes of host contact (i.e. indirect, direct, vector mediated) will have varying success rates and fitness costs, relative to the host-pathogen interaction. For example, infected vectors limited to one blood meal would benefit from feeding on a competent host but this may not be as critical for an endoparasite shedding many infective eggs in the environment simultaneously. In order for a pathogen to infect a new host species, it must overcome the species barrier, where by a pathogen may be less infectious in the new species and require a higher dose of the pathogen or other alteration to cause infection in

the new species (Klempner & Shapiro 2004). Changes that tend to overcome a species barrier have been documented in rabies, where the virus generated different genetic variants that resulted in host specific (adult versus neonate mice) pathogenicity levels (Morimoto et al. 1998). Other zoonotic pathogens (i.e. *Salmonella enterica*, *Escherichia coli* O157) have acquired genetic elements that increase virulence and the exploitation of new hosts (Besser et al. 2007; Conner et al. 1998). The ability of a pathogen to invade a new host population and transmit disease will depend on its transmission potential (R_o) and effective population size (Anderson & May 1991) and will be discussed in more detail in the next section.

Dynamics of Disease Epidemics at the Human-Animal Interface

Establishing the patterns and underlining mechanisms that drive disease emergence are challenging given the non-linear, multi-host interactions intrinsic to zoonotic diseases. Recently a classification scheme was published which delineates into five stages the evolutionary transformation of an animal pathogen (Stage 1) to a pathogen only of humans (Stage 5), with a zoonotic component represented in the intermediate stages (Stage 2-4) (Wolfe et al. 2007) . These stages are not progressive, as some pathogens (i.e. anthrax, plague) have maintained stable transmission cycles in the intermediary stages with no evidence of evolving specialization exclusively in humans. At each stage there are genetic, biological and social factors that influence whether a pathogen may move to an adjacent state. For example, in the transition between stages 1 and 2, an animal pathogen can infect a human but secondary human infections do not occur. Examples include Nipah virus, rabies and West Nile virus (WNV). Rabies displays a lot of genetic variability and demonstrated adaptation to a number of animal hosts but

its mode of pathogen transfer hinders its transmission among humans, who are unlikely to bite one another. Virus like West Nile does not progress beyond stage 2 because humans and some other mammals (e.g. horses) are generally dead end hosts not capable of developing the high viremic levels necessary to pass the virus on to other biting mosquitos (Campbell et al. 2002). Yellow fever described extensively earlier in this chapter is a classic example of a stage 4 pathogen. Phylogenetic relatedness of the spillover and recipient hosts is a major determinant of stage progression by a pathogen, as well is the frequency of species encounters (Antia et al. 2003). Additional details on the characteristics for each stage may be found in Figure 1.

Another way to consider the factors that influence cross-species spillover is to focus on the force of infection from animals to humans (Lloyd-Smith et al. 2009). We can distinguish each pathogen stage by focusing on the basic reproductive number (R_0), defined as the expected number of secondary infections from a susceptible population. Using this approach, we can differentiate between animal only ($R_0 = 0$), human only ($R_0 >1$) and zoonoses that have infected humans and will either be capable of sustaining the human cycle ($R_0 >1$) or die out ($R_0<1$). An interesting aspect of this approach is that movement between the intermediate (zoonotic) stages will be largely dependent on the spillover force of infection which is governed by the product of reservoir prevalence by reservoir-human contact rate by infection probability. The mode of pathogen transmission will influence the extent to which a combination of biological, agricultural and environmental factors (i.e. host density, vector abundance and competence, contact duration) effect the spillover force of infection (Lloyd-Smith et al. 2009).

Zoonotic Disease Emergence in Humans

"Zoonotic infectious disease emergence can be explained in part as a consequence of the disruption of natural ecological communities and the breakdown of naturally existing...host-parasite relationships that tend to regulate and stabilize species abundance" (Gillespie et al. 2005; Wilcox & Gubler 2005). The emergence of human pathogens from animal hosts is associated with a number of interacting diverse forces including, changes in land use or agricultural practices, altered host demographics and health, strength of public health systems, climate change, and globalization of travel and trade (Cleaveland et al. 2001; Daszak 2000; Fevre et al. 2006; Patz et al. 2005; Woolhouse & Gowtage-Sequeria 2006). Changing land use patterns can alter the number of reservoir hosts, increase the incidence of infection in animals, or bring animal hosts or disease vectors into closer contact with humans (Foley et al. 2005; Gillespie & Chapman 2006; Gillespie et al. 2005). Wildlife may move into urban areas as a result of degradation of their natural habitats and the availability of food near human dwellings (Saj et al. 2001). The AIDS epidemic has increased the population of immunocompromised and led to the recognition of some emerging opportunistic pathogens, such as *Cryptosporidium* (Tzipori 1988). Poor sanitation and waning vaccine campaigns may leave the young and immunologically naïve susceptible to preventable infectious diseases. Absence of laboratory diagnosis can provide opportunities for the spread of disease to go undetected and uncontrolled. Warmer climatic conditions have allowed disease vectors (i.e. arthropods) to survive the winter and may extend their transmission cycle and geographic range (Gagnon et al. 2002; Rogers & Randolph 2006). Increased rainfall has influenced the seasonal dynamics of outbreaks of Rift Valley fever and cholera (Harvell et al. 2002; Pascual et al. 2000).

The globalization of food production may contribute to zoonotic disease emergence by increasing the concentration, movement, and mixing of animals and animal products. Long-distance transport of animals has been associated with increased shedding of enteric pathogens (Bach et al. 2004). Large-scale farms and food processing facilities expose a greater number of people to a contaminated food source and foster the development of antimicrobial resistant bacteria (Altekruse et al. 1997; Sapkota et al. 2007; Tauxe 2002). Increased human mobility by modern transport means that the connected population is larger, so that a localized outbreak that in earlier times might have ended can now spread quickly to more susceptible hosts and cause a larger and more sustained epidemic (Black 1966; Mangili & Gendreau). These ecological changes over time and space will interact on some scale with host-pathogen dynamics (Schrag & Wiener 1995). The pathogen in turn may undergo changes that alter transmission patterns, becoming more virulent or better adapted to humans or animals (Levin 1996; Levins et al. 1994).

Zoonotic Disease Emergence in Non-Human Primates

Evidence of pathogen transmission to humans from wild primates (Calvignac-Spencer et al. 2012) and recognition of the risk for human pathogens to spread to wild primates (Köndgen et al. 2008; Palacios et al. 2011) has led to an awareness of the potential impact of zoonoses on nonhuman primate (NHP) health. Despite this potential for zoonotic transmission, few studies have examined how anthropogenic disturbance influences emerging diseases in NHPs. Recent studies conducted to understand how anthropogenic factors alter the disease dynamics of wild primate populations found that patterns of parasitism in wild primates may be influenced

by host characteristics (e.g. host ranging patterns, and diet) (Gillespie & Chapman 2006; Gillespie et al. 2005). In Kibale National Park (KNP), Uganda, red tail guenon (*Cercopithecus ascanius*), from logged forest had a higher prevalence and parasite species richness when compared to their counterparts in unlogged forest, red colobus (*Piliocolobus tephrosceles*) and black and white colobus (*Colobus guereza*). Behavioral studies found that only the red tail guenon from logged forest had increased ranging patterns and longer daily active periods that could contribute to the altered parasite patterns observed (Stickler 2004). A longer day and larger habitat range could result in increased contact opportunities with potentially infected habitat and conspecifics (Gillespie et al. 2005; Nunn et al. 2003) Logging was also found to decrease fruit tree densities that could result in changes in diet, contributing to animal stress, nutritional deficiency or increase the overlap of conspecifics (Freeland 1980; Gillespie & Chapman 2006)

In another study, researchers evaluated how parasite infection dynamics may be altered by changes in forest fragmentation in meta-populations of NHPs in KNP. The researchers found that while host density and fragment size did not account for substantial variation in parasite prevalence observed among red colobus (*Piliocolobus tephrosceles*), stump density as an index of habitat degradation, explained 85% of the variance observed in parasite prevalence (Gillespie & Chapman 2006). The authors hypothesize that stump density may be an indicator of increased human-NHP contact, and reduced food available and range size which can lead to increased stress and opportunities for parasite transmission between or within species (Chapman et al. 2003)

Increased human activity, including research, has also been found to alter patterns of parasitism in NHPs (Zommers et al. 2013). Research presence has both advantages and considerations for the health and well-being of wild primates. Research stations have been shown to positively impact primate survival (Tranquilli et al. 2012) but increased researcher presence in African parks has been linked to transmission of respiratory illness from humans to wild primates (Köndgen et al. 2008; Lonsdorf et al. 2011). In a study conducted in Budongo Forest, Uganda (Zommers et al. 2013), chimpanzees (*Pan troglodytes schweinfurthii*) that spend more time on the ground and along man-made trails had a higher parasite burden. The increased ground and trail use is speculated to increase contact with researchers and the soil substrate may be contaminated with infective parasite stages.

Human and Domesticated Animal Pathogens Impacting Endangered NHPs

NHP habitat loss is a result of expanding human population growth and activities in villages that share natural boundaries with forested areas where the NHP populations reside. The increased human-NHP contact and close genetic relatedness to primates may mean that higher rates of pathogen transmission to NHPs is probable (Gillespie et al. 2008). Recent studies have confirmed the transmission of potential pathogens from humans and domesticated animals to wild primates (Goldberg et al. 2008; Nizeyi et al. 2002; Rwego et al. 2008; Salyer et al. 2012). In these studies, wild primates residing in fragmented forest regions of Kibale and Bwindi Impenetrable National Parks or had routine exposure to humans and their livestock were colonized by bacteria that were genetically more similar to those carried by humans and livestock than the bacteria found in primates with minimal exposure to human activities (Goldberg et al. 2008; Rwego et

al. 2008). *Cryptosporidium* was also found among humans, primates and livestock in KNP. Strains from humans, livestock and primates living in close proximity to one another fell in one phylogenetic clade that clustered differently from two strains collected from primates in pristine forest (Salyer et al. 2012). In these studies, asymptomatic infections were common but it is not clear whether anthropogenic changes may alter transmission rates, host range and parasite virulence (Altizer et al. 2006; Altizer et al. 2003; Daszak 2000). A number of non-human primate (NHP) populations have seen increased morbidity and mortality from diseases, such as, Ebola, polio, respiratory and diarrheal diseases contributing to population declines in recent years (Bermejo et al. 2006; Graczyk et al. 2001; Köndgen et al. 2008; Walsh et al. 2003; Williams et al. 2008). Baseline data on patterns of infection in NHP populations is needed to understand the underlining disease processes and transmission mechanisms. The data can serve as a predictor of population health and manage disease risks in non-human primates and humans (Gillespie et al. 2008; Leendertz et al. 2006).

1.2 APPLYING THEORY TO EMPIRICAL FIELD WORK – MY DISSERTATION RESEARCH

Gombe National Park in Tanzania is home to a well-studied wild chimpanzee population that has been continuously monitored for over six decades (Goodall 1986). The park is located in Kigoma District, home to a human population of about 2,127,930 including refugees from Burundi and Democratic Republic of Congo (NBS 2012). Overall population growth rate in Tanzania is about 3% (WPR 2014). Because of the park's small size (35 km^2) the bordering forests outside the park were once critical in providing spillover habitat space for chimpanzee and other large mammal species (Wallis

& Lee 1999). The practice of slash-and-burn agriculture has contributed to a high rate of deforestation in Kigoma and almost a complete removal of forested land outside the park. The park border is permeable; villagers and their animals enter the park at the periphery and chimpanzees have been reported to raid crop fields in Mwamgongo village (I. Lipende, personal communication). Within the park, human occupancy includes researchers, members of the park authority, and their families. Water sources (e.g streams and Lake Tanganyika) are shared by NHPs, human residents and their domesticated animals. Residents use the lake and streams for bathing, washing clothes, and cooking utensils, while they also serve as water sources for baboons and chimpanzees (Wallis and Lee 1999). Infectious diseases are estimated to account for the majority of chimpanzee deaths (Lonsdorf et al. 2006). Park managers and researchers suspect that disease from humans poses a large risk to the sustainability of the primate population. With loss of park habitat due to human encroachment, the human-chimpanzee interface has grown, placing the chimpanzees at increased risk for disease spillover and outbreaks, such as polio and respiratory illnesses (Williams et al. 2008).

The intent of the next four chapters of this dissertation was to identify the ecological and epidemiological patterns of zoonotic disease emergence in a complex natural system. Broadly, this research focused on examining the impact of anthropogenic disturbance on zoonotic pathogen transmission to wild primates in a natural system. My studies were conducted in and around Gombe National Park, which is located in rural western Tanzania. The species of interest were nonhuman primates, specifically chimpanzees (*Pan troglodytes schweinfurthii*) and baboons (*Papio anubis*) in habitats experiencing varying degree of anthropogenic disturbance (i.e. forest fragmentation and

human contact), humans (*Homo sapiens*) and domesticated animals, dogs (*Canis lupus*) goats (*Capra hircus*), and sheep (*Ovis aries*). Additionally, specimens were analyzed to detect and characterize the presence of select zoonotic enteric pathogens: *Cryptosporidium* spp., *Salmonella*, *Shigella*, enterotoxigenic *E. coli* and *Vibrio*.

In Chapter 2, my research evaluated the utility of portable low-cost GPS technology combined with epidemiological data to assess the potential for introduction of *Cryptosporidium* into the Gombe wild chimpanzee population from the movement of domesticated animals. The relatively inexpensive and field friendly tracking tool I used in this study has been employed successfully to assess risk factors for the spread of dengue fever in humans (Vazquez-Prokopec et al. 2009). Zoonotic infectious diseases are one of the leading causes of chimpanzee morbidity and mortality in Gombe, and yet little is known about the sources of infection. Chapter 2 explores whether domesticated animals can serve as a source of pathogen spillover to endangered wildlife by capturing the recursive fine-scale mobility patterns of domesticated animals to establish the extent of domestic animal overlap with chimpanzee habitat. Distance and extent to which domesticated animals were found to move into the park were calculated. We assessed whether landscape changes as result of anthropogenic disturbance altered the natural habitat range of the chimpanzees. Fecal specimens were collected from domesticated animals and chimpanzees and examined for *Cryptosporidium*, a zoonotic enteric parasite common in domestic animals that also affects chimpanzee health. These lab data in combination with spatial epidemiology was used to look for evidence of hot spots for pathogen spillover.

Chapter 3 builds upon the empirical findings presented in Chapter 2 by

characterizing the epidemiology and ecology of *Cryptosporidium* in the Greater Gombe Ecosystem. This eukaryotic parasite is one of the most common causes of diarrhea in the developing world (Kotloff et al.), is capable of causing large outbreaks (Mac Kenzie et al. 1994), and an important contributor of morbidity and mortality in HIV infected persons (Cama et al. 2007). The genus is composed of a variety of different species with varying zoonotic potential (Xiao & Feng 2008). SIV infection occurs in the Gombe chimpanzees (Keele et al. 2009), but the ecology of *Cryptosporidium* in the Gombe community is poorly understood. We assessed whether *Cryptosporidium* might be an emerging pathogen in the NHP community and what the likely sources for the infection in NHPs might be (human, domestic animal, wildlife or water). We evaluated whether certain anthropogenic (i.e. human density), environmental (i.e. seasonality, water source) or population characteristics (i.e age, sex, community) increased ones risk for infection. We also subtyped the *Cryptosporidium* in positive specimens by sequencing techniques, to determine the relatedness of strains recovered from wildlife to those recovered from humans and domesticated animals, and whether the strains from NHPs were likely to be of anthropogenic origin. Chapter 3 concludes with a descriptive model of the ecology of *Cryptosporidium* in this system.

In Chapter 4, we build on the ecological theory that habitat overlap may increase the risk for exchange of antimicrobial resistance between humans and wildlife, by testing for the presence of antimicrobial resistance genes in feces of both groups. The presence of resistance genes is a proxy for bacterial spillover from humans to wildlife. We looked for genes conferring resistance to a class of antimicrobial agents commonly used in humans (sulfonamides) and to a less commonly used class (tetracyclines). Wildlife and

domestic animals are rarely treated with either agent class. We determined the prevalence of genes conferring resistance to sulfonamides and tetracycline in fecal specimens collected from human, livestock, and non-human primate populations and in drinking water sources. We examined the overlap in different resistance genes across groups, both as an indicator for potential disease spillover to identify potential reservoirs for antimicrobial resistance genes, and for the presumably host compatible bacteria that carry them. We analyzed human density, community structure and selected environmental features as possible risk factors affecting antimicrobial resistance spillover in this system. We considered the public health implications for antimicrobial resistance to commonly used drugs in humans and domestic animals in this community and the conservation concern for NHPS if these agents were to be used to treat them in extenuating circumstances.

Chapter 5 expands on the research in Chapter 4 to determine the epidemiology and ecology of several specific zoonotic enteric bacterial pathogens recovered from the Greater Gombe Ecosystem (GGE). Diarrheal diseases are a leading cause of mortality and morbidity among humans and domesticated animals and the causative pathogens have host ranges that extend to NHPs (Woolhouse & Gowtage-Sequeria 2006). These pathogens have a strong economic impact on animal trade and commerce. On a local scale, they can be transferred between humans and NHPs causing localized outbreaks, and contribute to morbidity and mortality, particularly in times of stress (Chapman et al. 2005). In addition, they can serve as sentinels for disease spillover (Wolfe et al. 1998). The cultivation of enteric bacterial pathogens from wild NHPs in rural settings is challenging, because it is difficult to collect, process and store suitable specimens.

Chapter 5 describes the considerable effort undertaken to preserve specimens and maintain a cold chain to ensure strain viability before shipment to a national reference lab. Standard culture methods in combination with molecular methods were used to recover *Salmonella*, *Shigella*, and *Vibrio* from the stools of humans, animals and wildlife. Virulence genes for enterotoxigenic *E. coli* (ETEC) were detected by PCR. Recovered strains were subject to phenotypic characterization: serotyping, antimicrobial susceptibility testing (AST) and genotyping using pulsed-field gel electrophoresis (PFGE), Inc typing to determine the compatibility group profile of plasmids, and whole genome sequencing (WGS). Culture, serotyping, and PCR were performed to assess the prevalence and subtypes of each pathogen recovered and to determine if there were commonalities among species, strains or groups. AST and plasmid profiling were performed to identify common resistance phenotypes/genotypes and the plasmid type associated with strains. A subset of *Salmonella* serotype Typhimurium strains were subject to WGS to assess if there were commonalities between groups, location, season or genetic mechanisms of antimicrobial resistance.

The empirical studies conducted in concert with foundational theories effectively extend our understanding of how zoonotic pathogens may be evolving and spilling into multiple hosts in the Greater Gombe Ecosystem and some anthropogenic and other wildlife forces that influence transmission potential in this local heterogeneous landscape.

1.3 FIGURES

Figure 1.1 Five stages and associated disease dynamics through which a pathogen of animals could evolve to cause disease exclusively in humans.

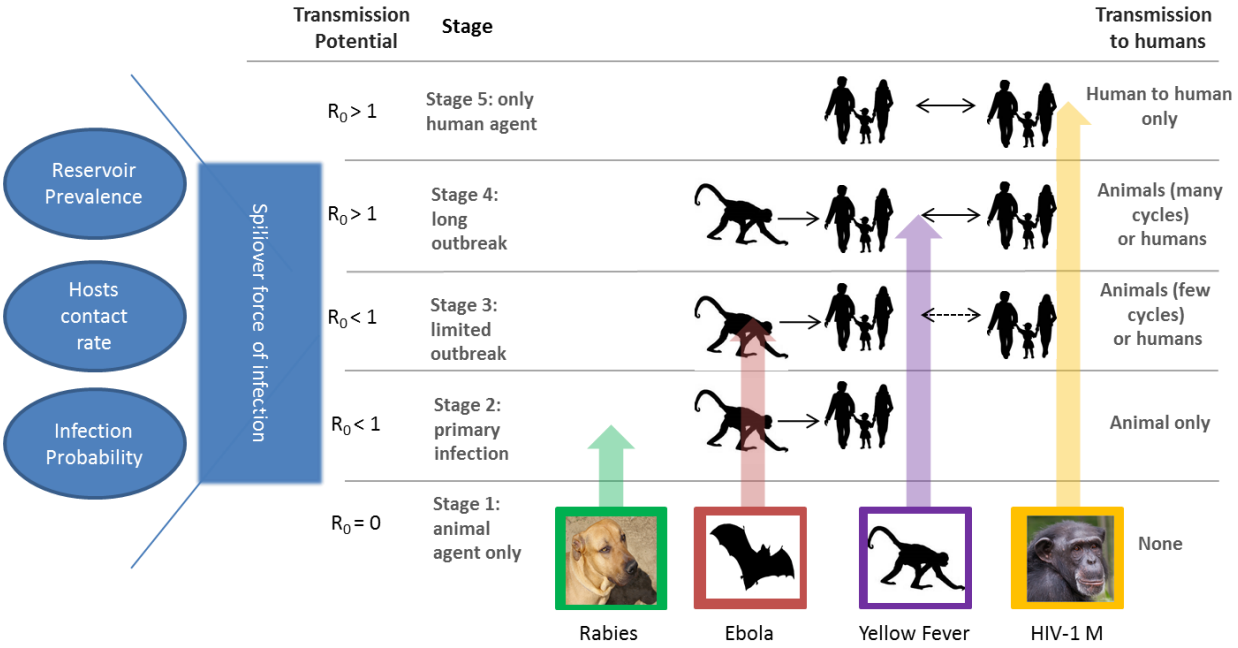


Image created based on data from Wolfe et al (2007) Nature: 447 and Lloyd-Smith (2009) Science: 326

1.4 TABLES

Table 1.1 Divergent characteristics of temperate and tropical human pathogens.

Characteristic	**Temperate Diseases (n=15)**	**Tropical Diseases (n=10)**	***p*-value**
Crowd disease of large human populations	10/15	0/10	$p<0.005$
Vector transmission route to humans	2/15	8/10	$p<0.005$
Duration of infection	12/15	3/10	$p<0.01$
Long-lasting or lifetime immunity	11/15	2/10	$p<0.009$
Non-human reservoir	3/15	7/10	$p<0.005$
Disease/pathogen examples	Diphtheria, Plague, Typhoid	Dengue, AIDS, Chagas' disease	N/A

Prepared from data presented in Wolfe, Dunavan & Diamond (2007), Nature 447: 279-283

1.5 REFERENCES

Altekruse, S. F., M. L. Cohen, and D. L. Swerdlow. 1997. Emerging foodborne diseases. Emerging Infectious Diseases **3**:285-293.

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. Ecology letters **9**:467-484.

Altizer, S., D. Harvell, and E. Friedle. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution **18**:589-596.

Anderson, R. M., and R. M. May 1991. Infectious diseases of humans. Oxford university press Oxford.

Antia, R., R. R. Regoes, J. C. Koella, and C. T. Bergstrom. 2003. The role of evolution in the emergence of infectious diseases. Nature **426**:658-661.

Bach, S. J., T. A. McAllister, G. J. Mears, and K. S. Schwartzkopf-Genswein. 2004. Long-Haul Transport and Lack of Preconditioning Increases Fecal Shedding of Escherichia coli and Escherichia coli O157:H7 by Calves. Journal of Food Protection **67**:672-678.

Barnett, E. D. 2007. Yellow fever: epidemiology and prevention. Clinical Infectious Diseases **44**:850-856.

Benavides, J. A., S. Godreuil, R. Bodenham, S. Ratiarison, C. Devos, M. O. Petretto, M. Raymond, and P. Escobar-Paramo. 2012. No Evidence for Transmission of Antibiotic-Resistant Escherichia coli Strains from Humans to Wild Western Lowland Gorillas in Lope National Park, Gabon. Applied and Environmental Microbiology **78**:4281-4287.

Bermejo, M., J. D. Rodríguez-Teijeiro, G. Illera, A. Barroso, C. Vilà, and P. D. Walsh. 2006. Ebola Outbreak Killed 5000 Gorillas. Science **314**:1564.

Besser, T. E., N. Shaikh, N. J. Holt, P. I. Tarr, M. E. Konkel, P. Malik-Kale, C. W. Walsh, T. S. Whittam, and J. L. Bono. 2007. Greater Diversity of Shiga Toxin-Encoding Bacteriophage Insertion Sites among Escherichia coli O157:H7 Isolates from Cattle than in Those from Humans. Applied and Environmental Microbiology **73**:671-679.

Black, F. L. 1966. Measles endemicity in insular populations: critical community size and its evolutionary implication. Journal of theoretical biology **11**:207-211.

Bradley, C. A., and S. Altizer. 2007. Urbanization and the ecology of wildlife diseases. Trends in ecology & evolution **22**:95-102.

Bryan, C. S., S. W. Moss, and R. J. Kahn. 2004. Yellow fever in the Americas. Infectious Disease Clinics of North America **18**:275-292.

Calvignac-Spencer, S., S. Leendertz, T. Gillespie, and F. Leendertz. 2012. Wild great apes as sentinels and sources of infectious disease. Clinical Microbiology and Infection **18**:521-527.

Cama, V. A., J. M. Ross, S. Crawford, V. Kawai, R. Chavez-Valdez, D. Vargas, A. Vivar, E. Ticona, M. Navincopa, J. Williamson, Y. Ortega, R. H. Gilman, C. Bern, and L. Xiao. 2007. Differences in clinical manifestations among Cryptosporidium species and subtypes in HIV-infected persons. J Infect Dis **196**:684-691.

Campbell, G. L., A. A. Marfin, R. S. Lanciotti, and D. J. Gubler. 2002. West Nile virus. The Lancet Infectious Diseases **2**:519-529.

Chapman, C. A., L. J. Chapman, K. D. Rode, E. M. Hauck, and L. R. McDowell. 2003. Variation in the nutritional value of primate foods: among trees, time periods, and areas. International Journal of Primatology **24**:317-333.

Chapman, C. A., T. R. Gillespie, and T. L. Goldberg. 2005. Primates and the Ecology of their Infectious Diseases: How will Anthropogenic Change Affect Host-Parasite Interactions? Evolutionary Anthropology: Issues, News, and Reviews **14**:134-144.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **356**:991-999.

Conner, C. P., D. M. Heithoff, S. M. Julio, R. L. Sinsheimer, and M. J. Mahan. 1998. Differential patterns of acquired virulence genes distinguish Salmonella strains. Proceedings of the National Academy of Sciences **95**:4641-4645.

Daszak, P. 2000. Emerging infectious diseases of wildlife - Threats to biodiversity and human health (vol 287, pg 443, 2000). Science **287**:1756-1756.

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

Davies, T. J., and A. B. Pedersen. 2008. Phylogeny and geography predict pathogen community similarity in wild primates and humans. Proceedings of the Royal Society B: Biological Sciences **275**:1695-1701.

Deem, S. L., W. B. Karesh, and W. Weisman. 2001. Putting Theory into Practice: Wildlife Health in Conservation

De la Teoría a la Práctica: la Salud de la Vida Silvestre en la Conservación. Conservation Biology **15**:1224-1233.

Diamond, J. M., and D. Ordunio 2005. Guns, germs, and steel. National Geographic.

Dobson, A. 2004. Population dynamics of pathogens with multiple host species. The American Naturalist **164**:S64-S78.

Fevre, E. M., B. Bronsvoort, K. A. Hamilton, and S. Cleaveland. 2006. Animal movements and the spread of infectious diseases. Trends in Microbiology **14**:125-131.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global Consequences of Land Use. Science **309**:570-574.

Freeland, W. 1980. Mangabey (Cerocebus albigena) movement patterns in relation to food availability and fecal contamination. Ecology:1297-1303.

Furuse, Y., A. Suzuki, and H. Oshitani. 2010. Origin of measles virus: divergence from rinderpest virus between the 11(th)and 12(th)centuries. Virology Journal **7**:52-52.

Gagnon, A., K. Smoyer-Tomic, and A. Bush. 2002. The El Niño Southern Oscillation and malaria epidemics in South America. International Journal of Biometeorology **46**:81-89.

Gao, F., E. Bailes, D. L. Robertson, Y. L. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature **397**:436-441.

Gillespie, T. R., and C. A. Chapman. 2006. Prediction of Parasite Infection Dynamics in Primate Metapopulations Based on Attributes of Forest Fragmentation. Conservation Biology **20**:441-448.

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

Gillespie, T. R., C. L. Nunn, and F. H. Leendertz. 2008. Integrative approaches to the study of primate infectious disease: Implications for biodiversity conservation and global health. American Journal of Physical Anthropology **137**:53-69.

Goldberg, T. L., T. R. Gillespie, I. B. Rwego, E. L. Estoff, and C. A. Chapman. 2008. In Forest Fragmentation as Cause of Bacterial Transmission among Primates, Humans, and Livestock, Uganda (vol 14, pg 1375, 2008). Emerging Infectious Diseases **14**:1825-1825.

Goodall, J. 1986. The Chimpanzees of Gombe: Patterns of Behavior Bellknap Press of the Harvard University Press Boston.

Graczyk, T. K., A. B. Mudakikwa, M. R. Cranfield, and U. Eilenberger. 2001. Hyperkeratotic mange caused by Sarcoptes scabiei (Acariformes: Sarcoptidae) in juvenile human-habituated mountain gorillas (Gorilla gorilla beringei). Parasitology research **87**:1024-1028.

Greenwood, M. 1935. Epidemics and Crowd-Diseases. An Introduction to the Study of Epidemiology. Epidemics and Crowd-Diseases. An Introduction to the Study of Epidemiology.

Grenfell, B. T., and A. P. Dobson 1995. Ecology of infectious diseases in natural populations. Cambridge University Press.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate Warming and Disease Risks for Terrestrial and Marine Biota. Science **296**:2158-2162.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-U994.

Keele, B. F., J. H. Jones, K. A. Terio, J. D. Estes, R. S. Rudicell, M. L. Wilson, Y. Y. Li, G. H. Learn, T. M. Beasley, J. Schumacher-Stankey, E. Wroblewski, A. Mosser, J. Raphael, S. Kamenya, E. V. Lonsdorf, D. A. Travis, T. Mlengeya, M. J. Kinsel, J. G. Else, G. Silvestri, J. Goodall, P. M. Sharp, G. M. Shaw, A. E. Pusey, and B. H. Hahn. 2009. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature **460**:515-519.

Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, and C. E. Mitchell. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature **468**:647-652.

Klempner, M. S., and D. S. Shapiro. 2004. Crossing the Species Barrier — One Small Step to Man, One Giant Leap to Mankind. New England Journal of Medicine **350**:1171-1172.

Köndgen, S., H. Kühl, P. K. N'Goran, P. D. Walsh, S. Schenk, N. Ernst, R. Biek, P. Formenty, K. Mätz-Rensing, and B. Schweiger. 2008. Pandemic human viruses cause decline of endangered great apes. Current Biology **18**:260-264.

Kotloff, K. L., J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. G. Faruque, A. K. M. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acácio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne, and M. M. Levine. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet **382**:209-222.

Krause, R. M. 1992. The origin of plagues: old and new. Science **257**:1073-1078.

Kurtenbach, K., K. Hanincová, J. I. Tsao, G. Margos, D. Fish, and N. H. Ogden. 2006. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat Rev Micro **4**:660-669.

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

Levin, B. R. 1996. The evolution and maintenance of virulence in microparasites. Emerging Infectious Diseases **2**:93-102.

Levin, S. A. 1992. THE PROBLEM OF PATTERN AND SCALE IN ECOLOGY. Ecology **73**:1943-1967.

Levins, R., T. Awerbuch, U. Brinkmann, I. Eckardt, P. Epstein, N. Makhoul, C. A. de Possas, C. Puccia, A. Spielman, and M. E. Wilson. 1994. The emergence of new diseases. American Scientist:52-60.

Lipsitch, M. 1997. Evolution in health and disease. Trends in microbiology **5**:303-305.

Lloyd-Smith, J. O., D. George, K. M. Pepin, V. E. Pitzer, J. R. Pulliam, A. P. Dobson, P. J. Hudson, and B. T. Grenfell. 2009. Epidemic dynamics at the human-animal interface. Science **326**:1362-1367.

Lonsdorf, E., C. Murray, E. Lonsdorf, D. Travis, I. Gilby, J. Chosy, J. Goodall, and A. Pusey. 2011. A Retrospective Analysis of Factors Correlated to Chimpanzee (Pan troglodytes schweinfurthii) Respiratory Health at Gombe National Park, Tanzania. EcoHealth **8**:26-35.

Lonsdorf, E. V., D. Travis, A. E. Pusey, and J. Goodall. 2006. Using retrospective health data from the Gombe chimpanzee study to inform future monitoring efforts. American Journal of Primatology **68**:897-908.

Mac Kenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierczak, D. G. Addiss, K. R. Fox, and J. B. Rose. 1994. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. New England journal of medicine **331**:161-167.

Mackenzie, J. S., D. J. Gubler, and L. R. Petersen. 2004. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat Med.

Mahaffy, A. 1949. Paper. The epidemiology of yellow fever in Central Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene **42**:511-530.

Mangili, A., and M. A. Gendreau. Transmission of infectious diseases during commercial air travel. The Lancet **365**:989-996.

McNeill, W. 2010. Plagues and peoples. Anchor.

Monath, T. P. 1989. The arboviruses: epidemiology and ecology. Volume V. CRC Press, Inc.

Morimoto, K., D. C. Hooper, H. Carbaugh, Z. F. Fu, H. Koprowski, and B. Dietzschold. 1998. Rabies virus quasispecies: implications for pathogenesis. Proceedings of the National Academy of Sciences **95**:3152-3156.

Mutebi, J.-P., and A. D. T. Barrett. 2002. The epidemiology of yellow fever in Africa. Microbes and Infection **4**:1459-1468.

NBS, N. B. o. S. 2012. The 2012 Population and Housing Census for United Republic of Tanzania.

Nizeyi, J. B., D. Sebunya, A. J. Dasilva, M. R. Cranfield, N. J. Pieniazek, and T. K. Graczyk. 2002. Cryptosporidiosis in people sharing habitats with free-ranging mountain gorillas (Gorilla gorilla beringei), Uganda. American Journal of Tropical Medicine and Hygiene **66**:442-444.

Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative tests of parasite species richness in primates. The American Naturalist **162**:597-614.

Palacios, G., L. J. Lowenstine, M. R. Cranfield, K. V. Gilardi, L. Spelman, M. Lukasik-Braum, J.-F. Kinani, A. Mudakikwa, E. Nyirakaragire, and A. V. Bussetti. 2011. Human metapneumovirus infection in wild mountain gorillas, Rwanda. Emerging infectious diseases **17**:711.

Pascual, M., J. A. Ahumada, L. F. Chaves, X. Rodó, and M. Bouma. 2006. Malaria resurgence in the East African highlands: Temperature trends revisited. Proceedings of the National Academy of Sciences **103**:5829-5834.

Pascual, M., X. Rodó, S. P. Ellner, R. Colwell, and M. J. Bouma. 2000. Cholera Dynamics and El Niño-Southern Oscillation. Science **289**:1766-1769.

Pasteur, L., J. Joubert, and C. Chamberland. 1878. The germ theory of disease. CR Hebdom Seances Acad Sci **86**:1037-1052.

Pasteur, M. 1881. On chicken cholera: study of the conditions of non-recidivation and of some other characteristics of this disease. Science:55-57.

Patz, J. A., D. Campbell-Lendrum, T. Holloway, and J. A. Foley. 2005. Impact of regional climate change on human health. Nature **438**:310-317.

Pongsiri, M. J., J. Roman, V. O. Ezenwa, T. L. Goldberg, H. S. Koren, S. C. Newbold, R. S. Ostfeld, S. K. Pattanayak, and D. J. Salkeld. 2009. Biodiversity Loss Affects Global Disease Ecology. BioScience **59**:945-954.

Reed, W., J. S. Carroll, and A. Agramonte. 1901. The etiology of yellow fever.: An additional note. Journal of the American Medical Association **XXXVI**:431-440.

Robert. E. Shope, J. M. M. 1997. Arboviruses. Plenum Medical Book Company, New York.

Rogers, D. J., and S. E. Randolph. 2006. Climate Change and Vector-Borne Diseases. Pages 345-381 in A. G. Simon I. Hay, and J. R. David, editors. Advances in Parasitology. Academic Press.

Rüegg, W. 2004. A history of the university in Europe: Volume 3, universities in the nineteenth and early twentieth centuries (1800–1945). Cambridge University Press.

Rwego, I. B., G. Isabirye-Basuta, T. R. Gillespie, and T. L. Goldberg. 2008. Gastrointestinal Bacterial Transmission among Humans, Mountain Gorillas, and Livestock in Bwindi Impenetrable National Park, Uganda. Conservation Biology **22**:1600-1607.

Saj, T. L., P. Sicotte, and J. D. Paterson. 2001. The conflict between vervet monkeys and farmers at the forest edge in Entebbe, Uganda. African Journal of Ecology **39**:195-199.

Salyer, S. J., T. R. Gillespie, I. B. Rwego, C. A. Chapman, and T. L. Goldberg. 2012. Epidemiology and Molecular Relationships of Cryptosporidium spp. in People, Primates, and Livestock from Western Uganda. Plos Neglected Tropical Diseases **6**.

Sapkota, A. R., L. Y. Lefferts, S. McKenzie, and P. Walker. 2007. What Do We Feed to Food-Production Animals? A Review of Animal Feed Ingredients and Their Potential Impacts on Human Health. Environmental Health Perspectives **115**:663-670.

Schrag, S. J., and P. Wiener. 1995. EMERGING INFECTIOUS-DISEASE - WHAT ARE THE RELATIVE ROLES OF ECOLOGY AND EVOLUTION. Trends in Ecology & Evolution **10**:319-324.

Smithburn, K., A. Haddow, and W. Lumsden. 1949. An outbreak of sylvan yellow fever in Uganda with Aëdes (Stegomyia) africanus Theobald as principal vector and insect host of the virus. Annals of tropical medicine and parasitology **43**:74.

Soper, F. L. 1935. Rural and Jungle Yellow Fever-A new Publie Health Problem in Colombia. Rural and Jungle Yellow Fever-A new Publie Health Problem in Colombia.

Soper, F. L. 1944. Yellow fever. Clinical tropical medicine. New York City: Paul B Hoeber:391-420.

Soper, F. L. 1967. Aedes aegypti and yellow fever. Bulletin of the World Health Organization **36**:521.

Soper, F. L., H. Penna, E. Cardoso, J. Serafim, M. Frobisher, and J. Pinheiro. 1933. Yellow fever without Aëdes aegypti. Study of a rural epidemic in the Valle do Chanaan, Espirito Santo, Brazil, 1932. American Journal of Epidemiology **18**:555-587.

Stickler, C. M. 2004. The effects of logging on primate-habitat interactions: a case study of redtail monkeys (Cercopithecus ascanius) in Kibale National Park, Uganda. University of Florida.

Subbarao, K., A. Klimov, J. Katz, H. Regnery, W. Lim, H. Hall, M. Perdue, D. Swayne, C. Bender, J. Huang, M. Hemphill, T. Rowe, M. Shaw, X. Xu, K. Fukuda, and N. Cox. 1998. Characterization of an Avian Influenza A (H5N1) Virus Isolated from a Child with a Fatal Respiratory Illness. Science **279**:393-396.

Tauxe, R. V. 2002. Emerging foodborne pathogens. International Journal of Food Microbiology **78**:31-41.

Taylor, L. H., S. M. Latham, and E. Mark. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London B: Biological Sciences **356**:983-989.

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

Tranquilli, S., M. Abedi-Lartey, F. Amsini, L. Arranz, A. Asamoah, O. Babafemi, N. Barakabuye, G. Campbell, R. Chancellor, and T. R. Davenport. 2012. Lack of conservation effort rapidly increases African great ape extinction risk. Conservation Letters **5**:48-55.

Tzipori, S. 1988. CRYPTOSPORIDIOSIS IN PERSPECTIVE. Advances in Parasitology **27**:63-129.

Vazquez-Prokopec, G. M., S. Stoddard, V. Paz-Soldan, A. Morrison, J. Vasquez-Belchoir, J. Elder, T. W. Scott, and U. Kitron. 2009. USEFULNESS OF COMMERCIALLY AVAILABLE GPS DATA-LOGGERS FOR TRACKING HUMAN MOVEMENT AND RISK OF DENGUE VIRUS INFECTION. American Journal of Tropical Medicine and Hygiene **81**:230-230.

Wallis, J., and D. R. Lee. 1999. Primate conservation: The prevention of disease transmission. International Journal of Primatology **20**:803-826.

Walsh, P. D., K. A. Abernethy, M. Bermejo, R. Beyersk, P. De Wachter, M. E. Akou, B. Huljbregis, D. I. Mambounga, A. K. Toham, A. M. Kilbourn, S. A. Lahm, S. Latour, F. Maisels, C. Mbina, Y. Mihindou, S. N. Obiang, E. N. Effa, M. P. Starkey, P. Telfer, M. Thibault, C. E. G. Tutin, L. J. T. White, and D. S. Wilkie. 2003. Catastrophic ape decline in western equatorial Africa. Nature **422**:611-614.

Walsh, P. D., R. Biek, and L. A. Real. 2005. Wave-like spread of Ebola Zaire. PLoS biology **3**:e371.

Whitlock, M. C. 1996. The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. American Naturalist:S65-S77.

Wilcox, B. A., and D. J. Gubler. 2005. Disease ecology and the global emergence of zoonotic pathogens. Environmental Health and Preventive Medicine **10**:263-272.

Williams, J. M., E. V. Lonsdorf, M. L. Wilson, J. Schumacher-Stankey, J. Goodall, and A. E. Pusey. 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:766-777.

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

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

Woolhouse, M. E., and S. Gowtage-Sequeria. 2006. Host range and emerging and reemerging pathogens. Page 192. Ending the War Metaphor:: The Changing Agenda for Unraveling the Host-Microbe Relationship-Workshop Summary. National Academies Press.

Woolhouse, M. E., D. T. Haydon, and R. Antia. 2005. Emerging pathogens: the epidemiology and evolution of species jumps. Trends in ecology & evolution **20**:238-244.

Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science **292**:1109-1112.

Woolhouse, M. E., J. P. Webster, E. Domingo, B. Charlesworth, and B. R. Levin. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nature genetics **32**:569-577.

WPR, W. P. R. 2014.

Xiao, L. H., and Y. Y. Feng. 2008. Zoonotic cryptosporidiosis. Fems Immunology and Medical Microbiology **52**:309-323.

Zommers, Z., D. W. Macdonald, P. J. Johnson, and T. R. Gillespie. 2013. Impact of human activities on chimpanzee ground use and parasitism (Pan troglodytes). Conservation Letters **6**:264-273.

CHAPTER 2

Global Positioning System Data-Loggers: A Tool to Quantify Fine-Scale Movement of Domestic Animals to Evaluate Potential for Zoonotic Transmission to an Endangered Wildlife Population

2.1 SUMMARY

Domesticated animals are an important source of pathogens to endangered wildlife populations, especially when anthropogenic activities increase their overlap with humans and wildlife. Recent work in Tanzania reports the introduction of *Cryptosporidium* into wild chimpanzee populations and the increased risk of ape mortality associated with SIVcpz-*Cryptosporidium* co-infection. Here we describe the application of novel GPS technology to track the mobility of domesticated animals (27 goats, 2 sheep and 8 dogs) with the goal of identifying potential routes for *Cryptosporidium* introduction into Gombe National Park. Only goats (5/27) and sheep (2/2) were positive for *Cryptosporidium*. Analysis of GPS tracks indicated that a crop field frequented by both chimpanzees and domesticated animals was a potential hotspot for *Cryptosporidium* transmission. This study demonstrates the applicability of GPS data-loggers in studies of fine-scale mobility of animals and suggests that domesticated animal–wildlife overlap should be considered beyond protected boundaries for long-term conservation strategies.

2.2 INTRODUCTION

Patterns of fine-scale animal mobility within heterogeneous landscapes have significant impact on foraging and reproductive success, inter and intra-specific competition, creation and persistence of meta-populations, gene flow and structure, and propagation of infectious diseases (Boyd 1996; Cross et al. 2010; Hanski 2012; Peakall et al. 2003; Plumb et al. 2009; Tilman & Kareiva 1997). Mobility depends on a complex repertoire of biological, behavioral and environmental processes including animal fitness, habitat selection, dispersion, foraging, social interactions, predator aversion, and reproduction, which may occur across multiple spatial and temporal scales (Levin 1992; McKinnon et al. 2010; Shaffer et al. 2006; Sterck et al. 2005). Within species, fine-scale mobility patterns vary with resource availability or habitat loss (Gillespie & Chapman 2001; Koh & Wilcove 2008).

At fine spatial scales (i.e., landscape level), animal mobility has historically been quantified by direct observation, mark-recapture studies or radio-telemetry (Craighead 1982; Hagen & Bull 2011; Kettlewell 1955). Direct observations and mark-recapture studies are labor intensive, affected by observer bias, and not suitable for some animal systems. Radio-telemetry, which relies on animals wearing radio transmitters overcomes some of these limitations; however, these systems still require close range observations that can influence animal behavior, limit the number of animals studied, and frequency of data captured (Biggins DE 2006c; Cooke et al. 2004).

Global positioning systems (GPS) determine the location of an individual on or above the Earth's surface 24 hours a day with a high level of accuracy (US Department of

Defense 2000). The large size of GPS collars has limited GPS telemetry studies to large animals with few individuals (Moen et al. 1996; Newman et al. 2009; Schwartz & Arthur 1999). However, recent reductions in the size and cost of GPS telemetry devices now make them attractive for ecological, biological and epidemiological studies of the interplay between a larger number of smaller wild and domesticated animals, their local environment, and spread of disease.

Domesticated animals play an important role in the persistence, amplification and transmission of zoonotic pathogens and serve as sources of pathogen spillover to endangered wildlife populations, especially when anthropogenic activities lead to habitat overlap between domesticated animals and wildlife (Daszak 2000; Jones et al. 2008). Epizootic diseases such as Chagas and Nipah virus infections cause morbidity and mortality in humans and wildlife; intervention strategies are hampered by the persistence of animal reservoirs, with recursive mobility and pathogen amplification capability (Gurtler et al. 2007; Parashar et al. 2000). Despite the critical role that domesticated animals play in disease dynamics, a lack of suitable tools to continuously track animal mobility has hindered the study of mechanisms driving zoonotic spillover and disease emergence.

Empirical evidence indicates that the endangered chimpanzee (*Pan troglodytes schweinfurthii*) population of Gombe National Park, Tanzania, is infected with enteric parasites associated with humans and domesticated animals (Gillespie et al. 2010). They have experienced SIV*cpz*-associated morbidity (Keele et al. 2009), which may be complicated by *Cryptosporidium* co-infection, as seen in human HIV-*Cryptosporidium* co-infections (Tzipori 1988). *Cryptosporidium* is a zoonotic gastrointestinal parasite of a

wide range of vertebrates, highlighting its transmission potential between host species (Hunter & Thompson 2005), from ingestion of contaminated food or water (Smith et al. 2007). *Cryptosporidium* is common in ruminants and completes its life cycle in the animal's intestines, shedding high numbers of infectious oocysts into the terrestrial environment (Fayer R 1997). Despite these health threats, few studies have examined the ecology of this pathogen in rural tropical forest systems characterized by high rates of overlap among humans, domesticated animals, and wildlife (Appelbee et al. 2005).

The present study aimed to 1) assess the utility of GPS telemetry to capture the mobility patterns of domesticated animals (dogs, goats and sheep) using portable GPS data-loggers and 2) establish putative *Cryptosporidium* transmission routes by determining the extent of infected domesticated animal overlap with chimpanzees.

2.3 MATERIAL and METHODS

Ethics Statement

This project was reviewed and approved by the Emory University Institutional Review Board (approval #: IRB00018856) under the Expedited review process per 45 CFR 46.110(3), Title 45 CFR Subpart D section 46.404, one parent consent, and 21 CFR 56.110 and the National Medical Research Institute, Dar Es Salaam Tanzania. All animal use followed the guidelines of the Weatherall Report on the use of non-human primates in research and was approved by the TanzaniaWildlife Research Institute, the Emory University Animal Care and Use Committee (protocol ID 087-2009), and Tanzania Commission for Science and Technology (permit number 2009-279-NA-2009-184).

Approval was also obtained from Tanzania National Parks (Permit number TNP/HQ/C10/13) to collect samples from wild chimpanzees. The researchers did not have any interactions with the chimpanzees at the park. All animals were sampled from households in Mwamgongo village. The owners of the animals provide verbal consent for the use of their animals for this study, and the verbal consent was documented. We have included the GPS coordinates for Mwamgongo village at the first mention of the village in the Methods section.

Study Site

Gombe National Park, (35 km^2) is located 16 km north of Kigoma on the shore of Lake Tanganyika in western Tanzania (4°40′S, 29°38′E) (Fig 1A), 1,500 m above sea-level [33]. Human presence in the park is restricted to researchers, tourists, park management, local field assistants, and associated families. The northern park boundary and adjacent village, Mwamgongo (4°40′S, 29°34’60′ E) are geographically isolated, accessible from Kigoma only by a 3-4 hour motorized boat trip or to the nearest villages by foot (distance 10 km; 3 hour travel time). Mwamgongo’s approximately 7,000 residents are fishermen and farmers of palm oil, maize and cassava (Pintea 2007). Domesticated animals include goats, sheep, Muscovy ducks, chickens and household dogs that are untethered with access to areas outside the village. There are no pigs due to religious preference. The area experiences bi-modal seasonality, with a wet season from October to mid-May with annual rainfall of 1,600 mm (Greater Outdoor Recreation Pages, 2010).

The park border is porous; people and animals follow the natural boundaries freely moving in and out of the park. Chimpanzee sightings have been reported east of

the village in an area used predominantly for agriculture, where villagers report spreading goat feces on crops as a deterrent to crop-raiding goats and chimpanzees (I. Lipende, personal communication). We focused our study at the park boundary and in the agricultural fields east of the village as these locations are the areas with the highest potential for human-domesticated animal-chimpanzee interaction (Fig 1B-D).

Study Enrollment and GPS Deployment

The study consisted of a demographic survey and two GPS deployment and diagnostic specimen collection periods during the dry (1 July - 15 August 2010) and wet (1 November -15 December) seasons to capture seasonal variation in animal movement patterns. A baseline demographic survey was performed in June 2010 to identify households within Mwamgongo village with at least one domesticated animal species: dog (*Canis lupus*), goat (*Capra hircus*) or sheep (*Ovis aries*), and to estimate the number of domesticated animals within the village. Each homeowner was informed of the study purpose and the data the GPS units would capture and asked for consent. Homeowners provided documented verbal consent for the use of their animals in this study. Movement data was captured using GPS data-loggers (Igot-U GT120, Mobile Action Technology Inc., Taipei, Taiwan) strapped to adjustable animal collars and placed visibly around the neck. We programmed units to capture data at 2 min intervals for eight consecutive days. A GPS unit exchange was scheduled after four days for functionality check, data download, and battery recharge. Efforts were made to survey the same animals in both seasons. If the same individual was not available, then a new individual was enrolled. We collected a fecal sample when an animal was collared.

Within Gombe National Park, we non-invasively collected fecal samples from known individuals of two communities of habituated chimpanzees. There was no direct interaction with the chimpanzees in the National Park. Kasekela, the larger community, is situated at the center of the park and has been studied continuously since 1960. Mitumba, the smaller Northern community, was habituated in the mid-1990s and is in proximity to Mwamgongo. The sample set comprised of 58 members of the Kasekela community and 26 members from Mitumba. Fecal samples were tested for *Cryptosporidium* spp. by molecular methods. Briefly, total nucleic acid was extracted from fecal samples (da Silva et al. 1999) using the FastDNA® spin kit (MP Biomedical, Solon, OH). DNA extracts were tested by polymerase chain reaction targeting 18S gene sequences specific for *Cryptosporidium* (Xiao et al. 1999). *Cryptosporidium* prevalence was evaluated using univariate Fisher's exact tests to determine significance between groups in SPSS version 20.0 (International Business Machines, Armonk, NY USA).

Data management and analysis

GPS data were downloaded by connecting each GPS unit via USB to a personal computer using @trip software (Mobile Action Technologies). Each individual was assigned a unique ID with data saved as .csv and .gpx files. Surveyed households were mapped by capturing a waypoint at the front door of each domicile. All experimental data was projected (UTM Zone 35S, WGS 1984 datum) and imported into ArcGIS 9.3 (ESRI, Redlands, CA). GPS mapping and local observations were utilized for park boundary demarcation and high-resolution satellite imagery (QuickBird, Digital Globe) used to determine the agricultural zone (663.5 km^2). Raw GPS point data was used to evaluate GPS battery performance with descriptive statistics and independent two-tailed Student t-

tests to evaluate significance. The positional point and line accuracy of the GPS units used in this study were previously found to be 4.4 m and 10.3 m respectively (Vazquez-Prokopec et al. 2009).

We limited analysis of mobility to data points captured during daylight hours (7:00-17:00 EAT; http://www.giasma.com/en) when animals were expected to be active. Average daily median and mean maximum distances traveled were calculated for each animal. A spatial join between points within the park and the village-park border was configured to calculate the median distance of park introgression. Data points were then aggregated to calculate the proportion of points within the park, the agricultural zone and the village perimeter (other areas outside the village but not within the park or agricultural zone). Statistical analyses were performed using Mann-Whitney U and Chi-square non-parametric tests in SPSS and SOFA Statistics version 1.3.2 (Paton-Simpson & Associates Ltd, Auckland, New Zealand) to assess differences between groups. Household mobility data was excluded from the analysis, as there have been no reports of chimpanzees entering the village.

2.4 RESULTS

Twenty-five households (96.1% of all contacted households) participated in the study. Sample sizes were determined as the number of animals collared over the estimated population size for each species. We collared 8 dogs (100% of village population), 2 sheep (20% of population), and 27 goats (18% of population). Longitudinal data collection was possible for all sheep, 75% of goats and 37.5% of dogs. We were unable to resample 3 individuals due to death (1 goat and 2 dogs), inability to recapture the animal (1 dog) or unavailability of household occupants (1 goat). A single

household declined enrollment due to concern that their animal (goat) may get stuck in grasses. Only one GPS collar (4%) was never retrieved due to the animal (dog) being reported stolen.

In total, 123 unique animal readings were captured by GPS. Eight (6.5%) generated < 24 hrs of data (range 0:00-22:00 hrs) due to programming error and were excluded from evaluation. The GPS units maintained an average battery life of 92:00 hrs (range: 27:20-123:30 hrs; (SD) = 16:40). Battery life was not affected by season with an average use of 95:50 hrs (range: 27:20-134:50) in dry season versus 89:60 hrs (range 37:40-123:30) during the wet season (*p*-value = 0.165). If the average unit held a battery life of 92 hours, then this could generate 2,760 signals per collar rotation given that the units were programmed to capture a point every two minutes. The average number of GPS points captured was 1,634 (60% coverage). Fewer signal points were generated during the wet season (1,501 points) versus dry (1,891), (independent Student's t- test, *p*-value 0.002).

Five goats (18.5%) and two sheep (100%) were positive for *Cryptosporidium*. All dogs were negative for the parasite. Infection status was not determined to affect the daily median distance of sheep (Mann-Whitney U = 31, *p*-value= 0.91) or goats (Mann-Whitney U = 561, *p*-value= 0.95) from their domicile (Table 1). Goats moved significantly further from home during the wet versus dry season (U = 8,935, *p*-value < 0.001) (Table 1). Small sample sizes prevented us to test whether such seasonal variation was observed in sheep or dogs. There was variation in the distance that dogs moved on a daily basis; one individual dog traveled approximately 4,500 m from its residence in the dry season compared to the canine daily median distance of 24.5 m (IQR: 15-4,565 m).

Dogs also had the greatest daily mean maximum distance traveled at 1,876 m in the dry season compared to 589 m for goats and 471 m for sheep (Figure 2).

All species moved into the national park with the median distance of introgression less than 50 m (dogs: median distance 37 m, IQR 20-80; goats median distance 20 m, IQR 11-39; sheep median distance 50 m, IQR 23-196). Of the three species, goats spent the most time in the park in the dry (35% of GPS points collected) and wet (35%) seasons (Figure 3; *p*-value <0.0001 for both seasons) compared to dogs (≤ 2% of GPS points) and sheep (≤ 1%). Outside the park, the crop fields were commonly visited by dogs and goats; in the dry season, 7% of GPS points were from seven goats that visited or moved through crop fields compared to 10 goats (14%) in the wet season. Four dogs (two per season) moved into these fields during the dry (2%) and wet (1%) seasons.

Goats infected with *Cryptosporidium* did not have different ranging patterns from those that were not infected ($X^2 = 0.157$, df =1 *p*-value 0.691). The aggregated GPS data showed that of the 5 goats with a specimen yielding *Cryptosporidium,* infection status did not alter ranging behavior; three goats were found to spend time in the park but not the crops fields while either infected or uninfected. A fourth goat, though *Cryptosporidium* positive in the dry season, maintained a ranging pattern in both seasons that included the park and crop fields. One infected goat was not found in either location. Infected goats made up 40% (dry season) and 25% (wet) of GPS points of all goats in the crop fields and contributed 2.7% (dry) and 5.6% (wet) of all goats points in the park (Figure 3). Small sample size made it difficult to discern significance of sheep ranging patterns based upon infection status. Both sheep collared were positive for *Cryptosporidium* in the wet season only. One sheep moved into the park during the wet season but not the dry

season. The other infected sheep stayed within the village perimeter. Neither sheep visited the crop fields. The prevalence of *Cryptosporidium* in the Mitumba and Kasekela chimpanzee communities was 15.4% and 20.6% but the difference between groups was not significant (Fisher's exact test (two-tailed) *p*-value 0.7660). Rates of chimpanzee crop raiding are unknown since monitoring does not occur outside park boundaries but villager sightings of Mitumba chimpanzees raiding the crop fields occur year round with greater frequency reported in the dry season.

2.5 DISCUSSION

The human-wildlife interface is permeable and regularly altered by relatively unknown ecological and epidemiological dynamics. Our study demonstrates that portable GPS technology allows rapid and concurrent characterization of fine-scale movement from multiple individuals in a population. This technology, in combination with clinical data, can also be used to identify the spatial arrangement of those individuals infected with an etiologic agent at a specific point in time, and determine whether their infection status may alter fine scale movement processes and / or be used to predict probabilities of disease spread over time. The GPS unit cost, battery life, durability, size and weight made it suitable for field deployment. The affordability of the unit ($50 USD each) allowed for tracking 42 animals (up to 12 animals per exchange) simultaneously for two consecutive 96-hour periods. Cost is a limiting factor when considering study design and number of individuals to sample, and GPS data-loggers like the ones used in this study can provide a reliable way for tracking fine-scale local movements of domesticated animals.

The size (L/W/H in mm; 44.5 x 28.5 x 13) and weight (20g) of the units minimized the likelihood of influencing animal movement and behavior and allows for the tracking of smaller animals. The durable, water resistant properties of the unit made it suitable for tracking during excessive rainfall. Strapping the unit to an adjustable, clip-on collar minimized investigator contact with study animals, thereby reducing stress. The low refusal rate by households invited to participate in the study indicated limited apprehension to the units and associated data collection. We collected 117 unique animal readings representing over 10,500 hours of animal mobility data. The only performance difference detected was the collection of fewer GPS points during the wet season, potentially due to factors affecting satellite signal loss such as greater canopy cover, excessive rainfall, or atmospheric conditions (Dussault et al. 1999).

The GPS data provide unique insights on the patterns of domesticated animal mobility and potential interaction with chimpanzees within the park and crop-raiding areas. Our study suggests that goats, due to their high overlap with chimpanzee habitats, are the most likely spillover host and that crop-raiding areas are the potential *Cryptosporidium* spillover hotspot into chimpanzee populations. The frequency of habitat overlap during the dry season is compelling as chimpanzees are more likely to raid crops as they seek out alternative food sources. Crop raiding is a frequently reported behavior among primates (Naughton-Treves et al. 1998). A Nigerian study reported that crop-raiding baboons were more likely to harbor the anthropogenic parasite *Balantidium coli* as compared to their less frequent raiding counterparts (Weyher et al. 2006). Interestingly, Gillespie et al. (Gillespie et al. 2010) found that *B. coli* only occurred in Mitumba chimpanzees and that overall chimpanzee parasite diversity was higher for

Mitumba chimpanzees compared to Kasekela chimpanzees. During the study period, it was also reported that villagers may spread animal feces on crops as a natural deterrent (I. Lipende, personal communication), which has the potential to indirectly perpetuate the spread of infection in this identified environmental hotspot.

Cryptosporidium was detected in both Mitumba and Kasekela but there was no significant difference in the prevalence of *Cryptosporidium* between groups. Although not statistically significant, it was surprising to find a moderate prevalence of *Cryptosporidium* in Kasekela community as compared to Mitumba. The Kasekela chimpanzees have less interaction with humans and no interaction with livestock as compared to the Mitumba community whose natural border with Mwamgongo village places them in contact with human and animal activities and at greater risk for zoonotic infections. These results suggest that different transmission cycles are operating in these groups. Additional molecular characterization of the positive samples is underway to determine the species of *Cryptosporidium* in this system to assess potential transmission pathways and sources for exposure.

Cryptosporidium is of global concern (Tzipori 1988) and capable of surviving in the environment for long periods. The parasite has been detected in other habituated primates. A bovine genotype was isolated from gorillas and people living in and around Bwindi Impenetrable National Park, Uganda (Graczyk et al. 2001; Nizeyi et al. 2002). Salyer et al. (Salyer et al. 2012) examined the molecular epidemiology of *Cryptosporidium* in wild primates, people and livestock in and around Kibale National Park in Uganda and found red colobus (*Procolobus badius*) and black-and-white colobus (*Colobus guereza*) infected with *Cryptosporidium parvum/C. hominis* that resembled that

of people and livestock at the forest edge, while red colobus in the forest interior were infected with a divergent subclade, suggesting the possibility of separate zoonotic and sylvatic cycles. These findings suggest that zoonotic transmission of *Cryptosporidium* can be frequent and occur with ease in tropical settings where people, livestock, and wild primates overlap.

Sheep were positive for *Cryptosporidium,* but their movement was predominantly restricted to the village, limiting their probability of contact with chimpanzees. It is of note that only two of the village sheep were tracked and their mobility patterns may not represent movement of all sheep in the village. Although the dogs tracked in this study were all negative for *Cryptosporidium,* they demonstrated substantial overlap with chimpanzees and may present other zoonotic risks to chimpanzees, such as rabies, foot and mouth disease and parasitic worms.

The findings from this research have local and broad implications. Although there was evidence of animal mobility into the park, the few animal points beyond the forest edge indicate limited park intrusion. Rather, the area where chimpanzees are known to raid crops was a potential hot spot for pathogen spillover due to high visitation rates by domestic animals and the dispersion of potentially infected animal feces. Application of animal feces to crops may perpetuate the transmission of foodborne pathogens (Newell et al. 2010). Pathogen introductions into the environment can occur in both the dry and wet seasons but actual spillover to chimpanzees was more likely to occur in the dry season.

The focus of conservation strategies traditionally has been on wildlife corridors to minimize habitat fragmentation and risk for population isolation (Rudnick et al. 2012).

These data suggest a need to monitor borders and edges, in wildlife management and park design. In this case, the application of integrated GPS/GIS technology was capable of identifying a novel area along the Mwamgongo/Gombe border most at risk from human and animal use that may impact the sustainability of vulnerable wildlife populations.

2.7 FIGURES

Figure 2.1 Gombe National Park and Mwamgongo Village, Tanzania. A. Location of study site within Tanzania. B-D Land use plans, chimpanzee sightings, park border, crop-raiding zone, village perimeter and animal mobility tracks of dogs, goats and sheep. Dot colors represent mobility pattern of each individual animal.

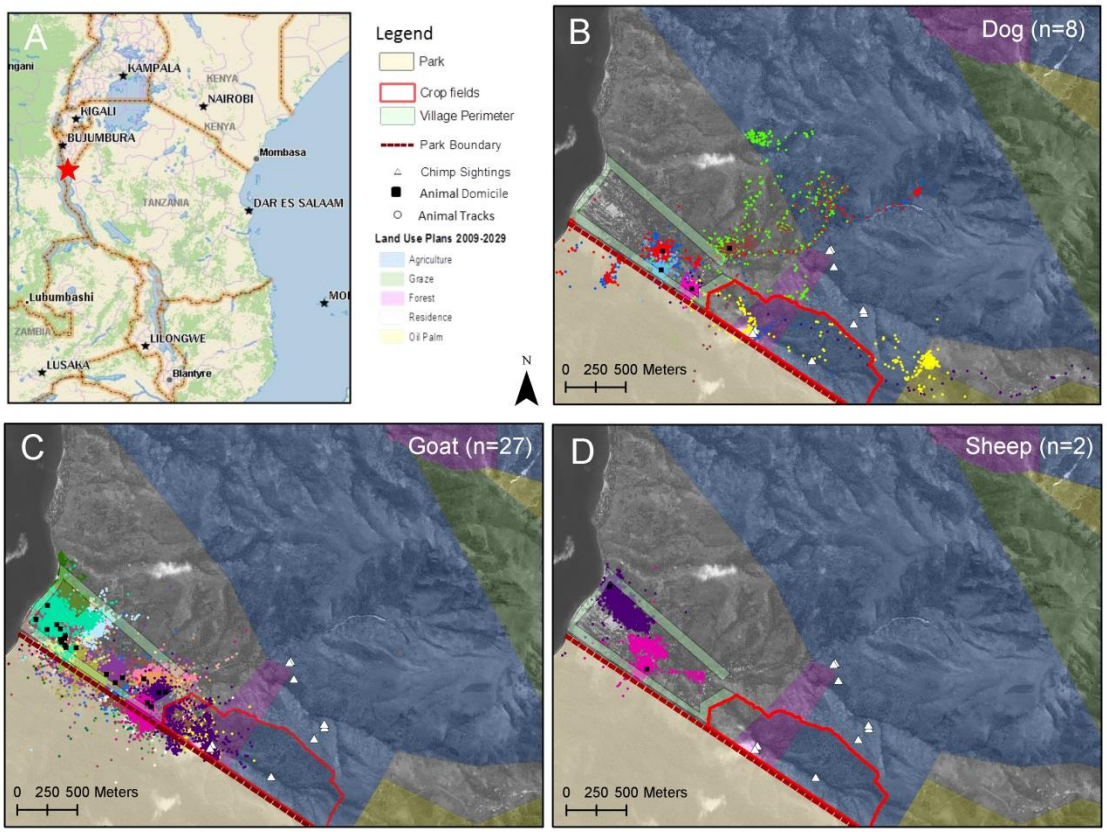


Figure 2.2 Mean maximum distance traveled from home (m) in dry and wet seasons by domesticated animal species in Mwamgongo village, Tanzania.

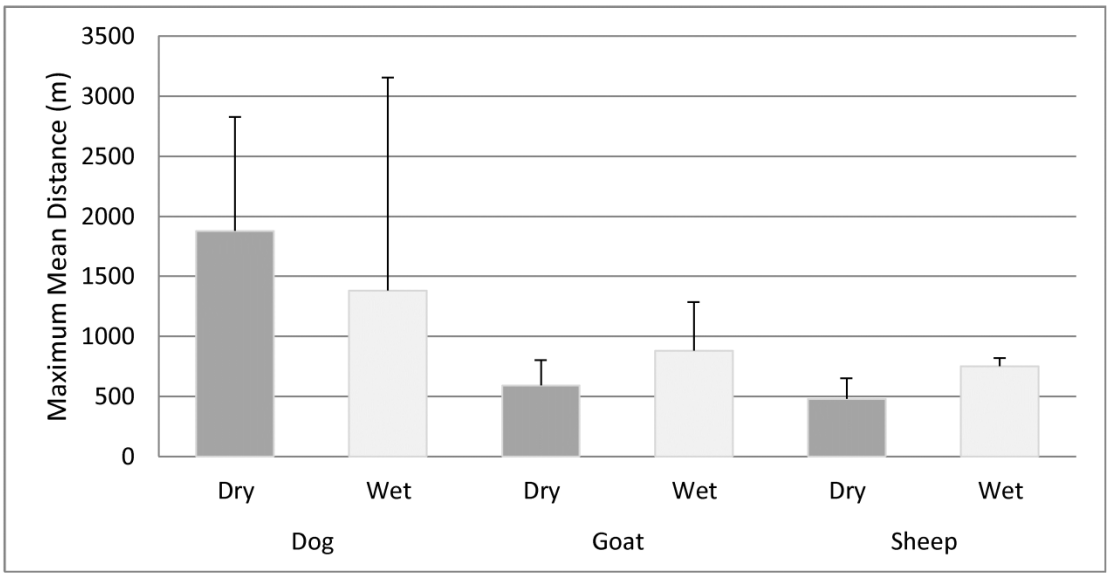
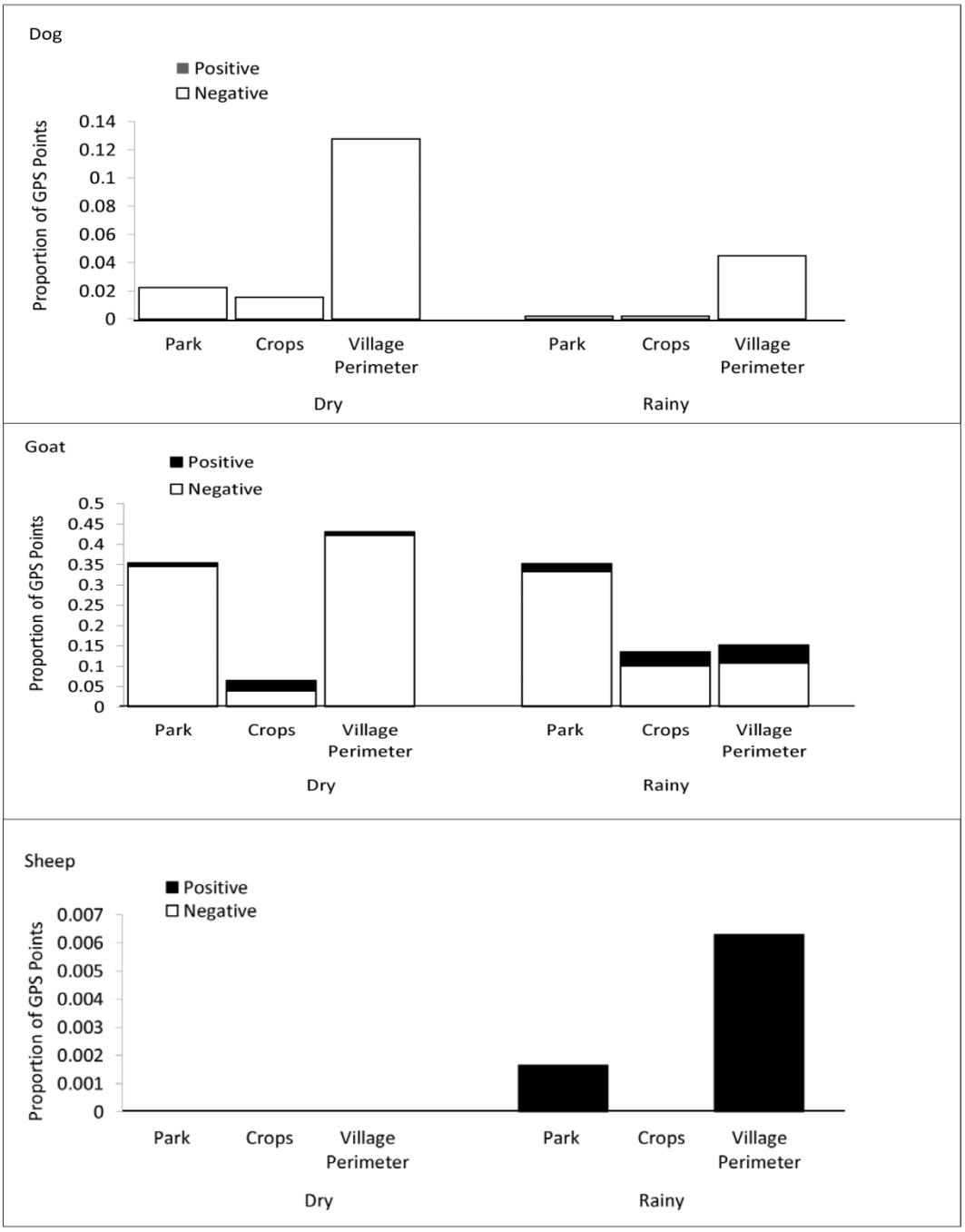


Figure 2.3 The proportion of GPS points from infected and uninfected domesticated animals in the defined study area in Mwamgongo village and Gombe National Park, Tanzania, during the dry and wet seasons. Infected animals (Goat: n = 5, Sheep: n =2, Dogs: n = 0)



2.7 TABLES

Table 2.1 Comparison of mobility patterns of domesticated animal species in Mwamgongo village adjacent to Gombe National Park, Tanzania.

	Daily distance traveled from domicile (m)		
Seasonality	**Dry**	**Wet**	***p*-value**
Dog (n=8)	24.5 (15-4564)	34 (13-2061)	$p = 0.382$
Goat (n=27)	63 (10-432)	127 (13-909)	$p = 0.001$*
Sheep (n=2)	204 (64-268)	185 (85-221)	$p = 0.091$
Infectious Status	***Cryptosporidium* Negative**	***Cryptosporidium* Positive**	***p*-value**
Goat (n=5)	67 (17-282)	85 (10-257)	$p = 0.095$
Sheep (n=2)	204 (64-268)	185 (85-221)	$p = 0.091$

Results are expressed as median with interquartile range in parentheses. *Statistically significant

2.8 REFERENCES

Appelbee, A. J., R. C. A. Thompson, and M. E. Olson. 2005. Giardia and Cryptosporidium in mammalian wildlife - current status and future needs. Trends in Parasitology **21**:370-376.

Biggins DE, M. B., Hanebury LR 2006c. Radio-telemetry for black-footed ferret research and monitoring. . Pages 175-189 in M. B. Roelle JE, Godbey JL and Biggins DE., editor. Recovery of the black-footed ferret - progress and continuing challenges. United States Geological Survey Scientific Investigations Report 2005-5293.

Boyd, I. L. 1996. Temporal scales of foraging in a marine predator. Ecology **77**:426-434.

Cooke, S. J., S. G. Hinch, M. Wikelski, R. D. Andrews, L. J. Kuchel, T. G. Wolcott, and P. J. Butler. 2004. Biotelemetry: a mechanistic approach to ecology. Trends in Ecology & Evolution **19**:334-343.

Craighead, F. C. 1982. Track of the grizzly Random House, New York, NY.

Cross, P. C., E. K. Cole, A. P. Dobson, W. H. Edwards, K. L. Hamlin, G. Luikart, A. D. Middleton, B. M. Scurlock, and P. J. White. 2010. Probable causes of increasing brucellosis in free-ranging elk of the Greater Yellowstone Ecosystem. Ecological Applications **20**:278-288.

da Silva, A. J., F. J. Bornay-Llinares, I. N. S. Moura, S. B. Slemenda, J. L. Tuttle, and N. J. Pieniazek. 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Molecular Diagnosis **4**:57-64.

Daszak, P. 2000. Emerging infectious diseases of wildlife - Threats to biodiversity and human health (vol 287, pg 443, 2000). Science **287**:1756-1756.

Dussault, C., R. Courtois, J. P. Ouellet, and J. Huot. 1999. Evaluation of GPS telemetry collar performance for habitat studies in the boreal forest. Wildlife Society Bulletin **27**:965-972.

Fayer R, S. C., Dubey JP. . 1997. The general biology of Cryptosporidium. Pages 1-42 in R. Fayer, editor. Cryptosporidium and Cryptospirodosis. CRC Press, Boca Raton, FL.

Gillespie, T. R., and C. A. Chapman. 2001. Determinants of group size in the red colobus monkey (Procolobus badius): an evaluation of the generality of the ecological-constraints model. Behavioral Ecology and Sociobiology **50**:329-338.

Gillespie, T. R., E. V. Lonsdorf, E. P. Canfield, D. J. Meyer, Y. Nadler, J. Raphael, A. E. Pusey, J. Pond, J. Pauley, T. Mlengeya, and D. A. Travis. 2010. Demographic and Ecological Effects on Patterns of Parasitism in Eastern Chimpanzees (Pan troglodytes schweinfurthii) in Gombe National Park, Tanzania. American Journal of Physical Anthropology **143**:534-544.

Great Outdoor Recreation Pages (2010) Gombe Stream National Park, Ecology and Climate. http://www.gorp.com/parks-guide/travel-ta-gombe-stream-national-park-sidwcmdev_053609.html. Access date: October 6, 2014

Graczyk, T. K., A. J. DaSilva, M. R. Cranfield, J. B. Nizeyi, G. Kalema, and N. J. Pieniazek. 2001. Cryptosporidium parvum Genotype 2 infections in free-ranging mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. Parasitology Research **87**:368-370.

Gurtler, R. E., M. C. Cecere, M. A. Lauricella, M. V. Cardinal, U. Kitron, and J. E. Cohen. 2007. Domestic dogs and cats as sources of Trypanosoma cruzi infection in rural northwestern Argentina. Parasitology **134**:69-82.

Hagen, I. J., and C. M. Bull. 2011. Home Ranges in the Trees: Radiotelemetry of the Prehensile Tailed Skink, Corucia zebrata. Journal of Herpetology **45**:36-39.

Hanski, I. 2012. Eco-evolutionary dynamics in a changing world. Pages 1-17 in R. S. Ostfeld, and W. H. Schlesinger, editors. Year in Ecology and Conservation Biology.

Hunter, P. R., and R. C. A. Thompson. 2005. The zoonotic transmission of Giardia and Cryptosporidium. International Journal for Parasitology **35**:1181-1190.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-U994.

Keele, B. F., J. H. Jones, K. A. Terio, J. D. Estes, R. S. Rudicell, M. L. Wilson, Y. Y. Li, G. H. Learn, T. M. Beasley, J. Schumacher-Stankey, E. Wroblewski, A. Mosser, J. Raphael, S. Kamenya, E. V. Lonsdorf, D. A. Travis, T. Mlengeya, M. J. Kinsel, J. G. Else, G. Silvestri, J. Goodall, P. M. Sharp, G. M. Shaw, A. E. Pusey, and B. H. Hahn. 2009. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature **460**:515-519.

Kettlewell, H. B. D. 1955. Selection experiments on industrial melanism. Heredity **9**:323-342.

Koh, L. P., and D. S. Wilcove. 2008. Is oil palm agriculture really destroying tropical biodiversity? Conservation Letters **1**:60-64.

Levin, S. A. 1992. THE PROBLEM OF PATTERN AND SCALE IN ECOLOGY. Ecology **73**:1943-1967.

McKinnon, L., P. A. Smith, E. Nol, J. L. Martin, F. I. Doyle, K. F. Abraham, H. G. Gilchrist, R. I. G. Morrison, and J. Bety. 2010. Lower Predation Risk for Migratory Birds at High Latitudes. Science **327**:326-327.

Moen, R., J. Pastor, Y. Cohen, and C. C. Schwartz. 1996. Effects of moose movement and habitat use on GPS collar performance. Journal of Wildlife Management **60**:659-668.

Naughton-Treves, L., A. Treves, C. Chapman, and R. Wrangham. 1998. Temporal patterns of crop-raiding by primates: linking food availability in croplands and adjacent forest. Journal of Applied Ecology **35**:596-606.

Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. van der Giessen, and H. Kruse. 2010. Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. International Journal of Food Microbiology **139**:S3-S15.

Newman, S. H., S. A. Iverson, J. Y. Takekawa, M. Gilbert, D. J. Prosser, N. Batbayar, T. Natsagdorj, and D. C. Douglas. 2009. Migration of Whooper Swans and Outbreaks of Highly Pathogenic Avian Influenza H5N1 Virus in Eastern Asia. PLoS ONE **4**:e5729.

Nizeyi, J. B., M. R. Cranfield, and T. K. Graczyk. 2002. Cattle near the Bwindi Impenetrable National Park, Uganda, as a reservoir of Cryptosporidium parvum and Giardia duodenalis for local community and free-ranging gorillas. Parasitology Research **88**:380-385.

Parashar, U. D., L. M. Sunn, F. Ong, A. W. Mounts, M. T. Arif, T. G. Ksiazek, M. A. Kamaluddin, A. N. Mustafa, H. Kaur, L. M. Ding, G. Othman, H. M. Radzi, P. T. Kitsutani, P. C. Stockton, J. Arokiasamy, H. E. Gary, L. J. Anderson, and I. Nipah Encephalitis Outbreak. 2000. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. Journal of Infectious Diseases **181**:1755-1759.

Peakall, R., M. Ruibal, and D. B. Lindenmayer. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, Rattus fuscipes. Evolution **57**:1182-1195.

Pintea, L. 2007. Applying satellite imagery and GIS for chimpanzee habitat change detection and conservation. University of Minnesota. University of Minnesota, St. Paul, Minnesota, USA.

Plumb, G. E., P. J. White, M. B. Coughenour, and R. L. Wallen. 2009. Carrying capacity, migration, and dispersal in Yellowstone bison. Biological Conservation **142**:2377-2387.

Rudnick DA, Ryan SJ, Beier P, Cushman SA, Dieffenbach F, Epps CW, Gerber LR, Hartter J, Jenness JS, Kintsch J, Merenlender AM, Perkl, RM, Preziosi, DV, Trombulak, SC (2012) The Role of Landscape Connectivity in Planning and Implementing Conservation and Restoration Priorities. Issues in Ecology, The Ecological Society of America Report no. 16:1-20

Salyer, S. J., T. R. Gillespie, I. B. Rwego, C. A. Chapman, and T. L. Goldberg. 2012. Epidemiology and Molecular Relationships of Cryptosporidium spp. in People, Primates, and Livestock from Western Uganda. Plos Neglected Tropical Diseases **6**.

Schwartz, C. C., and S. M. Arthur. 1999. Radio-tracking large wilderness mammals: integration of GPS and Argos technology. Ursus **11**:261-274.

Shaffer, S. A., Y. Tremblay, H. Weimerskirch, D. Scott, D. R. Thompson, P. M. Sagar, H. Moller, G. A. Taylor, D. G. Foley, B. A. Block, and D. P. Costa. 2006. Migratory shearwaters integrate oceanic resources across the Pacific Ocean in an endless summer. Proceedings of the National Academy of Sciences of the United States of America **103**:12799-12802.

Smith, H. V., S. M. Caccio, N. Cook, R. A. B. Nichols, and A. Tait. 2007. Cryptosporidium and Giardia as foodborne zoonoses. Veterinary Parasitology **149**:29-40.

Sterck, E. H. M., E. P. Willems, J. van Hooff, and S. A. Wich. 2005. Female dispersal, inbreeding avoidance and mate choice in Thomas langurs (Presbytis thomasi). Behaviour **142**:845-868.

Tilman, D., and P. Kareiva 1997. Spatial ecology: the role of space in population dynamics and interspecific interactions. Princeton University Press.

Tzipori, S. 1988. CRYPTOSPORIDIOSIS IN PERSPECTIVE. Advances in Parasitology **27**:63-129.

United States Department of Defense (2000) Global Positioning Systems Standard Positioning Service Performance Standard. http://www.gps.gov/systems/gps/performance/accuracy. Access date: September 20, 2013.

Vazquez-Prokopec, G. M., S. Stoddard, V. Paz-Soldan, A. Morrison, J. Vasquez-Belchoir, J. Elder, T. W. Scott, and U. Kitron. 2009. USEFULNESS OF COMMERCIALLY AVAILABLE GPS DATA-LOGGERS FOR TRACKING HUMAN MOVEMENT AND RISK OF DENGUE VIRUS INFECTION. American Journal of Tropical Medicine and Hygiene **81**:230-230.

Weyher, A. H., C. Ross, and S. Semple. 2006. Gastrointestinal parasites in crop raiding and wild foraging Papio anubis in Nigeria. International Journal of Primatology **27**:1519-1534.

Xiao, L. H., L. Escalante, C. F. Yang, I. Sulaiman, A. A. Escalante, R. J. Montali, R. Fayer, and A. A. Lal. 1999. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. Applied and Environmental Microbiology **65**:1578-1583.

CHAPTER 3

Epidemiology and Molecular Characterization of *Cryptosporidium* spp. in Humans, Wild Primates, and Domesticated Animals in the Greater Gombe Ecosystem, Tanzania

3.1 SUMMARY

Cryptosporidium is an important zoonotic parasite globally. Few studies have examined the ecology and epidemiology of this pathogen in rural tropical systems characterized by high rates of overlap among humans, domesticated animals, and wildlife. We investigated risk factors for *Cryptosporidium* infection and assessed cross-species transmission potential among people, non-human primates, and domestic animals in the Gombe Ecosystem, Kigoma District, Tanzania. A cross-sectional survey was designed to determine the occurrence and risk factors for *Cryptosporidium* infection in humans, domestic animals and wildlife living in and around Gombe National Park. Diagnostic PCR revealed *Cryptosporidium* infection rates of 4.3% in humans, 16.0% in non-human primates, and 9.6% in livestock. Local streams sampled were negative. DNA sequencing uncovered a complex epidemiology for *Cryptosporidium* in this system, with humans, baboons and a subset of chimpanzees infected with *C. hominis* subtype IfA12G2; another subset of chimpanzees infected with *C. suis*; and all positive goats and sheep infected with *C. xiaoi*. For humans, residence location was associated with increased risk of infection in Mwamgongo village compared to one camp (Kasekela), and there was an increased odds for infection when living in a household with another positive person. Fecal consistency and other gastrointestinal signs did not predict

Cryptosporidium infection. Despite a high degree of habitat overlap between village people and livestock, our results suggest that there are distinct *Cryptosporidium* transmission dynamics for humans and livestock in this system. The dominance of *C. hominis* subtype IfA12G2 among humans and non-human primates suggest cross-species transmission. Interestingly, a subset of chimpanzees was infected with *C. suis.* We hypothesize that there is cross-species transmission from bush pigs (*Potaochoerus larvatus*) to chimpanzees in Gombe forest, since domesticated pigs are regionally absent. Our findings demonstrate a complex nature of *Cryptosporidium* in sympatric primates, including humans, and stress the need for further studies.

3.2 INTRODUCTION

Cryptosporidium is one of the most important parasitic diarrheal agents in humans in the world, is among the top four causes of moderate-to-severe diarrheal disease in young children in developing nations, and is problematic as an opportunistic co-infection with HIV due to increased morbidity and mortality (Kotloff et al. 2013; Tzipori 1988). *Cryptosporidium* is well adapted to zoonotic, waterborne, and foodborne transmission, with a life cycle occurring in suitable hosts and transmission by the fecal-oral route (Smith et al. 2007). Zoonoses represent the majority of diseases emerging globally with potential to expand to new host systems, (Jones et al. 2008) yet despite these health threats, few studies have examined the ecology and epidemiology of this pathogen in rural tropical forest systems characterized by high rates of overlap among humans, domesticated animals, and wildlife (Appelbee et al. 2005; Leav et al. 2003).

In Tanzania, agriculture represents over a quarter of the national income and 80 percent of its labor force (US Counter Intelligence Agency 2013), but natural resources are declining, affected by desertification and soil degradation from recent droughts. This process has resulted in a high rate of loss of forest and woodland habitat (Ministry of Agriculture, Tanzania 2008). The resulting fragmented landscape increases human-wildlife contact in these areas, elevating the risk for disease transmission. The Greater Gombe Ecosystem (GGE), Tanzania, in particular, is vulnerable to habitat disturbance, and this has both ecological and financial implications since it is home to diverse wildlife, including endangered chimpanzees (*Pan troglodytes schweinfurthii*), that are important contributors to the national economy through tourism (US Counter Intelligence Agency 2013).

Gombe National Park, established in 1968, is a small 35-km^2 forest reserve located 16-km north of Kigoma in Western Tanzania (4°40′S 29°38′E). The park is 1500-m above sea-level with hills sloping westward from a rift escarpment to Lake Tanganyika (Wallis & Lee 1999). It is home to a number of non-human primate species, including baboons (*Pabio anubis*), and a well-known wild chimpanzee population studied continuously for over 50 years (Earnhardt JM 2003; Goodall 1986). There are three chimpanzee communities (Kasekela, Mitumba and Kalande); two of which, (Kasekela, and Mitumba) are habituated (Pusey et al. 2007). The habitat ranges of these two communities overlap slightly permitting opportunity for member contact. Their habitats have differing degrees of human encroachment (Pusey et al. 2008). Kasekela, the larger community (~ 65 individuals), is situated at the center of the park in less disturbed forest, whereas Mitumba, the smaller Northern community (~ 25 individuals), is in close

proximity to Mwamgongo (4°40′S, 29°34’60′ E), a village home to ~5000 inhabitants and their livestock. Another village borders the park to the South, but not along the Eastern ridge, due to high elevation and historic soil depletion. Human presence in the park is limited to researchers, tourists, park management staff, local field assistants and members of their families. The park border is not fenced and therefore villagers and their untethered animals (goats, sheep and dogs) are able to enter the park (Parsons MB 2014). Mitumba chimpanzees are frequently reported raiding agricultural fields to the east in the Northern village, Mwamgongo, especially during the dry season (I. Lipende, personal communication). There is little evidence that the chimpanzee population has emigrated outside its established habitat for over 20 years, and immigration events are rare.

Death from infectious diseases is the leading cause of mortality for Gombe chimpanzees (Lonsdorf et al. 2006; Williams et al. 2008). The chimpanzees have experienced SIVcpz-associated mortality and morbidity, with SIVcpz prevalence ranging between 9-18% and a 10-16-fold higher age-corrected death hazard for infected individuals (Keele et al. 2009). *Cryptosporidium* is of special concern in this chimpanzee population, as SIVcpz illness may be complicated by *Cryptosporidium* co-infection, and mirror clinical features observed in human HIV/*Cryptosporidium* co-infections (Tzipori 1988), that report *Cryptosporidium* infection rates from 8-30% (Colebunders et al. 1988; Tarimo et al. 1996). To improve our understanding of this relationship, and highlight potential management options, we investigated risk factors for *Cryptosporidium* infection and assessed cross-species transmission potential among people, non-human primates, and domestic animals in the GGE, Kigoma District, Tanzania.

3.3 MATERIALS and METHODS

Sample Frame

The study period occurred between March 2010 and February 2011. Paired fecal samples from humans and domestic animals were collected during the dry (July 1-August 15) and wet (November 1-December 15) seasons. Human subjects were either residents of Mwamgongo village (estimated population size (n) ~5000) or Gombe National Park (n ~100). A baseline demographic survey was performed in June 2010 to identify households within Mwamgongo village with at least one domestic animal species: dog (*Canis lupus*) n ~ 8, goat (*Capra hircus*), n ~ 150 or sheep (*Ovis aries*), n ~ 10. Twenty-five village households with domestic animals were randomly selected for study enrollment. Baboons (n ~ 198) were opportunistically sampled in Mitumba and Kasekela during these two collection periods. Chimpanzees (n ~ 90) were sampled in both Mitumba and Kasekela at quarterly intervals during the course of routine observational health monitoring [16].

Specimen Collection and Transport

Specimen cups were provided to enrolled village and park residents. Livestock specimens were aseptically collected by a village veterinary officer. Chimpanzee and baboon specimens were non-invasively collected from identified individuals as part of observational health monitoring. All fecal specimens were freshly voided and aseptically transferred to a screw cap plastic vial containing a 2.5% potassium dichromate solution (Fisher Scientific, Pittsburgh, PA). For baboon and non-human primate samples, care

was taken to avoid the collection of soil, foliage or water contaminants, by transferring the interior and top most portion of stool to a collection cup using a sterile wooden spatula or swab and avoiding the collection of fecal material in contact with the ground. Each vial was labeled with a unique identification number, and date of collection. Wildlife samples were additionally labeled with the name of the observer, location and animal name. Samples were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) and stored at 4^{o}C, and shipped in ice to Atlanta, GA United States.

Stream sampling

Approximately 1-liter of water was collected in 55-oz sterile Whirl-pak bags (Nasco, Fort Atkinson, WI) and filtered for protozoa using a 0.45-µm Millipore MF-Millipore cellulose ester filter mounted on the Millipore (Billerica, MA) filtration system (diameter 47-mm). When possible, filtration was done on one filter, but in extreme cases where turbidity was high, sequential filtration was performed using two filters. Using sterilized forceps, filters were aseptically transferred to 2-ml cryovials containing a 2.5% potassium dichromate solution. Due to the logistics of field sampling, opportunistic water samples were collected from low, middle and high points of 6 continual streams (dry and wet) and two seasonal streams (wet only). GPS coordinates were obtained using a GPSmap 60CSx from Garmin (Garmin International Inc. Olathe, KS) for each collection point to assist in identifying locations for repeat sampling and if necessary, to assign sampled streams to watersheds associated with specific human or chimpanzee groups.

DNA extraction, molecular detection and subtyping

Nucleic acid was extracted from all fecal specimens and water filters using the FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH) following the methods described (da Silva et al. 1999). DNA extracts were subsequently tested using a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) approach where a segment (~833 bp) of the *Cryptosporidium* SSU rRNA gene is amplified by nested PCR and then species and genotype diagnosis is made by restriction digestion of the secondary PCR product with *Ssp*I (New England BioLabs, Beverly, MA), and either *Vsp*I (Promega, Madison, WI) or *Mbo*II (New England BioLabs) (Lihua Xiao 2008; Xiao et al. 2001). Each sample was run in duplicate by PCR-RFLP analyses with appropriate controls. Specimens that were positive for *Cryptosporidium* by the SSU rRNA PCR were confirmed by DNA sequencing of the 18S PCR products (*C. suis, C. xiaoi and C. hominis*). A subset of specimens positive for *C. hominis* were also subtyped by sequencing the 60-kilodalton glycoprotein (GP60; ~900 bp) in both directions on an ABI 3130 Genetic Analyzer (Foster City, CA) (Alves et al. 2003). All sequences obtained were aligned with reference sequences using MEGA 6.0 or ClustalX software (http://www.clustal.org/) to identify *Cryptosporidium* species and *C. hominis* genotypes.

Human risk factor survey

A survey was administered to each human subject focusing on demography, gastrointestinal symptoms (presence or absence within the previous 4 weeks), medication usage, and water usage. To minimize response bias, surveys were administered by trained local field assistants in the national language (Swahili). Data were manually recorded on

paper forms, entered into spreadsheets in the computer program Microsoft Excel, and subsequently reviewed for accuracy.

Statistical analyses and control for sample bias

Results were tabulated and compared in Microsoft Excel (Redmond, WA). To control for sample bias we calculated infection rate as the proportion of individuals in each group positive for *Cryptosporidium* divided by the total number of individuals in each group examined (Gillespie et al. 2010). If a single individual sample was positive for *Cryptosporidium,* the subject was considered positive for the collection period (the season for most analyses). Statistical analyses were performed in SPSS version 20.0 (SPSS Inc., Chicago IL). Associations between human survey responses and infection status were compared using logistic regression for categorical binary data. Work history (agricultural fields or forest) were combined as a single factor in the final analysis. Odds ratios (OR) with 95% CI were calculated with significance set at 0.05 for all comparisons. Associations between chimpanzee demographic and observational health data and infection status were evaluated using a generalized estimating equation (GEE) method with exchangeable working correlation structure to account for repeat sampling of individuals. The Huber–White sandwich variance estimation technique was used to calculate confidence intervals (CI). In instances where cells contained less than 5 values, Fisher's exact tests were used to calculate *p* -values.

3.4 RESULTS

Six hundred and eighty-four fecal specimens were screened for *Cryptosporidium* including 254 human, 99 domestic animal (n=76 goat, n=14 sheep, n=9 dog) and 331 wildlife (n=251 chimpanzee, n=80 baboon) specimens. *Cryptosporidium* spp, were detected by PCR from 40 (5.8%) fecal samples but was not detected in any water samples (n=42). The infection rate of *Cryptosporidium* was highest among 21/131 (16.0%) nonhuman primates tested, compared to 7/73 (9.6%) livestock and 8/185 (4.3%) humans. No significant differences in frequency were observed between chimpanzees and baboons (Table 1, Fisher's exact test $p = 0.457$) or between the two chimpanzee communities (Table 1, Fisher's exact test $p = 0.7655$). Of the 8 cases of *Cryptosporidium* detected in humans, 7 (87.5%) resided in Mwamgongo village and one (12.5%) in Mitumba camp. No human cases were detected in the Kasekela camp. Sheep had the highest occurrence of *Cryptosporidium* (22%) compared to goat (9%) and dogs (0%) but small sample size prevented evaluation of significance.

We identified three species of *Cryptosporidium* (*C. hominis*, *C. suis* and *C. xiaoi*) in this population (Table 1) based on RFLP and sequence analyses of the SSU rRNA gene. *C. hominis* was detected in all human cases and all 7 cases from sheep and goats were *C. xiaoi*. Six of the 12 positive chimpanzees from Kasekela were genotyped as *C. suis* (a *Cryptosporidium* species predominantly associated with pigs but has been found in a few human cases). This species was not detected in the Mitumba chimpanzee community (Fisher's two-tailed exact test; *p*-value = 0.0537). The porcine species was also not found in the specimens from baboons, humans or domestic animals in the village. The remaining Kasekela chimpanzees (n=6) and the 4 Mitumba chimpanzees had

C. hominis. All baboons (n =5 individuals) also had *C. hominis*. GP60 subtyping of a subset (n = 16) of the *C. hominis* positive samples identified a common subtype IfA12G2 in humans and nonhuman primates. The subtype sequence was identical to two sequences in GenBank; a human *C. hominis* IfA12G2 sequence from South Africa (GenBank accession number JN867334) and a sequence recovered from an olive baboon in Kenya (GenBank accession number JF681172).

We used data from the survey to identify potential risk factors for *Cryptosporidium* infection (Table 2). Among 95 respondents (100%), villagers in Mwamgongo were at greater risk for infection when living with a person who was positive for *Cryptosporidium* (OR = 9.722; 95% CI 1.741-54.279; $p = 0.011$). Persons living with *Cryptosporidium*-positive livestock tended to have a greater odds of infection (OR = 4.750; 95% CI 0.944-23.908; $p = 0.059$). Other factors related to behaviors, including location, occupation (either agricultural or forestry), and not boiling water for consumption were not statistically significant. Although presence of clinical signs was not statistically significant, when reviewing survey data for the *Cryptosporidium* positive patients, 4/8 reported having diarrhea; 2 sought treatment at the village clinic (Flagyl and Paracetemol). Four of 8 households (50%) reported at least one additional member of the household experiencing gastrointestinal symptoms, including diarrhea and cramping. Interestingly, affected individuals did not report consuming water from an open source (100%), which appeared protective (OR = 0.156; 95% CI 0.085-2.875) but not statistically significant ($p = 0.162$). 4 % of study respondents reported boiling their water before use. No infected individuals reported boiling their drinking water. An association between season and *Cryptosporidium* infection was not observed in humans or non-

human primates (Tables 2 and 3). Chimpanzee demographic factors such as age and sex were not risk factors for *Cryptosporidium* infection and evidence of diarrhea was not a reliable predictor of *Cryptosporidium* illness. Kasekela chimpanzees tended to have a higher likelihood (OR = 7.062; 95% CI 0.398-125.251, $p = 0.07$) of infection with *C. suis* as compared to the Mitumba community (Table 3).

3.5 DISCUSSION

Of 90 humans residing in camps within Gombe National Park, only one *Cryptosporidium* infection was observed (1%). In contrast, *Cryptosporidium* infection in residents of Mwamgongo village reached 10% during the drier months. These frequencies are comparable to those reported in some studies from children and adults without HIV, ranging from 0-18% (Cegielski et al. 1999; Gomez Morales et al. 1995; Tumwine et al. 2005), though frequencies as high as 32% have been reported elsewhere (Salyer et al. 2012). Site and HIV prevalence would seem to be important factors in human occurrence rates. Although HIV testing was beyond the scope of this study, a recent country report (Tanzania Commission for AIDS (TACAIDS) 2008) indicates a low HIV prevalence (< 1%) from the Kigoma region where the study occurred. Infection was not statistically associated with gastrointestinal illness or stool consistency for humans or wildlife, findings consistent with earlier studies (Cama et al. 2007; Checkley et al. 1997; Gracenea et al. 2002; Houpt et al. 2005; Salyer et al. 2012). Surprisingly, unsafe drinking water (i.e. untreated/unboiled water, open water source) was not found to increase risk of *Cryptosporidium* infection, which could be the result of degradation or improper tapping of the water line, exposing water to environmental contamination (Onda et al. 2012). This warrants further study as consumption of contaminated ground

water has been repeatedly associated with *Cryptosporidium* infection (Akinbo et al. 2010; Bridgman et al. 1995).

Cryptosporidium was detected in baboons (11%) and the two chimpanzee communities (15-21%) at moderate frequencies. Similar frequencies of *Cryptosporidium* have been detected previously in other African primates. Habituated mountain gorillas in Bwindi Impenetrable National Park (BINP), Uganda had *Cryptosporidium* in (11%) of specimens sampled; 73% of positive specimens were detected from human-habituated gorillas (Nizeyi et al. 1999). Genetic characterization of this population determined that the gorillas and the local human community both carry *C. parvum* (Graczyk et al. 2001). Eleven percent of red colobus and black-and-white colobus in Kibale National Park (KNP), Uganda were infected with *Cryptosporidium;* genetic sequences from some humans and colobus from KNP were identical, while two strains from red colobus in the forest interior were infected with a divergent subclade, suggesting the possibility of separate zoonotic and sylvatic cycles (Salyer et al. 2012). These findings support the zoonotic spillover potential of *Cryptosporidium* among humans, wildlife and livestock.

Similar patterns have been observed with other directly or environmentally transmitted enteric pathogens. At KNP, *Giardia* was detected in red colobus in forest fragments (5.7%), but not detected in undisturbed forest (0%) (Salzer et al. 2007). Genetic analyses of recovered strains determined that red colobus were infected with *Giardia duodenalis* assemblages associated with humans and livestock, suggesting complex cross-species transmission (Johnston et al. 2010). *Escherichia coli* strains from gorillas in BINP and chimpanzees in KNP that overlapped with humans were more similar to strains collected from resident humans and livestock compared to strains

collected from gorillas and chimpanzees living in undisturbed forest (Rwego et al. 2008a; Rwego et al. 2008b). Additionally, in KNP, genetic similarity between human/livestock and primate bacteria increased three-fold with a moderate to high increase in anthropogenic disturbance of forest fragment. Bacteria harbored by humans and livestock were more similar to those of monkeys that entered human settlements to raid crops, than to bacteria of other primate species (Goldberg et al. 2008). These findings reinforce the notion that habitat overlap and anthropogenic disturbance increase the risk of interspecies transmission between wildlife, humans and livestock and that transmission can occur both via direct physical contact with or ingestion of contaminated feces and by indirect exposure via a shared (potentially contaminated) watershed. Although water samples screened in this study were negative for the parasite, waterborne outbreaks of *Cryptosporidium* as a result of human and animal fecal contamination are common (Aksoy U 2007; Baldursson & Karanis 2011; Persson K 2007). Watershed sampling for *Cryptosporidium* in this study was opportunistic using smaller volumes of water than advocated by standard screening protocols (Graczyk et al. 1997)and provided only limited inter-seasonal sampling(Davies et al. 2004; Hansen & Ongerth 1991; Jiang et al. 2005). Therefore, our negative results do not assure that waterborne transmission is not important in this system. Future studies using more comprehensive watershed sampling would help to resolve this aspect of *Cryptosporidium* transmission.

The results of our RFLP and sequence analyses of the SSU rRNA gene suggest multiple potential zoonotic pathways for *Cryptosporidium* transmission in this study system. The village data reinforces our understanding that species of *Cryptosporidium* vary in their zoonotic potential (Xiao & Feng 2008). Our data suggest that there is less

likelihood that the *C. xiaoi* affecting the livestock is capable of causing illness in humans, considering the close proximity of livestock to humans in this community [i.e., animals often residing in homes], where animal-human contact is quite high though it has been documented in other studies (Adamu et al. 2014). *Cryptosporidium hominis* was also not detected among the domesticated animals in this study, but *C. hominis* has been found in other studies to be a zoonotic species, affecting both humans and domesticated animals (Abeywardena et al. 2012; Rajendran et al. 2011). Homes with positive livestock had a tendency for increased risk of human infection suggesting contribution of environmental factors or behaviors that may place the household at increased risk.

We anticipated that the Kasekela chimpanzee community would have a lower occurrence of *Cryptosporidium* compared to Mitumba, which shares a natural border with Mwamgongo village. Although not statistically significant, there was a higher frequency of *Cryptosporidium* recovered in Kasekela. The occurrence of *C. hominis* was not statistically higher for Mitumba compared to Kasekela. However, the results demonstrated that 50% (6/12) of the *Cryptosporidium* recovered from the Kasekela community are *C. suis*, a species associated with pigs. Domestic pigs are not found in the park or village due to religious preference (predominantly Muslim communities). However, bush pigs (*Potaochoerus larvatus*), native to the Gombe forest habitat, are common in Kasekela but have not been observed frequently in Mitumba forest. Thus bush pigs may serve as the reservoir host for *C. suis* in this system. Chimpanzees may be infected through contact with this animal (i.e., chimpanzees occasionally consume bush pigs) or by indirect contact with infective feces on the forest floor or the contamination of shared water sources. This putative pathway of transmission is supported by the fact that

C. suis was not recovered in the Mitumba chimpanzee community, the baboons, domesticated livestock or village inhabitants. *C. suis* has zoonotic potential having been previously identified in an HIV+ patient in Lima, Peru, (Xiao et al. 2002)and from patients in Henan, China and England (Leoni et al. 2006; Wang et al. 2013). Similarly, (Salyer et al. 2012) found that while *Cryptosporidium* may be transmitted frequently among domesticated animals, humans and wildlife in areas of overlap, there may be host-parasite specific dynamics that occur in the absence of these regular interactions creating separate transmission cycles.

The appearance of the *C. hominis* species and its subtype IfA12G2C among the humans, baboons and chimpanzee communities demonstrates the zoonotic transmission potential of this parasite species among these closely related host species and points to a dominance of anthropozonotic transmission in this system. This subtype has been previously reported among captive olive baboons in Kenya (Li et al. 2011), and is prevalent in humans in Africa (Leav et al. 2002). Additionally, common primate behaviors may increase the likelihood for animal to human transmission. For example, baboons raid camp food reserves and homes, potentially transmitting etiologic agents via infected feces to humans. The Mitumba chimpanzees are reported to raid agricultural fields just outside the park boundaries, which can transmit diseases from the potentially contaminated feces of livestock or exposed human sewage.

Our results are based on small sample sizes, that if increased could alter the frequency and predictions of *Cryptosporidium* subtypes, infection and illness. The finding of *C. suis* in the Kasekela chimpanzee community is novel. We presume these chimpanzees experienced cross-species transmission from bush pigs in Gombe forest,

because domesticated pigs are absent from the area. Unfortunately, we do not have access to fecal specimens from the local bush pig population to compare to specimens recovered from Kasekela chimpanzees. Despite the high overlap observed between people and livestock in villages in this region, our results suggest that the transmission dynamics of *Cryptosporidium* for humans and livestock are distinct but the dominance of *C. hominis* in humans and non-human primates suggest the potential for cross-species transmission. Our findings highlight the complex nature of zoonotic parasite transmission and stress the need for further studies in similar systems.

3.6 TABLES

Table 3.1 Infection rate of *Cryptosporidium* species and *C. hominis* subtypes detected by species and location in and around Gombe National Park, Tanzania.

Host	Positive/Total	*Cryptosporidium* species detected (n)	*C. hominis* genotypes	Infection Rate (95% CI)
Humans				
Mwamgongo Village	7/95	*C. hominis* (7)	IfA12G2 (6/7)	0.07 (0.03-0.15)
Kasekela	0/58			0.0 (0.00-0.08)
Mitumba	1/32	*C. hominis* (1)	IfA12G2 (1/1)	0.03 (0.03-0.15)
Livestock				
Dog	0/8			0 (0.00-0.40)
Goat	5/56	*C. xiaoi* (5)		0.09 (0.03-0.20)
Sheep	2/9	*C. xiaoi* (2)		0.22 (0.03-0.60)
Non-human primates				
Baboon	5/47	*C. hominis* (5)	IfA12G2 (3/5)	0.11 (0.04-0.24)
Kasekela Chimpanzees				
All *Cryptosporidium*	12/58			0.21 (0.11-0.34)
C. hominis	6/58[a]	*C. hominis*(7)	IfA12G2 (2/7)	0.10 (0.04-0.22)
C. suis	7/58[a]	*C. suis* (6)		0.12 (0.05-0.23)
Mitumba Chimpanzees	4/26	*C. hominis* (4)	IfA12G2 (3/4)	0.15 (0.05-0.36)

[a]One individual was positive for both *C. suis* and *C. hominis*

Table 3.2 Risk factors for *Cryptosporidium* infection in people living in or adjacent to Gombe National Park, Tanzania.

			95% CI		
Variable	**n**	**OR**	**Lower**	**Upper**	***p***
Age (≤7 years)	179	0.83	0.09	7.27	0.87
Sex (female vs. male)	184	0.95	0.37	2.36	0.90
Seasonality: dry vs wet season	185	2.04	0.24	17.10	0.51
Location (Mwamgongo vs Mitumba)	127	0.41	0.04	3.42	0.40
Location (Mitumba vs Kasekela)	90	5.57	0.22	140.82	0.35
Location (Kasekela vs Mwamgongo)	**153**	**9.92**	**0.55**	**176.95**	**0.04**
Mwamgongo vs park resident	185	7.08	0.85	58.74	0.07
Experienced gastrointestinal symptoms	147	1.46	0.25	8.30	0.67
Used commercial or traditional medicine	173	0.55	0.03	10.19	0.47
Consumption of water from open water source	163	0.16	0.01	2.87	0.16
Work in agricultural fields or forest	171	3.53	0.66	18.73	0.13
Water not boiled before consumption	153	1.99	0.09	40.02	0.81
Live in household with another positive person (Mwamgongo)	**95**	**9.72**	**1.74**	**54.27**	**0.01**
Live in household with positive livestock (Mwamgongo)	95	4.75	0.94	23.90	0.05

Table 3.3 Risk factors for *Cryptosporidium* infection in chimpanzees in Gombe National Park, Tanzania

		All *Cryptosporidium*				*C. suis*				*C. hominis*			
			95% CI				95% CI				95% CI		
Variable	**n[a]**	**OR**	**Lower**	**Upper**	***p***	**OR**	**Lower**	**Upper**	***p***	**OR**	**Lower**	**Upper**	***p***
Age (≤10 years)	84	1.16	0.39	3.41	0.78	1.95	0.41	9.20	0.39	0.73	0.18	2.99	0.670
Sex (female vs. male)	84	0.42	0.12	1.41	0.16	0.18	0.02	1.50	0.11	0.71	0.17	2.98	0.647
Seasonality: (dry vs wet)	84	1.19	0.44	3.24	0.72	0.87	0.22	3.39	0.84	1.47	0.45	4.74	0.514
Location (Kasekela vs Mitumba)	84	0.93	0.27	3.18	0.91	7.06	0.39	125.25	0.07	2.35	0.61	8.99	0.211
Observed to have diarrhea	79	2.03	0.70	5.88	0.19	1.46	0.26	8.06	0.66	2.42	0.63	9.30	0.197

[a]Sample sizes may vary based on number of individual observations.

Binary logistic regression was used to calculate odds ratios, confidence interval and significance in most cases. Fisher's Exact test was used to calculate *p*-values when cells contained values less than 5.

3.7 REFERENCES

Abeywardena, H., A. R. Jex, M. J. Nolan, S. R. Haydon, M. A. Stevens, R. W. McAnulty, and R. B. Gasser. 2012. Genetic characterisation of Cryptosporidium and Giardia from dairy calves: Discovery of species/genotypes consistent with those found in humans. Infection Genetics and Evolution **12**:1984-1993.

Adamu, H., B. Petros, G. Q. Zhang, H. Kassa, S. Amer, J. B. Ye, Y. Y. Feng, and L. H. Xiao. 2014. Distribution and Clinical Manifestations of Cryptosporidium Species and Subtypes in HIV/AIDS Patients in Ethiopia. Plos Neglected Tropical Diseases **8**.

Akinbo, F. O., C. E. Okaka, R. Omoregie, T. Dearen, E. T. Leon, and L. Xiao. 2010. Molecular Characterization of Cryptosporidium spp. in HIV-infected Persons in Benin City, Edo State, Nigeria. Fooyin Journal of Health Sciences **2**:85-89.

Aksoy U, A. C., Sahin S , Usluca S, Yalcin G, KuralayF , Oral A. 2007. First reported waterborne outbreak of cryptospirodosis with *Cyclospora* co-infection in Turkey. Euro Surveillance **12**.

Alves, M., L. H. Xiao, I. Sulaiman, A. A. Lal, O. Matos, and F. Antunes. 2003. Subgenotype analysis of Cryptosporidium isolates from humans, cattle, and zoo ruminants in Portugal. Journal of Clinical Microbiology **41**:2744-2747.

Appelbee, A. J., R. C. A. Thompson, and M. E. Olson. 2005. Giardia and Cryptosporidium in mammalian wildlife - current status and future needs. Trends in Parasitology **21**:370-376.

Baldursson, S., and P. Karanis. 2011. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks - An update 2004-2010. Water Research **45**:6603-6614.

Bridgman, S. A., R. M. Robertson, Q. Syed, N. Speed, N. Andrews, and P. R. Hunter. 1995. Outbreak of cryptosporidiosis associated with a disinfected groundwater supply. Epidemiology and Infection **115**:555-566.

Cama, V. A., J. M. Ross, S. Crawford, V. Kawai, R. Chavez-Valdez, D. Vargas, A. Vivar, E. Ticona, M. Navincopa, J. Williamson, Y. Ortega, R. H. Gilman, C. Bern, and L. Xiao. 2007. Differences in clinical manifestations among Cryptosporidium species and subtypes in HIV-infected persons. J Infect Dis **196**:684-691.

Cegielski, J. P., Y. R. Ortega, S. McKee, J. F. Madden, L. Gaido, D. A. Schwartz, K. Manji, A. F. Jorgensen, S. E. Miller, U. P. Pulipaka, A. E. Msengi, D. H. Mwakyusa, C. R. Sterling, and L. B. Reller. 1999. Cryptosporidium, Enterocytozoon, and Cyclospora Infections in Pediatric and Adult Patients with Diarrhea in Tanzania. Clinical Infectious Diseases **28**:314-321.

Checkley, W., R. H. Gilman, L. D. Epstein, M. Suarez, J. F. Diaz, L. Cabrera, R. E. Black, and C. R. Sterling. 1997. Asymptomatic and Symptomatic Cryptosporidiosis: Their Acute Effect on Weight Gain in Peruvian Children. American Journal of Epidemiology **145**:156-163.

Colebunders, R., K. Lusakumuni, A. M. Nelson, P. Gigase, I. Lebughe, E. van Marck, B. Kapita, H. Francis, J. J. Salaun, and T. C. Quinn. 1988. Persistent diarrhoea in Zairian AIDS patients: an endoscopic and histological study. Gut **29**:1687-1691.

da Silva, A. J., F. J. Bornay-Llinares, I. N. S. Moura, S. B. Slemenda, J. L. Tuttle, and N. J. Pieniazek. 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Molecular Diagnosis **4**:57-64.

Davies, C. M., C. M. Ferguson, C. Kaucner, M. Krogh, N. Altavilla, D. A. Deere, and N. J. Ashbolt. 2004. Dispersion and transport of Cryptosporidium oocysts from fecal pats under simulated rainfall events. Applied and environmental microbiology **70**:1151-1159.

Earnhardt JM, R. S., Lonsdorf EV, Pusey AE. 2003. A demographic comparison of wild chimpanzees from Gombe and a managed population from North American zoos. American Journal of Primatology **60**:2.

Gillespie, T. R., E. V. Lonsdorf, E. P. Canfield, D. J. Meyer, Y. Nadler, J. Raphael, A. E. Pusey, J. Pond, J. Pauley, T. Mlengeya, and D. A. Travis. 2010. Demographic and Ecological Effects on Patterns of Parasitism in Eastern Chimpanzees (Pan troglodytes schweinfurthii) in Gombe National Park, Tanzania. American Journal of Physical Anthropology **143**:534-544.

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

Gomez Morales, M. A., C. Atzori, A. Ludovisi, P. Rossi, M. Scaglia, and E. Pozio. 1995. Opportunistic and non-opportunistic parasites in HIV-positive and negative patients with diarrhoea in Tanzania. Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) **46**:109-114.

Goodall, J. 1986. The Chimpanzees of Gombe: Patterns of Behavior Bellknap Press of the Harvard University Press Boston.

Gracenea, M., M. S. Gómez, J. Torres, E. Carné, and J. Fernández-Morán. 2002. Transmission dynamics of Cryptosporidium in primates and herbivores at the Barcelona zoo: a long-term study. Veterinary Parasitology **104**:19-26.

Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1997. Recovery of waterborne oocysts of Cryptosporidium from water samples by the membrane-filter dissolution method. Parasitology Research **83**:121-125.

Graczyk, T. K., A. J. DaSilva, M. R. Cranfield, J. B. Nizeyi, G. Kalema, and N. J. Pieniazek. 2001. Cryptosporidium parvum Genotype 2 infections in free-ranging

mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. Parasitology Research **87**:368-370.

Hansen, J. S., and J. E. Ongerth. 1991. Effects of time and watershed characteristics on the concentration of Cryptosporidium oocysts in river water. Applied and Environmental Microbiology **57**:2790-2795.

Houpt, E. R., O. Y. Bushen, N. E. Sam, A. Kohli, A. Asgharpour, C. T. Ng, D. P. Calfee, R. L. Guerrant, V. Maro, S. Ole-Nguyaine, and J. F. Shao. 2005. Short report: Asymptomatic Cryptosporidium hominis infection among human immundeficiency virus-infected patients in Tanzania. American Journal of Tropical Medicine and Hygiene **73**:520-522.

Jiang, J., K. A. Alderisio, and L. Xiao. 2005. Distribution of Cryptosporidium Genotypes in Storm Event Water Samples from Three Watersheds in New York. Applied and Environmental Microbiology **71**:4446-4454.

Johnston, A. R., T. R. Gillespie, I. B. Rwego, T. L. T. McLachlan, A. D. Kent, and T. L. Goldberg. 2010. Molecular Epidemiology of Cross-Species Giardia duodenalis Transmission in Western Uganda. Plos Neglected Tropical Diseases **4**.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-U994.

Keele, B. F., J. H. Jones, K. A. Terio, J. D. Estes, R. S. Rudicell, M. L. Wilson, Y. Y. Li, G. H. Learn, T. M. Beasley, J. Schumacher-Stankey, E. Wroblewski, A. Mosser, J. Raphael, S. Kamenya, E. V. Lonsdorf, D. A. Travis, T. Mlengeya, M. J. Kinsel, J. G. Else, G. Silvestri, J. Goodall, P. M. Sharp, G. M. Shaw, A. E. Pusey, and B. H. Hahn. 2009. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature **460**:515-519.

Kotloff, K. L., J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. G. Faruque, A. K. M. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acacio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne, and M. M. Levine. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet **382**:209-222.

Leav, B. A., M. Mackay, and H. D. Ward. 2003. Cryptosporidium species: New insights and old challenges. Clinical Infectious Diseases **36**:903-908.

Leav, B. A., M. R. Mackay, A. Anyanwu, R. M. O'Connor, A. M. Cevallos, G. Kindra, N. C. Rollins, M. L. Bennish, R. G. Nelson, and H. D. Ward. 2002. Analysis of

sequence diversity at the highly polymorphic Cpgp40/15 locus among Cryptosporidium isolates from human immunodeficiency virus-infected children in South Africa. Infection and Immunity **70**:3881-3890.

Leoni, F., C. Amar, G. Nichols, S. Pedraza-Diaz, and J. McLauchlin. 2006. Genetic analysis of Cryptosponidium from 2414 humans with diarrhoea in England between 1985 and 2000. Journal of Medical Microbiology **55**:703-707.

Li, W., N. M. Kiulia, J. M. Mwenda, A. Nyachieo, M. B. Taylor, X. C. Zhang, and L. H. Xiao. 2011. Cyclospora papionis, Cryptosporidium hominis, and Human-Pathogenic Enterocytozoon bieneusi in Captive Baboons in Kenya. Journal of Clinical Microbiology **49**:4326-4329.

Lihua Xiao, U. R. 2008. Cryptosporidium and Cryptosporidiosis. CRC Press, New York, USA.

Lonsdorf, E. V., D. Travis, A. E. Pusey, and J. Goodall. 2006. Using retrospective health data from the Gombe chimpanzee study to inform future monitoring efforts. American Journal of Primatology **68**:897-908.

Ministry of Agriculture Food Security and Cooperatives. The United Republic of Tanzania (2008) http://kilimo.go.tz/agricultural%20maps/Tanzania%20Soil%20Maps/Soil%20maps.htm.

Nizeyi, J. B., R. Mwebe, A. Nanteza, M. R. Cranfield, G. Kalema, and T. K. Graczyk. 1999. Cryptosporidium sp and Giardia sp infections in mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. Journal of Parasitology **85**:1084-1088.

Onda, K., J. LoBuglio, and J. Bartram. 2012. Global Access to Safe Water: Accounting for Water Quality and the Resulting Impact on MDG Progress. International Journal of Environmental Research and Public Health **9**:880-894.

Parsons MB, G. T. L., Travis D, Lipende L, Gilagiza B, Kamenya S, Pintea L, Vazquez-Prokopec GM. 2014. Global Positioning System Data-Loggers: A Tool to Quantify Fine-Scale Movement of Domestic Animals to Evaluate Potential for Zoonotic Transmission to an Endangered Wildlife Population. Plos ONE.

Persson K, S. B., de Jong B. 2007. An outbreak of cryptospirodosis at a day-care centre in Sweden. European Surveillance **12**.

Pusey, A. E., L. Pintea, M. L. Wilson, S. Kamenya, and J. Goodall. 2007. The contribution of long-term research at Gombe National Park to chimpanzee conservation. Conservation Biology **21**:623-634.

Pusey, A. E., M. L. Wilson, and D. A. Collins. 2008. Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:738-744.

Rajendran, P., S. S. R. Ajjampur, D. Chidambaram, D. Kattula, D. P. Rajan, H. Ward, and G. Kang. 2011. Short Report: Investigation of Potential Zoonotic

Transmission of Cryptosporidiosis in Southern India. American Journal of Tropical Medicine and Hygiene **85**:657-659.

Rwego, I. B., T. R. Gillespie, G. Isabirye-Basuta, and T. L. Goldberg. 2008a. High rates of Escherichia coli transmission between livestock and humans in rural Uganda. Journal of Clinical Microbiology **46**:3187-3191.

Rwego, I. B., G. Isabirye-Basuta, T. R. Gillespie, and T. L. Goldberg. 2008b. Gastrointestinal Bacterial Transmission among Humans, Mountain Gorillas, and Livestock in Bwindi Impenetrable National Park, Uganda. Conservation Biology **22**:1600-1607.

Salyer, S. J., T. R. Gillespie, I. B. Rwego, C. A. Chapman, and T. L. Goldberg. 2012. Epidemiology and Molecular Relationships of Cryptosporidium spp. in People, Primates, and Livestock from Western Uganda. Plos Neglected Tropical Diseases **6**.

Salzer, J. S., I. B. Rwego, T. L. Goldberg, M. S. Kuhlenschmidt, and T. R. Gillespie. 2007. Giardia sp and Cryptosporidium sp infections in primates in fragmented and undisturbed forest in western Uganda. Journal of Parasitology **93**:439-440.

Smith, H. V., S. M. Caccio, N. Cook, R. A. B. Nichols, and A. Tait. 2007. Cryptosporidium and Giardia as foodborne zoonoses. Veterinary Parasitology **149**:29-40.

Tanzania Commission for AIDS (TACAIDS), Z. A. C. Z., National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), Macro International Inc. . 2008. Tanzania HIV/AIDS and Malaria Indicator Survey 2007-98, Dar Es Salaam.

Tarimo, D. S., J. Z. Killewo, J. N. Minjas, and G. I. Msamanga. 1996. Prevalence of intestinal parasites in adult patients with enteropathic AIDS in north-eastern Tanzania. East African medical journal **73**:397-399.

Tumwine, J. K., A. Kekitiinwa, S. Bakeera-Kitaka, G. Ndeezi, R. Downing, X. Feng, D. E. Akiyoshi, and S. Tzipori. 2005. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. The American journal of tropical medicine and hygiene **73**:921-925.

Tzipori, S. 1988. CRYPTOSPORIDIOSIS IN PERSPECTIVE. Advances in Parasitology **27**:63-129.

Wallis, J., and D. R. Lee. 1999. Primate conservation: The prevention of disease transmission. International Journal of Primatology **20**:803-826.

Wang, L., H. Zhang, X. Zhao, L. Zhang, G. Zhang, M. Guo, L. Liu, Y. Feng, and L. Xiao. 2013. Zoonotic Cryptosporidium Species and Enterocytozoon bieneusi Genotypes in HIV-Positive Patients on Antiretroviral Therapy. Journal of Clinical Microbiology **51**:557-563.

Williams, J. M., E. V. Lonsdorf, M. L. Wilson, J. Schumacher-Stankey, J. Goodall, and A. E. Pusey. 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:766-777.

Xiao, L. H., C. Bern, M. Arrowood, I. Sulaiman, L. Zhou, V. Kawai, A. Vivar, A. A. Lal, and R. H. Gilman. 2002. Identification of the Cryptosporidium pig genotype in a human patient. Journal of Infectious Diseases **185**:1846-1848.

Xiao, L. H., C. Bern, J. Limor, I. Sulaiman, J. Roberts, W. Checkley, L. Cabrera, R. H. Gilman, and A. A. Lal. 2001. Identification of 5 types of Cryptosporidium parasites in children in Lima, Peru. Journal of Infectious Diseases **183**:492-497.

Xiao, L. H., and Y. Y. Feng. 2008. Zoonotic cryptosporidiosis. Fems Immunology and Medical Microbiology **52**:309-323.

US Counter Intelligence Agency (2013) "Tanzania". The World Factbook.

CHAPTER 4

Detection of antimicrobial resistance genes from fecal specimens of humans, animals and wild primates of Gombe National Park: evidence of spillover on a local scale

4.1 SUMMARY

Habitat overlap increases the risk for pathogenic bacterial exchange between humans and wildlife, threatening human health and wildlife conservation. Antimicrobial resistant bacteria can spread between wildlife, livestock, and humans, and serve as a possible proxy for disease spillover. In Gombe National Park, Tanzania, little is known about the prevalence of antimicrobial resistance. We determined the prevalence of genes conferring resistance to sulfonamides and tetracycline in fecal specimens collected from human, livestock, and non-human primate populations and in drinking water sources. We examined the overlap in resistance genes across groups as an indicator for potential disease spillover, and we evaluated risk factors for antimicrobial resistance spillover in this system. From March 2010 to February 2011, fecal specimens were collected from humans, livestock, and wildlife in dry and wet seasons from two park communities and a village outside the park. DNA extracted from specimens was tested for *sul1, sul2, tetA and tetB* genes. Fecal prevalence of sulfonamide resistance genes was highest among humans (74.3%), followed by wildlife (42.6%), then domestic animals (16.9%). Genes that encode tetracycline resistance were detected at much lower frequencies in all groups: humans (13.9%), wildlife (3.3%), and domesticated animals (5.6%). Differences in *sul* gene frequencies did not vary by location in humans nor in chimpanzees. Our findings

suggest that humans are the reservoir for antimicrobial resistance genes that spread to wildlife, regardless of human density. There was a strong statistical significance in the difference in frequency of *sul2* and *sul1* genes in humans ($p<0.001$) and chimpanzees ($p<0.001$); individuals positive for *sul2* were more likely to also be positive for *sul1* than those positive for *sul1* were to have *sul2*, supporting that these genes have different transfer mechanisms. Among chimpanzees, sex and age were not found to increase ones odds for having sulfonamide resistance genes. *Sul* resistance genes were detected from 4/22 (18.2%) of streams sampled; the *sul* frequency didn't vary by season. The prevalence of resistance genes in the chimpanzees is concerning; both as an indicator for pathogen spillover and in an extenuating circumstance where antimicrobial treatment may be administered. Sustainable interventions, including the promotion of safe water and hygiene activities, drug resistance monitoring and prudent antimicrobial use are needed to minimize the spread of antimicrobial resistance in this community.

4.2 INTRODUCTION

Antimicrobials have saved millions of lives worldwide, but the global emergence of drug resistance is compromising their usefulness. Antimicrobial usage in human and farm animal populations continues to grow globally, and provides strong selective pressure on bacteria for developing resistance (Byarugaba 2004). In resource limited settings, the sale of counterfeit and over the counter antimicrobials worsen the problem, as unregulated dispensing of antimicrobials results in inappropriate use, from either improper diagnosis or treatment regime (Bennish & Khan 2010). In these countries, suboptimal use of antimicrobials for disease prevention and treatment, broad administration in livestock and agriculture, and poor government oversight of their distribution are widespread (Byarugaba 2010). The lack of oversight is ubiquitous in health care settings, and in the community (Mitema 2010), where private shops and markets serve as resident dispensers increasing the risk of antimicrobial resistance developing.

Antimicrobial-producing bacteria occur in the environment (Martinez 2008; Riesenfeld et al. 2004) populating soil, aquatic plants and animals. The mixing of environmental strains with exogenous anthropogenic derived strains under clinical antimicrobial use or environmental pollutants create selective conditions that give rise to new resistant strains (Wellington et al. 2013). Resistant strains can emerge rapidly as a result of horizontal gene transfer of mobile genetic elements carrying antimicrobial resistance genes (Bartoloni et al. 2006). These strains can then potentially be transmitted between humans, animals, and wildlife who share the same habitat or resources.

Although, some research has examined the impact of antimicrobial resistance in wildlife, few have studied ape populations (Benavides et al. 2012). In one study, gorilla, human, and livestock bacterial isolates collected from within and around Bwindi Impenetrable National Park, Uganda harbored varying levels of resistance to at least one antimicrobial used by the local people and that drug resistance declined with decreasing shared habitat with humans (Rwego et al. 2008). This suggests that habitat overlap between species affects the dynamics of gastrointestinal bacterial transmission. A separate study examined patterns of gastrointestinal bacterial exchange among chimpanzees in Kibale National Park. *E. coli* isolates were compared by REP-PCR and antimicrobial susceptibility testing by disk diffusion. *E. coli* from chimpanzees were genetically more similar to those found in humans working in chimpanzee-directed research and tourism than those found in humans in the local population (Goldberg et al. 2007) suggesting that human interaction with wildlife facilitates bacterial transmission and antimicrobial resistance.

In Gombe National Park, Tanzania, disease is the leading cause of mortality in chimpanzees (*Pan troglodytes*). Park managers and researchers suspect that disease from humans poses a large risk to the sustainability of the primate population. With loss of park habitat due to human encroachment, the human-chimpanzee interface has grown, placing the chimpanzees at increased risk for disease spillover and outbreaks, such as polio and respiratory illnesses (Williams et al. 2008). Having been studied for over six decades, there is nearly complete lifespan data on this chimpanzee population (Earnhardt JM 2003; Goodall 1986). The two habituated populations encounter different degrees of

human contact —Mitumba, with ~ 25 individuals, is an edge community next to a growing village of Mwamgongo, while Kasekela, with ~ 65 individuals, is in the center of Gombe National Reserve (Pusey et al. 2008). The Mitumba group inhabits the northern part of the forest, sharing a park border with Mwamgongo village (population ~5000). With a narrow habitat range, and an adjacent highly dense human population (Gillespie et al. 2010), the Mitumba community is at particular risk for pathogen spillover from humans. The Kasekela group is located in a less disturbed forest setting, in the center of the park, where human residence is limited to researchers, members of the Tanzanian Park Authority, and their families. Because of Gombe park's status as one of the smallest wildlife reserves in Tanzania, the chimpanzee communities are at an elevated risk for disease (Lonsdorf et al. 2006). The habitat ranges of the two communities overlap only slightly, providing little opportunity for contact between the two.

Since antimicrobials are not regularly administered to the non-human primates at Gombe, antimicrobial resistance genes detected in gut flora in the primate population may provide evidence of bacterial spillover from the human and/or animal population. We chose to measure the prevalence of genes coding for resistance to sulfonamides, which is commonly used to treat diarrheal illness in the clinic and hospital setting and to tetracycline which was commonly used in past decades but much less so now. We selected genes *sul1*, *sul2*, *tetA*, *tetB* because they are the most common acquired determinants for resistance to those drugs among *Enterobactericiae* (Alekshun & Levy 2007; Bryan et al. 2004; Chopra & Roberts 2001). Fecal specimens collected from human, livestock, and non-human primate populations and drinking water sources were

tested for these resistance genes to evaluate their frequencies as indication of disease spillover and to identify potential risk factors for acquired resistance in chimpanzees.

4.3 MATERIALS AND METHODS

Study Site

Gombe National Park, Tanzania (4°41'59.97"S, 29°36'59.96"E), is a 35 km^2 forest reserve located 1500 m above sea-level (Wallis & Lee 1999) extending to Lake Tanganyika on the west and villages to the north and south (Figure 1). Some park resources are shared by the wildlife and village or camp residents. Residents use the lake and streams for bathing, washing clothes, and cooking utensils, while they also serve as water sources for baboons and chimpanzees (Wallis and Lee 1999). The park border is permeable; villagers and their animals enter the park at the periphery and chimpanzees have been reported to raid crop fields in Mwamgongo village (I. Lipende, personal communication).

Sample Collection Period

Fecal samples were collected in 2010 during the dry (July 1-August 15) and wet (November 1-December 15) seasons. Human subjects were either residents of Mwamgongo village or Gombe National Park; tourists were not sampled. Fecal samples were collected from humans and domestic animals: dogs (*Canis lupus*), goats (*Capra hircus*) and sheep (*Ovis aries*). Twenty-five village households with domestic animals were randomly selected for study enrollment. Park baboons were opportunistically sampled during these two collection periods. Chimpanzees in both Mitumba and

Kasekela were sampled during the course of routine observational health monitoring (Lonsdorf et al. 2006)

Specimen Collection and Transport

Specimen cups were provided to enrolled village and park residents with instructions. Livestock specimens were collected aseptically by a village veterinary officer. All fecal specimens were freshly voided and transferred aseptically to a screw cap plastic vial containing a 2.5% potassium dichromate solution (Fisher Scientific, Pittsburgh, PA). For wildlife samples, care was taken to avoid the collection of soil, foliage or water contaminants. Each vial was labeled with a unique identification number, and date of collection. Wildlife samples were also labeled with the name of the observer, location and animal name. Samples were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL), refrigerated and shipped at 4^{o}C to Atlanta, Georgia USA.

Water sampling

Water samples were collected from low, middle and high points of 6 permanent streams and two seasonal streams, using sterile gauze Moore swabs immersed overnight for 18-24 hours in flowing water. Swabs were transferred to sterile containers containing RNAlater (stored at ambient temperature) or tryptic soy broth with 20% glycerol (stored in a cryogenic storage dewar) prior to shipment to Atlanta, GA USA. GPS coordinates were obtained using a GPSmap 60CSx from Garmin (Garmin International Inc. Olathe, KS) for each collection point to assist in identifying locations for repeat sampling and if

necessary, to assign sampled streams to watersheds associated with specific human or chimpanzee groups.

Detection of Resistance Genes to Sulfonamide and Tetracycline

Nucleic acid was extracted from all fecal specimens, water samples and positive control samples using the FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH) following the methods described (da Silva et al. 1999). Total nucleic acid extractions were stored at -20ºC for working use and archived at -80º C. A multiplex polymerase chain reaction (PCR) was used to detect the presence of genes for sulfonamide resistance (*sul1*, *sul2*) and tetracycline resistance (*tetA*, *tetB*) genes (Chen et al. 2004). Each 25 µl PCR reaction consisted of 17.25 µl sterile distilled water, 2.5 µl of 10X PCR Buffer, 2.5 µl of deoxynucleoside triphosphates (2.5mM [each] dATP, dTTP, dCTP, dGTP), 0.5 µl of 25µM primer mix, 0.25 µl *Taq* polymerase and 2 µl of template in a 1.5 ml microcentrifuge tube. Sterile distilled water was used as a negative control, positive control strains were kindly provided by the Enteric Diseases Laboratory Branch, CDC, Atlanta, GA. *E. coli* strain DH0032 was used as the positive control for *sul1*, and *E. coli* strain DH3507 was the positive control for *sul2*. *Salmonella* strains 2013K-0573 and 2013K-1023 were positive control strains for *tetA* and *tetB* genes respectively. Ten microliters of the PCR product were electrophoresed on 1.5% SeaKem® LE Agarose (Lonza, Rockland, ME) gels. Gels were stained by adding 3 µl of ethidium bromide to the gel and 2 µl to the running buffer. Gel images were captured under UV exposure using a Gel Doc illumination system (Bio-Rad, Hercules, CA).

Data Analysis

Statistical analyses were performed with SPSS Statistics version 20 (International Business Machines, Armonk, NY USA). A chi-square test of independence was used to compare *sul* gene positivity by group, species or season. When sample size was too small, a Fisher's Exact Test was used. A McNemar's test was used to measure correlations in *sul* or *tet* gene positivity. Generalized estimated equations (GEE), were used to account for repeated sampling of individuals (range 1-3 specimens). GEEs with an exchangeable correlation matrix were performed to compare chimpanzee *sul* positivity with potential risk factors including group, age, sex, or season. Odds ratios and 95% confidence intervals were calculated for all cross-tabulations.

Ethics Statement

This project was reviewed and approved by the Emory University Institutional Review Board (approval #: IRB00018856) under the Expedited review process per 45 CFR 46.110(3), Title 45 CFR Subpart D section 46.404, one parent consent, and 21 CFR 56.110 and the Tanzanian National Institute for Medical Research Institute, Dar Es Salaam Tanzania, which approved oral consent due to low literacy rates. All adult subjects provided informed consent, and a parent or guardian of any child participant provided informed consent on their behalf. Oral informed consent was obtained by trained local field assistants and documented by witnessed notation on IRB-approved enrollment forms. All animal use followed the guidelines of the Weatherall Report on the use of non-human primates in research and was approved by the Tanzania Wildlife Research Institute and Tanzania Commission for Science and Technology (permit number 2009-279-NA-2009-184), and the Emory University Animal Care and Use

Committee (protocol ID 087-2009). Approval was also obtained from Tanzania National Parks (Permit number TNP/HQ/C10/13) to collect samples from wild chimpanzees. The researchers did not have any interactions with the chimpanzees in the park. All domesticated animals were sampled from households in Mwamgongo village. The owners of the domesticated animals provide verbal consent for the collection of fecal specimens for this study, and the verbal consent was documented.

4.4 RESULTS

Descriptive Statistics of All Individuals

The prevalence of sulfonamide resistance genes (*sul1* and/or *sul2*) were humans (74.3%), wildlife (42%) and domesticated animals (16.9%). The prevalence of tetracycline resistance genes (*tetA* and/or *tetB*) from fecal specimens were humans (13.9%), wildlife (3.30%) and domesticated animals (5.62%). Sulfonamide genes were found at a much higher frequency than tetracycline genes in humans (McNemar's 4.99; df =1; $p < 0.001$), wildlife (3.406; df =1; $p < 0.05$), and domestic animals (McNemar's 10.68; df =1; $p < 0.001$). This finding held for chimpanzees alone (McNemar's 2.64; df =1; $p < 0.05$) but was not observed in goats (McNemar's 0.800; df =1; $p = 0.371$) or baboons (McNemar's 0.363; df =1; $p < 0.546$). Significance was not evaluated in sheep or dogs due to small sample size.

There was no significant difference in the sulfonamide and tetracycline gene frequencies between the human groups nor between the chimpanzee groups; among humans, Mitumba (81.3%), Kasekela (78.7%) and Mwamgongo (69.1%) shared similar

sul gene frequencies detected in stool. 42.8% of Mitumba chimpanzees and 50% of Kasekela chimpanzees had *sul* genes present. However, we detected a higher frequency of *sul* genes from dogs (71.4%) that was comparable to humans but not seen in goats and sheep combined (Fisher's Exact Test $p = 0.001$). When comparing sulfonamide frequency between groups, the human population had a higher frequency of sulfonamides relative to chimpanzees ($X^2 = 16.734$, df = 1, $p < 0.05$), and to livestock ($X^2 = 80.771$, df = 1, $p < 0.05$). Chimpanzees had a higher frequency of sulfonamide genes when compared to all domestic animals ($X^2 = 18.426$, df = 1, $p < 0.05$).

Diversity of Sulfonamide and Tetracycline Genes in Fecal Specimens

We observed differences in the frequency of individual *sul* and *tet* genes detected from fecal specimens (Table 2). In every group, *sul2* was more frequently detected than *sul1*. There was a strong statistical significance in the difference between *sul2* and *sul1* prevalence in humans (McNemar's 5.88; df =1, $p<0.001$). Humans positive for *sul2* were more likely to also be positive for *sul1* (40.12%) than those positive for *sul1* were to have *sul2* (6.95%). The same trend was observed in chimpanzees: (McNemar's 6.81; df =1, $p<0.001$), with 34.67% for those with *sul2* to also have *sul1*, 1.33% for those with *sul1* to have *sul2* and domestic animals: (McNemar's 20.66; df =1, $p<0.001$, with 7.87% for those with *sul2* to also have *sul1* as compared to 2.25% for those with *sul1* to have *sul2*. However, the baboons showed no statistical significance in frequencies between the two sulfonamide genes (McNemar's 0.449; df =1, $p > 0.05$).

Within groups, individual tetracycline genes were detected at varying frequency (Table 2). Humans had a greater frequency of fecal specimens positive for *tetB* (11.76%) versus *tetA* genes (1.07%) (McNemar's 2.223, df =1, $p < 0.001$). Among wildlife, <6% of tetracycline genes were detected among chimpanzees and all were *tetB* positive only; baboons were negative for tetracycline genes tested. Both *tetA* and *tetB* genes were detected from goats. The frequency of *tet* genes in dogs mirrored those of humans.

Results of Water Sampling

Sul resistance genes were detected from 4/22 (18.2%) of streams sampled; all were permanent streams. No tetracycline resistance genes were detected in any streams sampled. Sulfonamide gene positivity did not vary by season (X^2 =0.512, df = 1, p >0.05). Positive sampling sites included Mitumba stream and Mpemba stream which runs through Mwamgongo village (Figure 1).

Risk Factors for Sulfonamide Resistance

We examined the association of several individual chimpanzee characteristics to see if any were correlated with carriage of sulfonamide resistance genes. We found that neither chimpanzee age, nor gender, nor location of residence was associated with sulfonamide gene carriage (Table 3). Using a chi-square test of independence, no statistically significant correlation was shown between humans harboring *sul* positivity

and living in their respective residence (p >0.05). The frequency of antimicrobial resistance determinants was also not found to vary by season in humans (X^2 =0.251, df = 1, p = .365) or chimpanzees (X^2 =0.006, df = 1, p = 0.563).

4.5 DISCUSSION

Sulfonamide resistance among all humans was high (74.3%), correlating well with the self-reported frequent use of this drug as compared to tetracycline resistance genes (13.9%), a less frequently used drug in this community. Even in the absence of regular sulfonamide treatment, between 40-50% of chimpanzee and baboon specimens were positive for sulfonamide resistance genes but less than 6% positive for tetracycline genes. Domestic animals had low *sul* resistance (16.9%), although the dog group, a human companion had frequencies comparable to humans (71.4%). These findings lend strong evidence to the influence of bacterial spillover between humans and wildlife. There was no statistically significant correlation between proximity to densely populated human areas and higher prevalence of *sul* genes in the chimpanzees. Despite the fact that Mitumba is a smaller community, has a narrower natural range that borders the high population density of Mwamgongo and the presence of researchers in their natural habitat, these individuals had a comparable prevalence of sulfonamide and tetracycline genes to the Kasekela community. Since human density does not seem to be a risk factor for wildlife acquiring antimicrobial resistance determinants, it may be that human interaction is the strongest risk factor. Though contrary to our predictions, these results

present interesting questions to consider in understanding the distribution, spread and maintenance of antimicrobial resistance in Gombe.

A number of possibilities could explain the high prevalence of *sul* positivity identified in Kasekela. Although Kasekela (n~100) is far less dense than Mwamgongo, the residents of this camp consist of researchers and members of the Tanzanian National Park Authority, who have a consistent income and can afford to purchase a broader variety of antimicrobials compared to the average village resident who may not have access to these health resources (Byarugaba 2004; Okeke et al. 2007). Additionally, park staff may feel pressured to take antimicrobials when they feel ill as symptomatic researchers and field staff would not be allowed into the park. Kasekela was also the first chimpanzee group to be habituated, and human acceptance may increase camp visits and food theft (Gillespie, Lipende, personal communication). While efforts are made in the camp to secure food, and contain excrement; these systems are not perfect and may break down or residual excrement may leech into the natural environment during periods of heavy rain. Chimpanzees may come in contact with food or human feces, placing them at risk to acquire plasmid mediated resistance genes via the fecal-oral route. Trash pits are typically buried less than 2 m below the surface, easily accessible to raiding baboons and/or other forest animals (Wallis & Lee 1999). These explanations would be consistent with findings from Bwindi Impenetrable National Park, Uganda, where local antimicrobial use was accountable for the frequency and class of antimicrobial resistance genes recovered from gorillas (Rwego et al. 2008). In another study, baboons who had daily contact with unprocessed human refuse in Amboseli National Park, Kenya had a

higher proportion of antimicrobial resistant bacteria compared to those baboons living in undisturbed habitat (Rolland et al. 1985).

Alternatively, other modes of gene acquisition and transmission besides direct contact with humans could be promoting antimicrobial resistance in wildlife populations. Although the sample sizes were small, the high prevalence of *sul* genes in the dog population (77.7%) may increase the risk for antimicrobial resistance gene transmission to wildlife. Dogs have been shown to move into the park and frequent crop fields in the village observed to regularly be raided by chimpanzees (Parsons MB 2014). Antimicrobial resistance genes could be acquired either naturally or indirectly from environmental sources, such as soil and water. Sulfonamide antimicrobials have a high excretion rate in humans and animals (Thiele-Bruhn & Beck 2005). They can accumulate in the environment and have been shown to leach from the soil into groundwater systems (Hamscher et al. 2005), a finding that was not observed when evaluating the behavior of tetracyclines after repeated fertilization with liquid manure. The differences in soil mobility based on different sorption coefficients and the availability of the two drugs may explain differences in the frequencies of these genes detected from the Gombe streams. The two locations where *sul2* genes were detected are regularly used by camp (Mitumba stream) and village (Mwamgongo stream) people for bathing, washing of food and utensils. Since humans, baboons, chimpanzees, and domestic animals share these streams that all flow in to Lake Tanganyika (Wallis & Lee 1999), the possibility of encountering sulfonamides in the environment may be higher. (Mariano et al. 2009) conducted a case-control study in Kruger National Park, South Africa which clearly

showed that impala drinking from antimicrobial infected rivers were at a higher odds risk for infection with resistant gut flora relative to their unexposed counterparts.

It is important to consider that there may be other unrecognized factors. A study in Gabon that analyzed the phylogenetic and antimicrobial profile of *E. coli* strains from humans, livestock and wildlife, including gorillas, found no evidence of transmission of antibiotic resistant *E. coli* strains from humans to gorillas (Benavides et al. 2012). The strains were distinct suggesting that horizontal gene transfer or naturally acquired resistance might be responsible instead of bacterial transmission from humans.

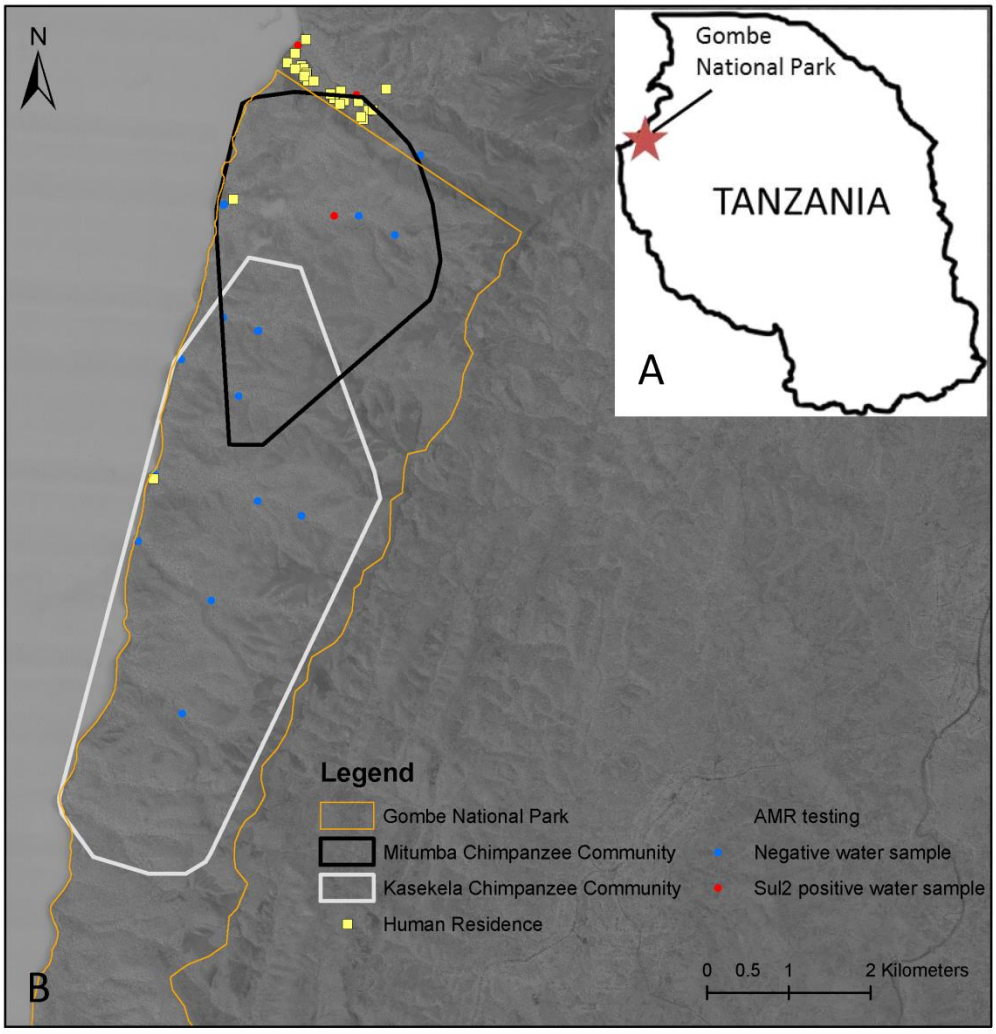
Across all species, there was a higher prevalence of the *sul2* gene and our results show that being positive for *sul2* coincided with also being positive for *sul1*. Although, both genes of resistance code for a similar mechanism, they have distinct transfer mechanisms which may explain the variation in frequencies observed. The *sul1* gene is typically linked to other resistance genes in class 1 integrons but the *sul2* gene is located on small nonconjugative plasmids or large transmissible multi-resistance plasmids (Byarugaba 2010). Other studies have seen similar trends with *sul2* prevalence higher than *sul1*, and that even in the absence of sulfonamide use there is no drastic decrease in the decline of resistance genes in the community (Enne et al. 2001). A molecular characterization of the genetic determinants highlighted that *sul2* was encoded on a rather larger plasmid then previously reported and that this plasmid carried genes to a number of other antimicrobials, suggesting that the reduced resistance does not occur due to the genetic linkage of the *sul2* gene to other resistance determinants on the plasmid that may be under positive selection (Enne et al. 2001).

Sulfonamide resistance among all humans correlated well with the frequently reported use of this drug to treat gastroenteritis and other diarrheal ailments by villagers and camp residents. This is concerning as the findings suggest potential treatment failures, where the drug or dosage may be ineffective or an antimicrobial wasn't necessary for treatment due to a lack of awareness. Sulfonamide antimicrobials have been shown to lose their efficacy after a few years of widespread use (Enne et al. 2001) through the development of resistance genes. In this system, domesticated livestock, chimpanzees and baboons are not routinely treated with antimicrobials. It is worrisome that resistance to commonly used antimicrobials in the human population may already exist in wildlife and behooves testing recovered gastrointestinal bacteria from these groups for antimicrobial resistance genes. Should antimicrobials be a necessary solution to treat a vulnerable wildlife population, such as the Gombe chimpanzees, it would be essential to know what drugs are effective.

Though antimicrobial resistance genes from areas of high human density have not shown a significant association with the presence of these genes in wildlife, humans are still the likely reservoir for exposing wildlife to drug-resistant bacteria and antimicrobial resistance genes. Habitat reduction from human encroachment will continue to increase potential contact with humans and increase the risk for disease transmission (Williams et al. 2008). Practical and sustainable recommendations, including the promotion of locally driven safe water and hygiene practices, antimicrobial resistance monitoring and prudent antibiotic use (Bennish & Khan 2010; Moeller et al. 2013) are needed to minimize the spread of disease and resistance.

4.6 FIGURES

Figure 4.1 Gombe National Park study site. A. Location of Gombe National Park in Tanzania. B. Home ranges for the chimpanzee groups studied; Mitumba and Kasekela and water sampling efforts of park and village streams.



4.7 TABLES

Table 4.1 Prevalence of sulfonamide and tetracycline resistant genes detected in fecal specimens from humans, wildlife and domesticated animals in the Greater Gombe Ecosystem, Tanzania.

Group	Species/Source	n	Sulfonamide genes (*sul1*, *sul2*)		Tetracycline genes (*tetA*, *tetB*)	
			positive	Prevalence (95% CI)	positive	Prevalence (95% CI)
Wildlife	Mitumba Chimpanzee	21	9	0.428 (0.226-0.656)	1	0.048 (0.002-0.259)
	Kasekela Chimpanzee	54	27	0.500 (0.363-0.637)	3	0.055 (0.014-0.163)
	Baboons	47	16	0.340 (0.213-0.494)	0	0.000 (0.000-0.945)
	All Chimpanzee	75	36	0.480 (0.364-0.598)	4	0.053 (0.017-0.138)
	ALL WILDLIFE	**122**	**52**	**0.426 (0.338-0.519)**	**4**	**0.033 (0.010-0.087)**
Human	Mitumba people	32	26	0.813 (0.630-0.921)	8	0.250 (0.121-0.438)
	Kasekela people	61	48	0.787 (0.660-0.878)	9	0.148 (0.074-0.267)
	Mwamgongo people	94	65	0.691 (0.587-0.780)	9	0.096 (0.0474-0.178)
	ALL HUMAN	**187**	**139**	**0.743 (0.673-0.803)**	**26**	**0.139 (0.094-0.199)**
Domestic Animal	Dog	7	5	0.714 (0.303-0.949)	1	0.142 (0.008-0.580)
	Goat	69	7	0.101 (0.045-0.203)	4	0.058 (0.019-0.149)
	Sheep	13	3	0.230 (0.062-0.540)	0	0.000 (0.000-0.283)
	ALL DOMESTIC ANIMAL	**89**	**15**	**0.169 (0.100-0.266)**	**5**	**0.056 (0.021-0.132)**
Water	Stream sites	21	4	0.190 (0.063-0.426)	0	0.000 (0.000-0.192)
	Pipe	1	0	0.000 (0.000-0.945)	0	0.000 (0.000-0.945)
	ALL WATER	**22**	**4**	**0.182 (0.060-0.410)**	**0**	**0.000 (0.000-0.604)**

Table 4.2 Proportion of sulfonamide and tetracycline genes identified among fecal specimens collected from humans, wildlife and domestic animals in and around Gombe National Park, Tanzania.

		Frequency of genes detected from fecal specimens by PCR						
		number of individuals positive for gene (percentage)						
Group	n	*sul1 only*	*sul2 only*	*sul1/sul2*	*tetA only*	*tetB only*	*tetA/tetB*	Negative for genes tested
Wildlife								
Mitumba Chimpanzee	21	0 (0)	7 (33.33)	2 (9.52)	0 (0)	1 (4.76)	0 (0)	12 (57.14)
Kasekela Chimpanzee	54	1 (1.85)	19 (35.19)	7 (12.96)	0 (0)	3 (5.56)	0 (0)	27 (50.00)
Baboon	47	4 (8.51)	9 (19.15)	3 (6.38)	0 (0)	0 (0)	0 (0)	31 (65.95)
All Chimpanzee	75	1 (1.33)	26 (34.7)	9 (12.0)	0 (0)	4 (5.33)	0 (0)	39 (52.0)
ALL WILDLIFE	**122**	5 (4.10)	35 (28.69)	12 (9.84)	0	4 (3.29)	0	70 (57.38)
Humans								
Mitumba people	32	1 (3.13)	15 (46.88)	10 (31.25)	0 (0)	8 (25.0)	0 (0)	4 (12.5)
Kasekela people	61	5 (8.19)	30 (49.18)	13 (21.31)	1 (1.64)	6 (9.84)	2 (3.28)	12 (19.67)
Mwamgongo people	94	7 (7.45)	30 (31.91)	28 (29.79)	1 (1.06)	8 (8.51)	0 (0)	29 (30.85)
ALL HUMAN	**187**	13 (6.95)	75 (40.11)	51 (27.27)	2 (1.07)	22 (11.76)	2 (1.07)	45 (24.06)
Domestic Animal								
Dog	7	0 (0)	2 (28.57)	3 (42.86)	0 (0)	1 (14.29)	0 (0)	2 (28.57)
Goat	69	1 (1.45)	4 (5.80)	2 (2.90)	3 (4.35)	1 (1.45)	0 (0)	61 (88.41)
Sheep	13	1 (7.69)	1 (7.69)	1 (7.69)	0 (0)	0 (0)	0 (0)	10 (76.92)
ALL DOMESTIC ANIMAL	**89**	2 (2.25)	7 (7.87)	6 (6.74)	3 (3.37)	2 (2.25)	0 (0)	73 (82.02)
Environmental								
Stream sites	21	0 (0)	4	0 (0)	0 (0)	0 (0)	0 (0)	17 (80.95)
Pipe	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100.00)
ALL WATER	**22**	0 (0)	4 (18.18)	0 (0)	0 (0)	0 (0)	0 (0)	18 (81.81)

Table 4.3 Risk factors for sulfonamide resistance in chimpanzees in Gombe National Park, Tanzania

		Sulfonamide positivity			
Variable	**n**	**OR**	**Lower**	**Upper**	***p***
Location (Kasekela vs Mitumba)	75	1.62	0.68	3.84	0.26
Sex (female vs male)	75	1.44	0.68	3.08	0.34
Age (≤10 years)	75	1.33	0.61	2.88	0.46
Seasonality (rainy vs dry season)	75	1.94	0.94	4.02	0.07

4.8 REFERENCES

Alekshun, M. N., and S. B. Levy. 2007. Molecular Mechanisms of Antibacterial Multidrug Resistance. Cell **128**:1037-1050.

Bartoloni, A., M. Benedetti, L. Pallecchi, M. Larsson, A. Mantella, M. Strohmeyer, F. Bartalesi, C. Fernandez, E. Guzman, Y. Vallejos, A. L. Villagran, H. Guerra, E. Gotuzzo, F. Paradisi, T. Falkenberg, G. M. Rossolini, and G. Kronvall. 2006. Evaluation of a rapid screening method for detection of antimicrobial resistance in the commensal microbiota of the gut. Transactions of the Royal Society of Tropical Medicine and Hygiene **100**:119-125.

Benavides, J. A., S. Godreuil, R. Bodenham, S. Ratiarison, C. Devos, M. O. Petretto, M. Raymond, and P. Escobar-Paramo. 2012. No Evidence for Transmission of Antibiotic-Resistant Escherichia coli Strains from Humans to Wild Western Lowland Gorillas in Lope National Park, Gabon. Applied and Environmental Microbiology **78**:4281-4287.

Bennish, M. L., and W. A. Khan 2010. What the Future Holds for Resistance in Developing Countries.

Bryan, A., N. Shapir, and M. J. Sadowsky. 2004. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical Escherichia coli strains isolated from diverse human and animal sources. Applied and environmental microbiology **70**:2503-2507.

Byarugaba, D. K. 2004. A view on antimicrobial resistance in developing countries and responsible risk factors. International Journal of Antimicrobial Agents **24**:105-110.

Byarugaba, D. K. 2010. Mechanisms of Antimicrobial Resistance. Pages 15-26 in A. J. Sosa, D. K. Byarugaba, C. F. AmabileCuevas, P. R. Hsueh, S. Kariuki, and I. N. Okeke, editors. Antimicrobial Resistance in Developing Countries. Springer, New York.

Chen, S., S. H. Zhao, D. G. White, C. M. Schroeder, R. Lu, H. C. Yang, P. F. McDermott, S. Ayers, and J. H. Meng. 2004. Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats. Applied and Environmental Microbiology **70**:1-7.

Chopra, I., and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews : MMBR **65**:232-260 ; second page, table of contents.

da Silva, A. J., F. J. Bornay-Llinares, I. N. S. Moura, S. B. Slemenda, J. L. Tuttle, and N. J. Pieniazek. 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Molecular Diagnosis **4**:57-64.

Earnhardt JM, R. S., Lonsdorf EV, Pusey AE. 2003. A demographic comparison of wild chimpanzees from Gombe and a managed population from North American zoos. American Journal of Primatology **60**:2.

Enne, V. I., D. M. Livermore, P. Stephens, and L. M. C. Hall. 2001. Persistence of sulphonamide resistance in Escherichia coli in the UK despite national prescribing restriction. Lancet **357**:1325-1328.

Gillespie, T. R., E. V. Lonsdorf, E. P. Canfield, D. J. Meyer, Y. Nadler, J. Raphael, A. E. Pusey, J. Pond, J. Pauley, T. Mlengeya, and D. A. Travis. 2010. Demographic and Ecological Effects on Patterns of Parasitism in Eastern Chimpanzees (Pan troglodytes schweinfurthii) in Gombe National Park, Tanzania. American Journal of Physical Anthropology **143**:534-544.

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

Goodall, J. 1986. The Chimpanzees of Gombe: Patterns of Behavior Bellknap Press of the Harvard University Press Boston.

Hamscher, G., H. T. Pawelzick, H. Hoper, and H. Nau. 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. Environmental Toxicology and Chemistry **24**:861-868.

Lonsdorf, E. V., D. Travis, A. E. Pusey, and J. Goodall. 2006. Using retrospective health data from the Gombe chimpanzee study to inform future monitoring efforts. American Journal of Primatology **68**:897-908.

Mariano, V., C. McCrindle, B. Cenci-Goga, and J. Picard. 2009. Case-control study to determine whether river water can spread tetracycline resistance to unexposed Impala (Aepyceros melampus) in Kruger National Park (South Africa). Applied and environmental microbiology **75**:113-118.

Martinez, J. L. 2008. Antibiotics and antibiotic resistance genes in natural environments. Science **321**:365-367.

Mitema, E. 2010. The Role of Unregulated Sale and Dispensing of Antimicrobial Agents on the Development of Antimicrobial Resistance in Developing Countries. Pages 403-411 in A. d. J. Sosa, D. K. Byarugaba, C. F. Amábile-Cuevas, P.-R. Hsueh, S. Kariuki, and I. N. Okeke, editors. Antimicrobial Resistance in Developing Countries. Springer New York.

Moeller, A. H., M. Shilts, Y. Y. Li, R. S. Rudicell, E. V. Lonsdorf, A. E. Pusey, M. L. Wilson, B. H. Hahn, and H. Ochman. 2013. SIV-Induced Instability of the Chimpanzee Gut Microbiome. Cell Host & Microbe **14**:340-345.

Okeke, I. N., O. A. Aboderin, D. K. Byarugaba, K. K. Ojo, and J. A. Opintan. 2007. Growing problem of Multildrug-Resistant enteric pathogens in Africa. Emerging Infectious Diseases **13**:1640-1646.

Parsons MB, G. T. L., Travis D, Lipende L, Gilagiza B, Kamenya S, Pintea L, Vazquez-Prokopec GM. 2014. Global Positioning System Data-Loggers: A Tool to Quantify Fine-Scale Movement of Domestic Animals to Evaluate Potential for Zoonotic Transmission to an Endangered Wildlife Population. Plos ONE.

Pusey, A. E., M. L. Wilson, and D. A. Collins. 2008. Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:738-744.

Riesenfeld, C. S., R. M. Goodman, and J. Handelsman. 2004. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environmental Microbiology **6**:981-989.

Rolland, R., G. Hausfater, B. Marshall, and S. Levy. 1985. Antibiotic-resistant bacteria in wild primates: increased prevalence in baboons feeding on human refuse. Applied and environmental microbiology **49**:791-794.

Rwego, I. B., G. Isabirye-Basuta, T. R. Gillespie, and T. L. Goldberg. 2008. Gastrointestinal Bacterial Transmission among Humans, Mountain Gorillas, and Livestock in Bwindi Impenetrable National Park, Uganda. Conservation Biology **22**:1600-1607.

Thiele-Bruhn, S., and I. C. Beck. 2005. Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere **59**:457-465.

Wallis, J., and D. R. Lee. 1999. Primate conservation: The prevention of disease transmission. International Journal of Primatology **20**:803-826.

Wellington, E. M. H., A. B. A. Boxall, P. Cross, E. J. Feil, W. H. Gaze, P. M. Hawkey, A. S. Johnson-Rollings, D. L. Jones, N. M. Lee, W. Otten, C. M. Thomas, and A. P. Williams. 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infectious Diseases **13**:155-165.

Williams, J. M., E. V. Lonsdorf, M. L. Wilson, J. Schumacher-Stankey, J. Goodall, and A. E. Pusey. 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:766-777.

CHAPTER 5

Epidemiology and ecology of zoonotic enteric pathogens in Gombe National Park based upon phenotypic and genotypic characterization of bacterial strains from humans, domesticated animals and wild primates

5.1 SUMMARY

Diarrheal disease is a leading cause of illness and death globally however in developing countries disease specific estimates are usually unavailable. Non-typhoidal *Salmonella*, *Shigella* and *Enterotoxigenic E. coli* are important zoonotic enteric pathogens capable of infecting humans, domestic animals and wildlife. Nonhuman primates (NHPs) are particularly vulnerable to infectious diseases of humans because of similar genetic make-up. A major driver in disease emergence in wild NHP is habitat loss as a result of expanding population growth and anthropogenic activities in villages that share natural boundaries with forested areas where these wild NHP populations reside. In Gombe National Park, home to a well-studied nonhuman primate population of wild chimpanzees, there have been population declines attributed to infectious diseases, including polio, respiratory and diarrheal diseases. The aim of our study was to describe the epidemiology and ecology of several specific zoonotic enteric bacterial pathogens recovered from humans, domestic animals and NHPs in the Greater Gombe Ecosystem (GGE). Bacterial culture and diagnostic PCR of fecal specimens identified Enterotoxigenic *E. coli* (ETEC) in humans 42/268 (15.7%), domesticated animals 13/99

(13.1%) and NHPs 12/208 (5.7%). *Salmonella* was isolated from all three groups: NHPs 8/208 (3.9%), humans 14/268 (5.2%) and domesticated animals 5/99 (5.1%), representing serotypes Typhimurium, Heidelberg, Enteritidis and Uganda. *Shigell*a was isolated from NHPs 3/208 (1.4%) and humans 7/268 (2.6%), from which we serogrouped *S. flexneri*, *S. boydii*, and *S. sonnei*. Strains of *S. flexneri* 2a and *S. boydii* 1 were found in both humans and NHPs. Sixteen of 17 (94%) of the human and nonhuman primate strains of *Salmonella* Typhimurium showed greater than 82% genotypic similarity and clustered together by pulsed-field gel electrophoresis (PFGE). Three of four, (75%) of the domesticated animal strains of *Salmonella* Typhimurium were different PFGE genotypes that clustered far from the human and NHP isolates. *Salmonella* strains recovered from NHPs were resistant to the same antimicrobials as the human isolates, specifically ampicillin, streptomycin, sulfisoxazole, trimethoprim-sulfamethoxazole (ASSuST) and contained genetic determinants *aadB, aadA1, blaTEM-1B, blaOXA-1, sul1, dfrA23* associated with these drug classes although they are not exposed to these drugs suggesting that they had an anthropogenic source. The *Shigella* isolates from both humans and NHPs were also multidrug resistant (ASSuST) with additional resistance to chloramphenicol and tetracycline. Further study is needed but the data suggests strain sharing between humans and NHPs in Gombe and that the *Salmonella* and *Shigella* are likely of human origin.

5.2 INTRODUCTION

The 2004 Global Burden of Disease update estimates that diarrheal diseases account for 4.6 billion diarrheal episodes and 2,163,000 deaths annually (WHO 2008). One of the challenges to determining regional or country specific estimates is the lack of

laboratory based surveillance in the developing world where the incidence of diarrhea is high and one of the top five causes of death in young children (Boschi-Pinto et al. 2010). In areas where population-based, laboratory-based studies have occurred, enteric bacterial pathogens, including *Shigella*, non-typhoidal *Salmonella*, and Enterotoxigenic *E. coli* (ETEC) are a frequent cause of diarrheal illness in sub-Saharan Africa (Kotloff et al. 2013; Morpeth et al. 2009). Determining the etiology of diarrheal illness is important to ensure appropriate patient management, and the availability of isolates to test for drug susceptibility when indicated for severe bacterial infections. In some instances, it may be possible to refer strains to a reference laboratory for subtyping and gene characterization to aid in epidemiological investigations, identify genetic mechanisms of resistance and pathogenicity, or detect the emergence of new strains, food vehicle or a novel host (Conner et al. 1998; Jackson et al. 2013; Kariuki et al. 2006; Rasko et al. 2011; Sheth et al. 2011; Ye et al. 2010).

Emerging infectious diseases account for a growing proportion of all infectious diseases impacting humans – currently more than 10%. Over 60% have zoonotic sources with the majority caused by pathogens that originated in wildlife (Jones et al. 2008). African examples include HIV and Rift Valley fever that have spread to humans from domesticated animals or non-human primates (Daubney et al. 1931; Gao et al. 1999). Zoonotic disease emergence in humans is attributed to a number of factors including, host susceptibility, altered land use patterns, and changing human behaviors (Daszak 2000; Wolfe et al. 2005). In Africa, HIV infection has been shown to increase human susceptibility and disease severity to some zoonotic pathogens ((Crump et al. 2011; MADHI et al. 2000; Mor & Tzipori 2008). In many rural areas of sub-Saharan Africa,

growing human populations are moving into areas with extensive overlap with wildlife or have to venture into these areas to find water or fuel wood. This places people in closer contact with wildlife where the risk of wildlife zoonotic emergence is high. (Chomel et al. 2007; Daszak et al. 2001; Taylor et al. 2001).

Humans are not the only species susceptible to the emergence of infectious diseases as a result of these altered landscape patterns. Nonhuman primates (NHP) may be particularly vulnerable to the infectious disease of humans because of our close genetic relatedness. A major driver in disease emergence in wild NHP is habitat loss as a result of expanding population growth and anthropogenic activities (e.g. logging, agricultural intensification) in villages that share natural boundaries with forested areas where these wild NHP populations reside (Gillespie & Chapman 2006; Gillespie et al. 2005; Zommers et al. 2013). In Gombe National Park, home to a well-studied population of wild chimpanzees, there has been a dramatic population decline predominantly attributed to infectious diseases (Williams et al. 2008). Retrospective health monitoring of the population suggests that respiratory and diarrheal diseases contribute to this decline (Lonsdorf et al. 2006). However the causes of diarrheal disease in these wild NHPs have received little study. Many enteric bacterial pathogens have host ranges that extend to NHP (Woolhouse & Gowtage-Sequeria 2006). On a local scale, they can be transferred between humans and NHP causing localized outbreaks, morbidity and mortality, particularly in times of stress (Chapman et al. 2005).

The aim of our study was to describe the epidemiology and ecology of several specific zoonotic enteric bacterial pathogens recovered from the Greater Gombe Ecosystem (GGE). We used a combination of traditional culture techniques and

molecular tools to assess the prevalence and subtypes of each bacterial pathogen recovered and to determine if there were commonalities among bacterial species, strains or groups found in human and non-human populations.

5.3 MATERIALS and METHODS

Study Site

Gombe National Park (35 km^2) is located on the shore of Lake Tanganyika in the Kigoma region of western Tanzania (4°40′S, 29°38′E) (Wallis & Lee 1999). The park is home to a globally renowned wild chimpanzee population that has been studied continuously for over six decades (Goodall 1986; Pusey et al. 2007). There are three chimpanzee communities (Kasekela, Mitumba and Kalande); two of which, (Kasekela and Mitumba) are habituated to human contact. The habitat ranges of these two habituated communities overlap slightly permitting opportunity for member contact (Earnhardt JM 2003). There is little evidence that the chimpanzees have emigrated outside the established habitat for over 20 years, and immigration events are rare. Their habitats have differing degrees of human encroachment. Kasekela, the larger community (~ 65 individuals), is situated at the center of the park in less disturbed forest, whereas Mitumba, the smaller Northern community (~ 25 individuals), is in close proximity to a nearby village, Mwamgongo (4°40′S, 29°34’60′ E), that is home to ~7000 inhabitants and their domesticated animals (goat, sheep and dogs). Villagers and their untethered animals are able to enter the unfenced park (Parsons et al. 2014). The Mitumba chimpanzees are frequently reported raiding the agricultural fields in Mwamgongo especially during the dry season (I. Lipende, personal communication). Human residents of the park itself include researchers, tourists, park management, local field assistants,

and associated families. The park boundary and Mwamgongo are geographically isolated, accessible from Kigoma only by motorized boat (a distance 20km, 3 hour journey each way) or to the nearest villages by foot (distance 10 km; ~3 hour travel time).

Study Timeframe

The study occurred between March 2010 and February 2011. A baseline demographic survey was performed in June 2010 to identify households within Mwamgongo village with at least one domesticated animal species: dog (*Canis lupus*), goat (*Capra hircus*), or sheep (*Ovis aries*). Twenty-five village households with domesticated animals were randomly selected for study enrollment. Fecal specimens were collected from humans and domesticated animals during dry (July 1-August 15) and wet (November 1-December 15) seasons. Human subjects were either residents of Mwamgongo village or Gombe National Park. Chimpanzees were sampled in both Mitumba and Kasekela during the course of routine observational health monitoring (Lonsdorf et al. 2006). The Kalande population was not sampled. Park baboons were opportunistically sampled during these two collection periods.

Specimen Collection and Transport

For human study enrollment, fecal specimens were collected from a population cross section irrespective of health status. Local clinical officers explained the intent of the study and asked for informed consent. Once informed consent was granted, the local health officer provided each study participant with a single use specimen container. Study participants were asked to defecate in the cup and return it promptly to be placed in a large cooler that contained ice packs, located near to each residence. The specimen

containers were retrieved from the camp and village and delivered to the field lab within 12-16 hours. Domestic animal specimens were aseptically collected by a village veterinary officer. Chimpanzee and baboon specimens were non-invasively collected from identified individuals as part of observational health monitoring. For bacterial testing by culture, two sterile swabs were immersed in the fecal material and transferred to pre-chilled Cary-Blair transport medium (Remel Microbiology, Thermo Fisher Scientific Lenexa, KS). An aliquot of freshly voided stool was also aseptically transferred to a screw cap plastic vial containing a 2.5% potassium dichromate preservative solution (Fisher Scientific, Pittsburgh, PA) for molecular testing. For NHP samples, care was taken to avoid the collection of soil, foliage or water contaminants, by transferring the interior and top most portion of stool to the collection of fecal material in contact with the ground. Each vial was labeled with a unique identification number, and date of collection. NHP samples were additionally labeled with the name of the observer, location and the name of the individual. . Cary-Blair transport specimens were stored short-term (6-8 weeks) at $\geq$ -80^{o}C in a 20 liter dewar of liquid nitrogen. Potassium dichromate preserved samples were stored at 4^{o}C. All specimens were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) and maintained at comparable temperature (range -80^{o}C to 4^{o}C) during shipment and final storage at the testing laboratory in Atlanta, GA USA.

Bacterial culture, serotyping and virulence testing

Fecal swabs in Cary-Blair transport medium were cultured for *Salmonella, Shigella* and *Vibrio* species by standard techniques (Nataro et al. 2007). Isolates of *Salmonella* and *Shigella* were confirmed using serotype specific antisera (Denka Seiken

Co. LTD, Tokyo, Japan) by slide agglutination for O-somatic (lipopolysaccharide) antigens and tube agglutination (H-flagellar antigen – *Salmonella* only). *Shigella* isolates were screened for the presence of the invasion plasmid (*ipaH*) by PCR (Sethabutr et al. 1993). To look for the presence of heat stable (ST) and heat labile (LT) toxin virulence genes of ETEC (Qadri et al. 2005) , nucleic acid was extracted from preserved fecal specimens using the FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH) following methods described (da Silva et al. 1999). DNA extracts were subsequently tested by PCR following published methods (Schultsz et al. 1994). Resulting products were run on a 1% agarose gel (Lonza Rockland, ME USA) stained with ethidium bromide, UV imaged and captured using a Gel Doc XR System (Bio-Rad, Hercules, CA).

Antimicrobial susceptibility testing and plasmid PCR-based replicon typing

Susceptibility testing of *Salmonella* and *Shigella* isolates was performed by the disk diffusion method for the following antimicrobials: ampicillin, chloramphenicol, ceftriaxone, ciprofloxacin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline and trimethoprim-sulfamethoxazole in accordance with recommendations of the Clinical Laboratory Standards Institute (CLSI) (CLSI 2011) . *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Antimicrobial resistance was defined by CLSI interpretive criteria for *Enterobacteriaceae* (CLSI 2012). Plasmid PCR-based replicon typing (PBRT) was performed as previously described (Johnson & Nolan 2009). Incompatibility (Inc) typing classifies plasmids by their ability to stably coexist with other plasmids in a bacterial strain. This trait is dependent on replication machinery therefore plasmid replicon type

distinguishes Inc groups, and the terms Inc and *Rep* type to describe plasmids in strains are synonymous (Johnson & Nolan 2009).

Molecular Subtyping by Pulsed-Field Gel Electrophoresis (PFGE) and Whole Genome Sequencing (WGS)

Molecular subtyping by pulsed field gel electrophoresis (PFGE) was performed as described for *Salmonella enterica* spp (Ribot et al. 2006) and *Shigella* strains (Pichel et al. 2012). DNA fingerprints were captured using a Gel Doc XR system and analyzed in BioNumerics, version 6.5 (Applied Maths, Saint-Martens-Latem, Belgium). Gels were normalized by aligning the molecular bands of the *Salmonella* Braenderup size standard in each lane with the global H9812 size standard in the database (Hunter et al. 2005). Dendograms were generated for comparison of genotypic similarities in the DNA fingerprints using unweighted pair group method with arithmetic mean (UPGMA) analysis with optimization and tolerance window set to 1.5%.

Genomic DNA was extracted using the 5 Prime ArchivePure DNA Cell Kit (5 Prime Inc. Gaithersburg, MD USA). DNA libraries were prepared using the Nextera XT Library Preparation Kit (Illumina, CA, USA) using a modified library preparation protocol. Briefly, tagmentation and dual indexing were done according to manufacturer's protocol, post-PCR clean-up was done using 0.5x AMPure beads to post-PCR volume, normalization was done using Qubit 2.0 (Life Technologies, Grand Island, NY USA) and pooled, then sequenced using 2x250bp chemistry on the MiSeq (Illumina, CA, USA). Sequences were trimmed and de novo assembled in CLC Genomics Workbench version 7.5.1 (CLC bio; Qiagen Aarhus, Denmark). Fasta sequences were uploaded to the ResFinder 2.1 https://cge.cbs.dtu.dk/services/ResFinder/ and PlasmidFinder 1.2 https://cge.cbs.dtu.dk/services/PlasmidFinder/ for in silico detection of acquired antibiotic

resistance genes or plasmids among sequenced *Salmonella* and *Shigella* isolates (Carattoli et al. 2014; Zankari et al. 2012). The threshold for reporting a match between a gene or plasmid in the database and the input genome was set to be 90% identity for ResFinder and 95% identify for PlasmidFinder. ResFinder and PlasmidFinder are free software tools based on a database that covers many classes of antimicrobial resistance agents (aminoglycoside, betalactamase, fluoroquinolone, fosfomycin, fusidic acid, glycopeptide, macrolide-lincosamide-streptograminB, phenicol, rifampicin, sulphoamide, tetracycline, and trimethophorim), which are searched using BLAST (Altschul et al. 1997).

Data Management and Analysis

Results were tabulated and compared in Microsoft Excel (Redmond, WA). Statistical analyses were performed with SPSS Statistics version 20 (International Business Machines, Armonk, NY USA). A chi-square test of independence was used to compare bacterial culture or ETEC frequency by group, species or season, with two-tailed significance set at 0.05 for all comparisons. In instances where cells contained less than five values, Fisher's exact tests were used to calculate *p* -values.

5.4 RESULTS

Descriptive statistics for all groups

Five hundred and seventy-five fecal specimens were tested for *Salmonella*, *Shigella* and *Vibrio* spp. by culture and ETEC by PCR (Table 1). The specimens were collected from 268 human, 99 domesticated animals (n=76 goat, n=14 sheep, n=9 dog)

and 208 NHPs (n=127 chimpanzee, n=81 baboon). Twenty-seven *Salmonella enterica* strains were identified: 8 (3.9%) from 208 NHP specimens, 14 (5.2%) of 268 from humans and 5 (5.1%) of 99 from domesticated animals. Four serotypes of *Salmonella* were found: Typhimurium (21), Heidelberg (3), Enteritidis (2) and Uganda (1). *Salmonella* Typhimurium was the most ubiquitous serotype, identified within each group from multiple communities (Table 1). *Salmonella* Heidelberg was recovered from humans in Mwamgongo and Mitumba. *Salmonella* Enteritidis was found in a single chimpanzee in Kasekela and from a human in Mwamgongo village. A single case of *Salmonella* Uganda was recovered from a goat in Mwamgongo village. Ten *Shigell*a strains were identified: 3 (1.4%) from 208 NHPs (1.44 %) and 7 (2.6%) of 268 humans. The 10 *Shigella* strains identified represented three of the four serogroups of *Shigella* (*S. flexneri* [5] *S. boydii* [4] and *S. sonnei* [1]). Within serogroups, we identified *S. flexneri* serotypes 1a and 2a and *S. boydii* 1 and 2 (Table 1). All 10 *Shigella* strains were positive for the *Shigella* spp *ipaH* gene. No specimen yielded *Vibrio* spp., including *V. cholerae*. Virulence genes of ETEC (*ST, LT*) were detected in each group: 42 (15.7%) from 268 humans, 13 (13.1%) of 99 domesticated animals and 12 (5.8%) of 208 NHPs.

Salmonella and *Shigella* combined were not recovered more frequently from sub-adult (≥10 years) chimpanzees or old (>30 years) chimpanzees compared to prime (11-30 years) members (Chi-square 1.814; df = 1, *p*-value 0.278). Bacterial recovery rates did not differ by season; all groups wet versus dry (Chi-square statistic 2.98, df = 1, *p-value* = 0.084). There was no significant difference in frequency of *Salmonella* or *Shigella* strains detected between the two chimpanzee communities (Chi-square 0.179; df =1,

Fisher's exact test $p = 0.505$), nor between human residence; Kasekela or Mitumba camp versus Mwamgongo village (Chi-square 0.349, df =1, *p*-value 0.362).

Antimicrobial resistance of enteric bacterial isolates

Among the 21 *Salmonella* Typhimurium isolates we identified five different resistance patterns. The most common pattern, among 10/21 (47.6%) *Salmonella* Typhimurium was resistance to ampicillin, streptomycin, sulfisoxazole, trimethoprim-sulfamethoxazole (ASSuTmp-Su). This pattern was observed in chimpanzees (4), humans (5) and a domesticated animal (1). Three human (14.3%) *Salmonella* Typhimurium isolates exhibited resistance to chloramphenicol in addition. The remaining eight Typhimurium isolates had different resistance profiles: one was pan-susceptible (baboon), three were resistant to streptomycin (domesticated animals), two were resistant to ampicillin, streptomycin, and sulfisoxazole (a baboon and a human), and two were resistant to streptomycin, sulfisoxazole and trimethoprim-sulfamethoxazole (a chimpanzee and a human). Both *Salmonella* Enteritidis isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, trimethoprim-sulfamethoxazole, and tetracycline (ACSSuTmp-SuT). All three *Salmonella* Heidelberg isolates were pan-susceptible to the drugs tested. The *Salmonella* Uganda isolate only showed resistance to sulfisoxazole. Differences in antimicrobial resistance were observed between the *Salmonella* and *Shigella* isolates recovered (Figure 1). All seven *Shigella* isolates recovered from humans were resistant to ACSSuTmp-SuT. The majority of the *Shigella* strains recovered from NHPs had a comparable resistance phenotype to the human isolates except for a *Shigella boydii* 2 recovered from a chimpanzee that was susceptible to chloramphenicol and tetracycline (ASSuTmp-Su).

None of the bacterial strains were found to be resistant to nalidixic acid, ciprofloxacin or ceftriaxone.

Plasmid typing

Plasmid incompatibility typing by PCR identified the following plasmid replicon types in *Salmonella* Typhimurium (FIIA), Enteritidis (FIIA), *Shigella boydii* 1 (P-1), *Shigella boydii* 2 (FIIA) and *Shigella sonnei* (I1). *Rep* plasmids were not detected from the *Salmonella* ser. Heidelberg, ser. Uganda or surprisingly, the *Shigella flexneri* strains. Based on these findings, representative isolates of *Shigella* and *Salmonella* isolates were selected for WGS for the sequence-based detection of plasmids and antimicrobial resistance determinants.

Strain characterization by PFGE and WGS

All *27 Salmonella* strains were PFGE subtyped by primary enzyme *Xba*I and similar *Salmonella* strains (n= 22) were further subtyped by secondary enzyme (*Bln*I). Subtyping indicated there was substantial similarity among the strains isolated from humans and NHP's and less so with the strain isolated from the domesticated animals. By PFGE, *Salmonella* Typhimurium grouped into two genotype clusters. Cluster I comprised 17 (81%) of the 21 *Salmonella* Typhimurium including all of the human and all but one of the nonhuman primate isolates, with 82% genotypic similarity to one another (Figure 2). . Cluster II included three isolates, all from domesticated animals. Within cluster I there were two pairs of Typhimurium isolates with indistinguishable PFGE patterns. One pair of isolates came from a chimpanzee and human that both resided in Mitumba; both had pattern combination (*Xba*I/*Bln*I: K/C). A second pair of

isolates with indistinguishable PFGE patterns was from humans residing in Mwamgongo village and Mitumba camp (*Xba*I/*Bln*I: M/F). Also within cluster I was a single goat isolate (*Xba*I/*Bln*I: I/C), though the other *Salmonella* Typhimurium isolated from the domesticated animals clustered genotypically far from the human and NHP isolates. A Typhimurium strain isolated from a baboon had a different genotypic pattern (*XbaI*: D) that clustered with 65% similarity to other Typhimurium strains; this isolate was also susceptible to antimicrobials tested. All three *Salmonella* Heidelberg isolates were recovered from humans in Mwamgongo and Mitumba and had indistinguishable PFGE patterns by two enzymes (*Xba*I/*Bln*I: C/H). The two *Salmonella* Enteritidis isolates were similar but not indistinguishable genotypes; one came from a human and one from a chimpanzee.

Shigella strains were subtyped by single enzyme analysis using *Xba*I. The *Shigella* isolates identified from humans and NHPs were also genotypically similar by PFGE within specific serotypes (Figure 3). No strains were found to be indistinguishable from one another but very similar patterns (90-95% pattern similarity) were observed between a pair of *Shigella flexneri* 2a strain from a human and a Kasekela chimpanzee and between a pair of *Shigella boydii* 2 strains from a Mitumba chimpanzee and a human from Mwamgongo.

We performed WGS on 13 isolates: Typhimurium n =9, Enteritidis n =1, *Shigella* n = 3). We found that the antimicrobial resistance gene sequence data correlated well with the phenotype. Among the *Salmonella* isolates, Resfinder confirmed the presence of the FIIA plasmids and identified additional FIB plasmids in all ten *Salmonella* tested.One Typhimurium strain carried additional Q1 and Col156 plasmids. In the three *Shigella*

isolates selected for WGS, we detected FIIA plasmids in two strains (*Shigella flexneri* 2a and *Shigella boydii* 2), and Col plasmids in two strains (*Shigella flexneri* 1a and *Shigella boydii* 2)

Using ResFinder to test the nine *Salmonella* Typhimurium isolates with similar PFGE genotypes (90-95% similarity) and antimicrobial resistance phenotypes, we identified genetic determinants associated with resistance to specific drug classes: beta-lactamases (e.g. ampicillin) *(blaTEM-1B, blaOXA-1)* aminoglycosides (e.g. streptomycin) *(aadB, aadA1),* sulfonamides (e.g. sulfisoxazole) *(sul1),* and trimethoprim *(dfrA23*). A chloramphenicol resistant strain of *Salmonella* Typhimurium carried a phenicol resistance gene (*catA1).* A representative single strain of *Salmonella* Enteritidis was sequenced for comparison and found to carry some of the same genes (*aadB, aadA1, blaTEM-1B, blaOXA-1, sul1, dfrA23*) and additional genes: aminoglycosides (*strA, strB),* sulfonamides *(sul2),* tetracyclines *(tetA)* and trimethoprim *(dfrA7).* The serotype Enteritidis isolate also carried a different chloramphenicol determinant (*catA2*) from that *Salmonella* Typhimurium isolate.

WGS of single strains of *Shigella flexneri* 1a and 2a and *Shigella boydii* 2 were found to carry resistance genes more similar to the *Salmonella* Enteriditis compared to the *Salmonella* Typhimurium: beta-lactams (*blaOXA-1)* aminoglycosides (*strA, strB, aadB, aadA1*) phenicols (*catA1*), sulfonamides *(sul1, sul2),* trimethoprims (*dfrA1, dfrA23*), and tetracyclines (*tetB*), Both the NHP primate *Shigella boydii 2* isolate and two *Shigella flexneri* from humans had similar aminoglycoside resistance genes (*strA, strB, aadA1*). The NHP *Shigella boydii* 2 isolate also carried genes for sulfonamides (*sul1*)

and trimethroprim (*dfrA23*) that were not detected in the two human *Shigella flexneri* but these genes were seen in both human and NHP *Salmonellae* strains.

5.5 DISCUSSION

We identified three bacterial enteric agents that are circulating in the Greater Gombe Ecosystem among humans, domesticated animals and NHPs. Similar *Salmonella* and *Shigella* were found in both human and NHP populations, and for *Salmonella*, the circulation may also include domesticated animals. Among the humans, the isolation rates of *Salmonella* (14/268) and *Shigella* (7/268) are comparable to some studies; *Salmonella* (1/309) and *Shigella* (13/209) (Gascón et al. 2000) and *Salmonella* 15/280 and *Shigella* 7/280 (Moyo et al. 2011) but lower than other studies; *Salmonella* (166/1094) (Berkley et al. 2005) and *Salmonella* (11/47) and *Shigella* (6/47) (Musiime et al. 2009). These differences could be attributed to study location (rural versus urban setting), host demographics (age, HIV prevalence, clinical severity), sample size and study design. Our study was small and sampled all ages, rather than being limited to children under 5 where the burden of enteric illness is greatest (Walker et al. 2013). In addition, the rates of HIV are lower in the rural Kigoma region ($\leq$ 2%) relative to urban settings (~5.7%) in Tanzania (Tanzania Commission for AIDS (TACAIDS) 2008). Previous studies have shown a correlation between HIV infection and greater susceptibility to these two pathogens (Acheson & Hohmann 2001; Baer et al. 1999; Gordon et al. 2002).

Shigella flexneri was the most frequent species of *Shigella* we identified. *S. flexneri* 2a is one of the most common serotypes in the developing world (Kotloff et al.).

The host range of *Shigella* is limited to primates. *Salmonella* Typhimurium and *Salmonella* Enteritidis are both frequently identified serotypes of humans and animals globally. They have a relatively broad host range that includes humans, livestock and poultry. Clinical disease may range from asymptomatic carriage, gastroenteritis or a more life threatening illness (Gordon 2008; Herikstad et al. 2002). Clones of *Salmonella* Enteriditis have been linked to specific hosts (i.e. chicken) and multiple waves of *Salmonella* Typhimurium have been shown to disseminate rapidly (e.g. DT104 globally, ST313 in Africa), and carry antimicrobial resistance genes to multiple drug classes (Frech et al. 2003; Kingsley et al. 2009; Ribot et al. 2002).

ETEC, a pathogen of animals and humans that causes acute watery diarrhea, is characterized by heat-labile (LT) and heat-stable (ST) enterotoxins (Nataro & Kaper 1998). Few studies have looked at ETEC in NHPs but genetic evidence of the LT virulence gene for this pathogen recently was reported at high frequencies in lemurs and humans in Madagascar (Bublitz et al. 2014). In Gombe, ETEC was detected in all groups but the distribution of virulence genes was different across groups. ST-ETEC has been associated with severe diarrhea in humans, including children under the age of 5 years (Kotloff et al. ; Okeke 2009). The pathogen exhibits host specificity with domesticated animals and humans found to be infected with different serotypes of ETEC that have distinct colonizing factors (CF), essential for adherence and colonization of the intestinal mucosa. The high specificity in humans and domesticated animals is mainly due to the specific recognition between bacterial colonization factors and the epithelial receptors during host-parasite interaction (Blanco et al. 1991). A limitation of our study is that the virulence determinants of ETEC were detected by PCR; isolates are not available to

identify serotype and CF type making it difficult to further delineate the ecology of what strains might be circulating among the humans, domesticated animals and humans.

Earlier studies in natural settings report similar frequencies of *Salmonella* and *Shigella* to what we observe in Gombe among wild NHPs (Kalema 1995; Nizeyi et al. 2001) and lemurs in Madagascar (Bublitz et al. 2014). In these studies, there is evidence for bacterial spillover to primates from humans as a result of increased habitat disturbance and human activity in close proximity to primate habitat. Our study provides molecular evidence for strain similarities among *Salmonella* and *Shigella* isolates recovered from humans and NHPs in Gombe. The PFGE data suggests anthropogenic factors are resulting in pathogen spillover between humans and NHPs in Gombe. Both the *Salmonella* and *Shigella* strains recovered from the humans and NHPs had similar genotypes, with one pair of *Salmonell*a Typhimiurum isolates recovered from a chimpanzee and human genotypically indistinguishable by PFGE. Differences in genotypic clusters or in isolation rates of *Salmonella* and *Shigella* were not observed between primate groups (Mitumba versus Kasekela) or human groups (camp versus village) suggesting that the strains are being shared across the host populations. The S*almonella* strains from the domesticated animals were not as closely related to the humans and NHPs suggesting that these strains may have been acquired from other sources, show varying host specificity or some other yet to be determined explanation. PFGE using one or two enzymes to establish strain relatedness has been used with success in many foodborne outbreak investigations and instances where there is a strong epidemiological link between strains (CDC 2008; Jay et al. 2007; Painter et al. 2015). However the results still need to be interpreted with caution as strain genetic relatedness

does not necessary imply phylogenetic relatedness. For the purposes of this study, where humans, domesticated animals and NHPs are in close proximity to one another PFGE provided adequate discrimination to account for differences between strains.

Zoonotic transmission of enteric bacteria has been documented in other NHP studies. In Kibale National Park (KNP), Uganda, researchers found that chimpanzees had bacteria genetically more similar to strains from humans involved in chimpanzee-research and tourism then humans from a local village (Goldberg et al. 2007). Another study conducted in KNP found that *E. coli* from local livestock and humans within the same community were genetically quite similar and that a lack of hand washing increased the likelihood of having genetically similar human *E. coli* strains to livestock compared to human counterparts who did wash their hands before eating (Rwego et al. 2008a). There is also evidence that other non-bacterial zoonotic enteric pathogens, such as *Cryptosporidium* and *Giardia* that originated in humans or livestock may be spilling into NHPs populations with increased human and animal activities in close proximity to NHPs (Graczyk et al. 2001; Nizeyi et al. 2002a; Nizeyi et al. 2002b; Parsons et al. 2015; Salyer et al. 2012; Salzer et al. 2007). All these studies highlight the complexity of enteric disease dynamics in natural systems and the need for further study.

The strains in our study were found to be resistant to a number of antimicrobials (ASSuTmp-Su) and to some extent chloramphenicol and tetracycline ACSSuTmp-SuT), representing different drug classes. This multi-drug phenotype is one of the most commonly reported in sub-Saharan Africa based on a recent summation of various studies (Leopold et al. 2014). Both of these multi-drug resistance phenotypes were detected among bacterial strains from humans and NHPs, and to less extent, domesticated

animals. Gombe NHPs are not routinely administered antibiotics for treatment of infections. Therefore, this is strong evidence for the spillover of pathogens and resistance genes from humans to NHPs, with little participation from domesticated animals. Two NHP studies in Uganda at Kibale National Park (KNP) and in Bwindi Impenetrable National Park (BINP) had comparable findings (Goldberg et al. 2007; Rwego et al. 2008b) . In KNP, *E. coli* from humans and chimpanzees were resistant to a least one locally available antibiotic and resistance was higher to local antibiotics in both groups then to an antibiotic not available for use. In BINP, drug resistance in gorillas was found to decline proportionately with decreasing degree of habitat overlap with humans. An informal assessment of antimicrobial use and availability conducted in Mwamgongo village in 2010 indicated that fluoroquinolones were seldom administered by the village dispensary or at the hospital for human illness. Ceftriaxone was only available at the local hospital via IV and reported to be reserved for sexually transmitted infections. The most common antimicrobials reported for use in the treatment of diarrheal infections were ampicillin, trimethoprim-sulfamethoxazole, Penicillin, amoxicillin and to a less extent tetracycline based on availability and cost. It is of note that the commonly administered drugs are those to which we found drug resistance. From a conservation perspective, this is concerning for NHPs if these agents were to be used to treat them in extenuating circumstances. Resistance will complicate treatment but the solution is not to administer more drugs (e.g. ciprofloxacin, ceftriaxone) for general treatment. Resistance is likely to develop rapidly to these drugs which need to be reserved for the most severe human and animal infections. The promotion of locally driven safe water

and hygiene practices, and prudent antibiotic use (Bennish & Khan 2010; Moeller et al. 2013) may help minimize the spread of human disease and resistance.

We used a recently developed analytic tool to assess whole genome sequences (WGS) for the presence of specific resistance genes (i.e. ResFinder) (Carattoli et al. 2014; Zankari et al. 2012). We were able to identify genetic resistance determinants that are circulating among bacterial strains in Gombe. Based on Blast comparison, the resistance genes and plasmid sequences identified in this system are common among *Enterobacterciae*, and shared by lateral gene transfer (LGT) on virulence plasmids, type 1 integrons and mediated by transposons (Alton & Vapnek 1979; Amyes & Towner 1990; Luck et al. 2001; Villa & Carattoli 2005). Although there were differences in the genes identified from *Salmonella* and *Shigella* species, it is not easy to delineate differences in acquistion mechanisms. LGT occurs extensively in prokaryotes, particularly *Shigella*, which can acquire these genes directly from other bacteria or by natural uptake from the environment (Levy & Marshall 2004; Stokes & Gillings 2011)

There are a number of anthropogenic drivers that may be promoting the exchange of bacteria between humans and NHPs in Gombe. The increase in NHP research in Gombe has been previously linked to increases in NHP disease outbreaks thought to be of human origin (Lonsdorf et al. 2006; Pusey et al. 2008). The chimpanzees are habituated to humans and this acceptance may increase camp visits and food theft (Lipende, personal communication). Although practices are in place to secure food, and contain excrement; these systems are not perfect and may break down. Residual excrement may leech into the natural environment, including streams during periods of heavy rain. These streams and the lake are used by people for bathing, washing of food and utensils

and are also water sources for the NHPs. Trash pits are typically buried less than 2 m below the surface, easily accessible to raiding baboons and/or other forest animals (Wallis & Lee 1999). The chimpanzees are also known to crop-raid in Mwamgongo village, where villagers report spreading feces on crops. This behavior places them in closer contact to humans, their livestock and potentially infected feces (Parsons MB 2014). The epidemiology of disease in Gombe is complex and additional research is needed to understand how this interspecies transmission is occurring.

We used a combination of traditional and molecular methods in combination with standardized health surveillance data to understand the epidemiology and ecology of select zoonotic enteric pathogens in the Greater Gombe Ecosystem to ascertain the frequency and genotypes of these pathogens circulating in human, domesticated animal and wild primate populations. The data suggest anthropogenic factors result in enteric bacterial strain sharing between humans and NHPs in Gombe. Exchange between humans and non-human primates appear to be common, with less exchange between either and domesticated animals. The exchange includes bacterial pathogens that are highly resistant to the antimicrobial agents in common use in the human population, suggesting that an important part of the pathogen exchange is from humans to NHPs. Additional research is needed to expand our understanding of the role of pathogen exchange in primate ecology and illness, the behaviors that lead to this exchange, and to assess how anthropogenic effects influence the zoonotic potential of pathogens to wildlife (Gillespie et al. 2008).

Ethics Statement

This project was reviewed and approved by the Emory University Institutional Review Board (approval #: IRB00018856) under the Expedited review process per 45 CFR 46.110(3), Title 45 CFR Subpart D section 46.404, one parent consent, and 21 CFR 56.110 and the National Medical Research Institute, Dar Es Salaam Tanzania. All animal use followed the guidelines of the Weatherall Report on the use of non-human primates in research and was approved by the Tanzania Wildlife Research Institute, the Emory University Animal Care and Use Committee (protocol ID 087-2009), and Tanzania Commission for Science and Technology (permit number 2009-279-NA-2009-184). Approval was also obtained from Tanzania National Parks (Permit number TNP/HQ/C10/13) to collect samples from wild chimpanzees. The researchers did not have any interactions with the chimpanzees at the park. All animals were sampled from households in Mwamgongo village. The owners of the animals provide verbal consent for the use of their animals for this study, and the verbal consent was documented.

5.6 FIGURES

Figure 5.1 Comparison of antimicrobial resistance in *Salmonella* (A) and *Shigella* (B) strains recovered from nonhuman primates, humans and domestic animals in and around Gombe National Park, Tanzania.

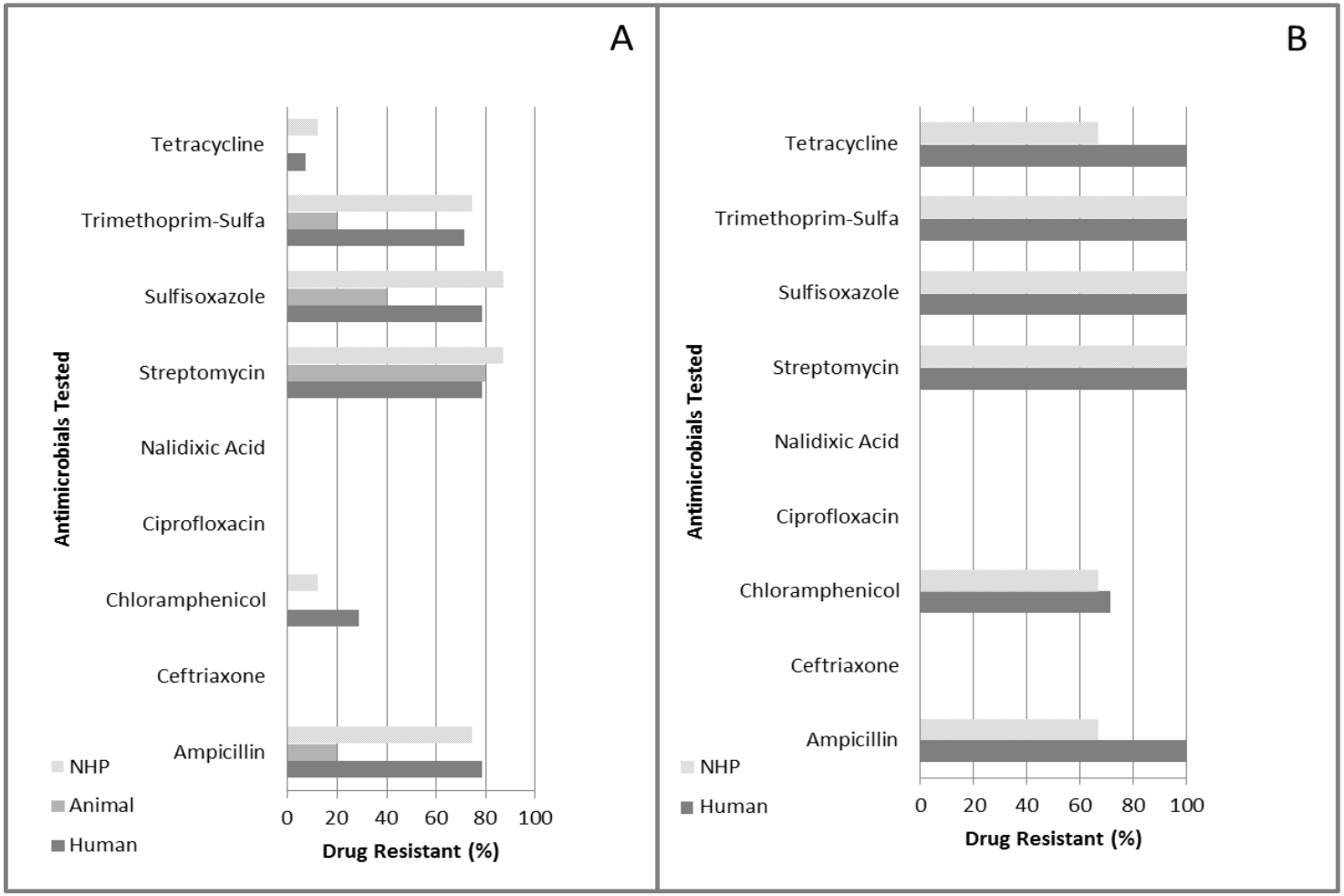
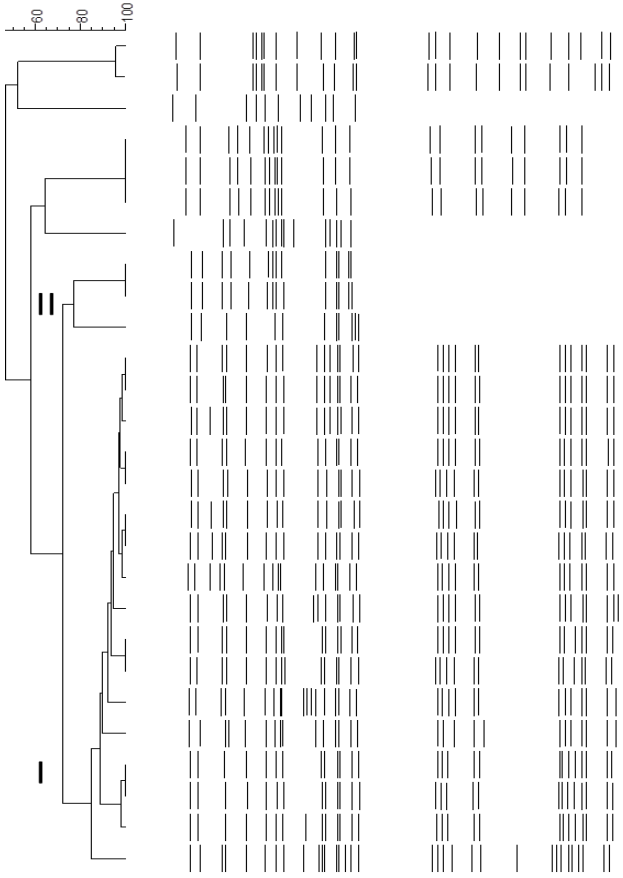
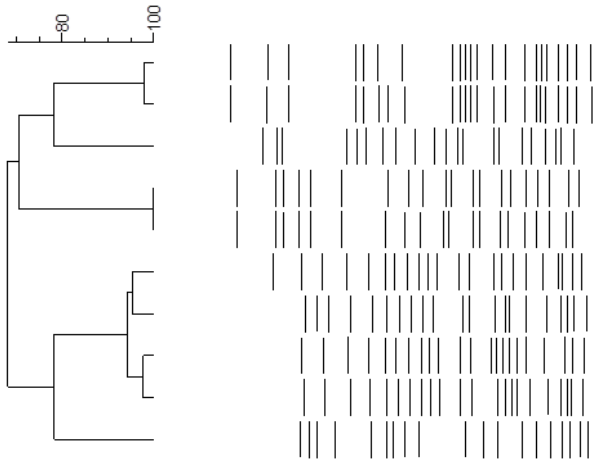


Figure 5.2 PFGE dendrogram of *Salmonella* isolates (n=27) recovered from humans, domesticated animals and non-human primates in and around Gombe National Park, Tanzania to illustrate pattern similarities between strains. The dendrogram is a composite analysis of primary (*XbaI*) and secondary (*BlnI*) enzymes weighted equally. Letter designations were assigned to highlight strains with different PFGE patterns. Strains with a letter-number designation have either a single band shift or single band difference relative to the parent letter strain. Isolates with an (*) were selected for whole genome sequencing.



	Specimen ID	*XbaI* Pattern	*BlnI* Pattern	Location	Group	Serotype
*	GM20101326	XbaI Pattern A	BlnI Pattern I	Kasekela	Chimpanzee	Enteriditis
	GM20101635	XbaI Pattern A1	BlnI Pattern I1	Mwamgongo	Human	Enteriditis
	GM20101402	XbaI Pattern B		Mwamgongo	Goat	Uganda
	GM20101238	XbaI Pattern C	BlnI Pattern H	Mitumba	Human	Heidelberg
	GM20101293	XbaI Pattern C	BlnI Pattern H	Mwamgongo	Human	Heidelberg
	GM20101362	XbaI Pattern C	BlnI Pattern H	Mwamgongo	Human	Heidelberg
	GM20101513	XbaI Pattern D			Baboon	Typhimurium
	GM20101322	XbaI Pattern E		Mwamgongo	Sheep	Typhimurium
	GM20101573	XbaI Pattern E		Mwamgongo	Sheep	Typhimurium
	GM20101318	XbaI Pattern F		Mwamgongo	Goat	Typhimurium
*	GM20101140	XbaI Pattern I	BlnI Pattern C	Mwamgongo	Goat	Typhimurium
	GM20101614	XbaI Pattern I	BlnI Pattern C	Mwamgongo	Human	Typhimurium
*	GM20101641	XbaI Pattern J	BlnI Pattern C	Mwamgongo	Human	Typhimurium
*	GM20101018	XbaI Pattern G	BlnI Pattern C	Kasekela	Chimpanzee	Typhimurium
*	GM20101370	XbaI Pattern G	BlnI Pattern D	Kasekela	Human	Typhimurium
*	GM20101456	XbaI Pattern K	BlnI Pattern C	Mitumba	Chimpanzee	Typhimurium
	GM20101684	XbaI Pattern K	BlnI Pattern C	Mitumba	Human	Typhimurium
	GM20101049	XbaI Pattern K1	BlnI Pattern C	Mitumba	Chimpanzee	Typhimurium
	GM20101532	XbaI Pattern H	BlnI Pattern C1	Kasekela	Chimpanzee	Typhimurium
*	GM20101171	XbaI Pattern N	BlnI Pattern A		Baboon	Typhimurium
	GM20101242	XbaI Pattern N	BlnI Pattern B	Mitumba	Human	Typhimurium
*	GM20101034	XbaI Pattern L	BlnI Pattern C	Kasekela	Chimpanzee	Typhimurium
	GM20101194	XbaI Pattern P	BlnI Pattern E	Kasekela	Human	Typhimurium
	GM20101237	XbaI Pattern M	BlnI Pattern F	Mitumba	Human	Typhimurium
*	GM20101289	XbaI Pattern M	BlnI Pattern F	Mwamgongo	Human	Typhimurium
	GM20101337	XbaI Pattern M1	BlnI Pattern F	Mwamgongo	Human	Typhimurium
*	GM20101355	XbaI Pattern O	BlnI Pattern G	Mwamgongo	Human	Typhimurium

Figure 5.3 PFGE dendrogram of *Shigella* isolates (n=10) recovered from humans, and non-human primates in and around Gombe National Park, Tanzania to illustrate pattern similarities between strains. Letter designations were assigned to highlight strains with different PFGE patterns. Strains with a letter-number designation have either a single band shift or single band difference relative to the parent letter strain. Isolates with an (*) were selected for whole genome sequencing.



PFGE-Xbal	Specimen ID	Location	Group	Serotype	*Xba*I Pattern
	GM20101687	Mitumba	Human	boydii 1	Xba-I Pattern A
	GM20101715		Baboon	boydii 1	Xba-I Pattern A1
	GM20101261	Mwamgongo	Human	sonnei	Xba-I Pattern B
*	GM20101039	Mitumba	Chimpanzee	boydii 2	Xba-I Pattern C
	GM20101296	Mwamgongo	Human	boydii 2	Xba-I Pattern C1
	GM20101268	Mwamgongo	Human	flexneri 2a	Xba-I Pattern D
	GM20101280	Mwamgongo	Human	flexneri 2a	Xba-I Pattern E
	GM20101228	Mitumba	Human	flexneri 2a	Xba-I Pattern E1
*	GM20101414	Kasekela	Chimpanzee	flexneri 2a	Xba-I Pattern E2
*	GM20101378	Mwamgongo	Human	flexneri 1a	Xba-I Pattern F

5.7 TABLES

Table 5.1 Proportion of fecal specimens positive for bacterial enteric pathogens tested by traditional culture or PCR from non-human primates, humans and domesticated animals of Gombe National Park, Tanzania.

	Non-Human Primates			**Humans**			**Domesticated Animals**		
	Kasekela Chimpanzee	Mitumba Chimpanzee	Baboons	Kasekela	Mitumba	Mwamgongo	Goat	Sheep	Dogs
Number of specimens tested (n)	91	36	81	76	39	153	76	14	9
Bacterial culture									
Salmonella ser. Typhimurium	3 (3.29)	2 (5.55)	2	2 (2.63)	3 (7.69)	5 (3.26)	2 (2.63)	2 (7.14)	0
Salmonella ser. Heidelberg	0	0	0	0	1 (2.56)	2 (1.31)	0	0	0
Salmonella ser. Enteritidis	1 (1.09)	0	0	0	0	1 (0.65)	0	0	0
Salmonella ser. Uganda	0	0	0	0	0	0	1 (1.31)	0	0
All *Salmonella* species	**4 (4.39)**	**2 (5.55)**	**2 (2.47)**	**2 (2.63)**	**4 (10.3)**	**8 (5.23)**	**3 (3.95)**	**2 (7.14)**	**0**
Shigella flexneri	1 (1.09)	0	0	0	1 (2.56)	3 (1.96)	0	0	0
Shigella boydii	0	1 (2.78)	1 (1.23)	0	1 (2.56)	1 (0.65)	0	0	0
Shigella sonnei	0	0	0	0	0	1 (0.65)	0	0	0
All *Shigella* species	**1 (1.09)**	**1 (2.78)**	**1 (1.23)**	**0**	**2 (5.13)**	**5 (3.26)**	**0**	**0**	**0**
Vibrio spp.	0	0	0	0	0	0	0	0	0
PCR for ETEC virulence factors									
ETEC (ST-only or ST/LT)	2 (2.19)	1 (2.78)	0	10 (13.16)	6 (15.38)	18 (11.76)	2 (2.63)	0	1 (1.11)
ETEC (LT only)	5 (5.49)	1 (2.78)	3 (3.70)	2 (2.63)	2 (5.13)	4 (2.61)	8 (10.52)	2 (7.14)	0
All ETEC	**7 (7.69)**	**2 (5.55)**	**3 (3.70)**	**12 (15.78)**	**8 (20.51)**	**22 (14.38)**	**10 (13.15)**	**2 (7.14)**	**1 (1.11)**

5.8 REFERENCES

Acheson, D., and E. L. Hohmann. 2001. Nontyphoidal Salmonellosis. Clinical Infectious Diseases **32**:263-269.

Alton, N. K., and D. Vapnek. 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9.

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research **25**:3389-3402.

Amyes, S., and K. Towner. 1990. Trimethoprim resistance; epidemiology and molecular aspects—a review based upon a Symposium held on 19 April 1989 at the 4th European Congress of Clinical Microbiology, Nice, France. Journal of medical microbiology **31**:1-1.

Baer, J. T., D. J. Vugia, A. L. Reingold, T. Aragon, F. J. Angulo, and W. Z. Bradford. 1999. HIV infection as a risk factor for shigellosis. Emerging infectious diseases **5**:820.

Bennish, M. L., and W. A. Khan 2010. What the Future Holds for Resistance in Developing Countries.

Berkley, J., B. Lowe, I. Mwangi, T. Williams, E. Bauni, S. Mwarumba, C. Ngetsa, M. Slack, S. Njenga, C. Hart, K. Maitland, M. English, K. Marsh, and J. Scott. 2005. Bacteremia among children admitted to a rural hospital in Kenya. New Engl J Med **352**:39 - 47.

Blanco, J., M. Blanco, J. I. Garabal, and E. A. González. 1991. Enterotoxins, colonization factors and serotypes of enterotoxigenic Escherichia coli from humans and animals. Microbiologia (Madrid, Spain) **7**:57-73.

Boschi-Pinto, C., M. Young, and R. E. Black. 2010. The child health epidemiology reference group reviews of the effectiveness of interventions to reduce maternal, neonatal and child mortality. International Journal of Epidemiology **39**:i3-i6.

Bublitz, D. C., P. C. Wright, F. T. Rasambainarivo, S. J. Arrigo-Nelson, J. R. Bodager, and T. R. Gillespie. 2014. Pathogenic enterobacteria in lemurs associated with anthropogenic disturbance. American Journal of Primatology:n/a-n/a.

Carattoli, A., E. Zankari, A. Garcìa-Fernandez, M. Volby Larsen, O. Lund, L. Villa, F. M. Aarestrup, and H. Hasman. 2014. PlasmidFinder and pMLST: in silico detection and typing of plasmids. Antimicrobial Agents and Chemotherapy.

Chapman, C. A., T. R. Gillespie, and T. L. Goldberg. 2005. Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? Evolutionary Anthropology **14**:134-144.

Chomel, B. B., A. Belotto, and F.-X. Meslin. 2007. Wildlife, Exotic Pets, and Emerging Zoonoses. Emerging Infectious Diseases **13**:6-11.

CLSI, C. a. L. S. I. 2012. Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement. , Wayne, PA.

CLSI, C. L. S. I. 2011. Performance standards for antimicrobial disk susceptibility tests; approved standard M2-A11, 11th ed. CLSI, Wayne, PA.

Conner, C. P., D. M. Heithoff, S. M. Julio, R. L. Sinsheimer, and M. J. Mahan. 1998. Differential patterns of acquired virulence genes distinguish Salmonella strains. Proceedings of the National Academy of Sciences **95**:4641-4645.

Centers for Disease Control and Prevention. 2008. Outbreak of Salmonella serotype Saintpaul infections associated with multiple raw produce items--United States, 2008. MMWR. Morbidity and mortality weekly report **57**:929.

Crump, J. A., H. O. Ramadhani, A. B. Morrissey, W. Saganda, M. S. Mwako, L.-Y. Yang, S.-C. Chow, S. C. Morpeth, H. Reyburn, B. N. Njau, A. V. Shaw, H. C. Diefenthal, J. F. Shao, J. A. Bartlett, and V. P. Maro. 2011. Invasive Bacterial and Fungal Infections Among

Hospitalized HIV-Infected and HIV-Uninfected Adults and Adolescents in Northern Tanzania. Clinical Infectious Diseases **52**:341-348.

da Silva, A. J., F. J. Bornay-Llinares, I. N. S. Moura, S. B. Slemenda, J. L. Tuttle, and N. J. Pieniazek. 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Molecular Diagnosis **4**:57-64.

Daszak, P. 2000. Emerging infectious diseases of wildlife - Threats to biodiversity and human health (vol 287, pg 443, 2000). Science **287**:1756-1756.

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

Daubney, R., J. Hudson, and P. Garnham. 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep cattle and man from East Africa. The Journal of Pathology and Bacteriology **34**:545-579.

Earnhardt JM, R. S., Lonsdorf EV, Pusey AE. 2003. A demographic comparison of wild chimpanzees from Gombe and a managed population from North American zoos. American Journal of Primatology **60**:2.

Frech, G., C. Kehrenberg, and S. Schwarz. 2003. Resistance phenotypes and genotypes of multiresistant Salmonella enterica subsp. enterica serovar Typhimurium var. Copenhagen isolates from animal sources. Journal of Antimicrobial Chemotherapy **51**:180-182.

Gao, F., E. Bailes, D. L. Robertson, Y. L. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature **397**:436-441.

Gascón, J., M. Vargas, D. Schellenberg, H. Urassa, C. Casals, E. Kahigwa, J. J. Aponte, H. Mshinda, and J. Vila. 2000. Diarrhea in Children under 5 Years of Age from Ifakara, Tanzania: a Case-Control Study. Journal of Clinical Microbiology **38**:4459-4462.

Gillespie, T. R., and C. A. Chapman. 2006. Prediction of Parasite Infection Dynamics in Primate Metapopulations Based on Attributes of Forest Fragmentation. Conservation Biology **20**:441-448.

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

Gillespie, T. R., C. L. Nunn, and F. H. Leendertz. 2008. Integrative approaches to the study of primate infectious disease: Implications for biodiversity conservation and global health. American Journal of Physical Anthropology **137**:53-69.

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

Goodall, J. 1986. The Chimpanzees of Gombe: Patterns of Behavior Bellknap Press of the Harvard University Press Boston.

Gordon, M. A. 2008. Salmonella infections in immunocompromised adults. Journal of Infection **56**:413-422.

Gordon, M. A., H. T. Banda, M. Gondwe, S. B. Gordon, M. J. Boeree, A. L. Walsh, J. E. Corkill, C. A. Hart, C. F. Gilks, and M. E. Molyneux. 2002. Non-typhoidal salmonella bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. Aids **16**:1633-1641.

Graczyk, T. K., A. J. DaSilva, M. R. Cranfield, J. B. Nizeyi, G. Kalema, and N. J. Pieniazek. 2001. Cryptosporidium parvum Genotype 2 infections in free-ranging mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. Parasitology Research **87**:368-370.

Herikstad, H., Y. Motarjemi, and R. Tauxe. 2002. Salmonella surveillance: a global survey of public health serotyping. Epidemiology and infection **129**:1-8.

Hunter, S. B., P. Vauterin, M. A. Lambert-Fair, M. S. Van Duyne, K. Kubota, L. Graves, D. Wrigley, T. Barrett, and E. Ribot. 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. Journal of Clinical Microbiology **43**:1045-1050.

Jackson, B. R., P. M. Griffin, D. Cole, K. A. Walsh, and S. J. Chai. 2013. Outbreak-associated Salmonella enterica serotypes and food commodities, United States, 1998–2008. Emerging infectious diseases **19**:1239.

Jay, M. T., M. Cooley, D. Carychao, G. W. Wiscomb, R. A. Sweitzer, L. Crawford-Miksza, J. A. Farrar, D. K. Lau, J. O'Connell, A. Millington, R. V. Asmundson, E. R. Atwill, and R. E. Mandrell. 2007. Escherichia coli O157:H7 in Feral Swine near Spinach Fields and Cattle, Central California Coast. Emerging Infectious Diseases **13**:1908-1911.

Johnson, T. J., and L. K. Nolan. 2009. Plasmid replicon typing. Pages 27-35. Molecular Epidemiology of Microorganisms. Springer.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-U994.

Kalema, G. 1995. Epidemiology of the intestinal parasite burden of mountain gorillas in Bwindi Impenetrable Forest National Park. British Veterinary Zoological Society Newsletter **14**:4-6.

Kariuki, S., G. Revathi, N. Kariuki, J. Kiiru, J. Mwituria, and C. Hart. 2006. Characterisation of community acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. BMC Microbiology **6**:101.

Kingsley, R. A., C. L. Msefula, N. R. Thomson, S. Kariuki, K. E. Holt, M. A. Gordon, D. Harris, L. Clarke, S. Whitehead, and V. Sangal. 2009. Epidemic multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. Genome research **19**:2279-2287.

Kotloff, K. L., J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. G. Faruque, A. K. M. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acacio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne, and M. M. Levine. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet **382**:209-222.

Kotloff, K. L., J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. G. Faruque, A. K. M. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acácio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne, and M. M. Levine. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet **382**:209-222.

Leopold, S. J., F. van Leth, H. Tarekegn, and C. Schultsz. 2014. Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review. Journal of Antimicrobial Chemotherapy **69**:2337-2353.

Levy, S., and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med **10**:S122 - S129.

Lonsdorf, E. V., D. Travis, A. E. Pusey, and J. Goodall. 2006. Using retrospective health data from the Gombe chimpanzee study to inform future monitoring efforts. American Journal of Primatology **68**:897-908.

Luck, S. N., S. A. Turner, K. Rajakumar, H. Sakellaris, and B. Adler. 2001. Ferric dicitrate transport system (Fec) of Shigella flexneri 2a YSH6000 is encoded on a novel pathogenicity island carrying multiple antibiotic resistance genes. Infection and immunity **69**:6012-6021.

MADHI, S. A., K. PETERSEN, A. MADHI, A. WASAS, and K. P. KLUGMAN. 2000. Impact of human immunodeficiency virus type 1 on the disease spectrum of Streptococcus pneumoniae in South African children. The Pediatric Infectious Disease Journal **19**:1141-1147.

Moeller, A. H., M. Shilts, Y. Y. Li, R. S. Rudicell, E. V. Lonsdorf, A. E. Pusey, M. L. Wilson, B. H. Hahn, and H. Ochman. 2013. SIV-Induced Instability of the Chimpanzee Gut Microbiome. Cell Host & Microbe **14**:340-345.

Mor, S. M., and S. Tzipori. 2008. Cryptosporidiosis in Children in Sub-Saharan Africa: A Lingering Challenge. Clinical Infectious Diseases **47**:915-921.

Morpeth, S. C., H. O. Ramadhani, and J. A. Crump. 2009. Invasive Non-Typhi Salmonella Disease in Africa. Clinical Infectious Diseases **49**:606-611.

Moyo, S., N. Gro, M. Matee, J. Kitundu, H. Myrmel, H. Mylvaganam, S. Maselle, and N. Langeland. 2011. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. BMC Pediatrics **11**:19.

Musiime, V., I. Kalyesubula, D. Kaddu-Mulindwa, and J. Byarugaba. 2009. Enteric Bacterial Pathogens in HIV-Infected Children With Acute Diarrhea in Mulago Referral and Teaching Hospital, Kampala, Uganda. Journal of the International Association of Physicians in AIDS Care (JIAPAC).

Nataro, J. P., C. A. Bopp, P. I. Fields, J. B. Kaper, and N. A. Strockbine. 2007. Escherichia, Shigella and Salmonella in P. R. Murray, editor. Manual of Clinical Microbiology. ASM, Washington, D.C.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic escherichia coli. Clinical microbiology reviews **11**:142-201.

Nizeyi, J. B., M. R. Cranfield, and T. K. Graczyk. 2002a. Cattle near the Bwindi Impenetrable National Park, Uganda, as a reservoir of Cryptosporidium parvum and Giardia duodenalis for local community and free-ranging gorillas. Parasitology Research **88**:380-385.

Nizeyi, J. B., R. B. Innocent, J. Erume, G. Kalema, M. R. Cranfield, and T. K. Graczyk. 2001. Campylobacteriosis, salmonellosis, and shigellosis in free-ranging human-habituated mountain gorillas of Uganda. Journal of Wildlife Diseases **37**:239-244.

Nizeyi, J. B., D. Sebunya, A. J. Dasilva, M. R. Cranfield, N. J. Pieniazek, and T. K. Graczyk. 2002b. Cryptosporidiosis in people sharing habitats with free-ranging mountain gorillas (Gorilla gorilla beringei), Uganda. American Journal of Tropical Medicine and Hygiene **66**:442-444.

Okeke, I. N. 2009. Diarrheagenic Escherichia coli in sub-Saharan Africa: Status, Uncertainties and Necessities.

Painter, J. E., A. T. Walker, J. Pytell, M. T. Nua, S. Soliai-Lemusu, E. Mintz, I. Ali, M. Parsons, H. Martin, and M. Beach. 2015. Notes from the Field: Outbreak of Diarrheal Illness Caused by Shigella flexneri-American Samoa, May-June 2014. MMWR. Morbidity and mortality weekly report **64**:30-30.

Parsons, M., D. Travis, E. V. Lonsdorf, I. Lipende, D. Roellig, A. Collins, S. Kamenya, Z. Hongwei, L. Xiao, and T. R. Gillespie. 2015. Epidemiology and Molecular Characterization of Cryptosporidium spp. in Humans, Wild Primates and Domesticated Animals in the Greater Gombe Ecosystem, Tanzania. Plos Neglected Tropical Diseases ***Accepted***.

Parsons, M. B., T. R. Gillespie, E. V. Lonsdorf, D. Travis, I. Lipende, B. Gilagiza, S. Kamenya, L. Pintea, and G. M. Vazquez-Prokopec. 2014. Global Positioning System Data-Loggers: A Tool to Quantify Fine-Scale Movement of Domestic Animals to Evaluate Potential for Zoonotic Transmission to an Endangered Wildlife Population. PLoS ONE **9**:e110984.

Parsons MB, G. T. L., Travis D, Lipende L, Gilagiza B, Kamenya S, Pintea L, Vazquez-Prokopec GM. 2014. Global Positioning System Data-Loggers: A Tool to Quantify Fine-Scale Movement of Domestic Animals to Evaluate Potential for Zoonotic Transmission to an Endangered Wildlife Population. Plos ONE.

Pichel, M., S. P. Brengi, K. L. Cooper, E. M. Ribot, S. Al-Busaidy, P. Araya, J. Fernández, T. I. Vaz, K. M. Kam, and M. Morcos. 2012. Standardization and international multicenter validation of a PulseNet pulsed-field gel electrophoresis protocol for subtyping Shigella flexneri isolates. Foodborne pathogens and disease **9**:418-424.

Pusey, A. E., L. Pintea, M. L. Wilson, S. Kamenya, and J. Goodall. 2007. The contribution of long-term research at Gombe National Park to chimpanzee conservation. Conservation Biology **21**:623-634.

Pusey, A. E., M. L. Wilson, and D. A. Collins. 2008. Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:738-744.

Qadri, F., A.-M. Svennerholm, A. Faruque, and R. B. Sack. 2005. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clinical microbiology reviews **18**:465-483.

Rasko, D. A., D. R. Webster, J. W. Sahl, A. Bashir, N. Boisen, F. Scheutz, E. E. Paxinos, R. Sebra, C.-S. Chin, D. Iliopoulos, A. Klammer, P. Peluso, L. Lee, A. O. Kislyuk, J. Bullard, A. Kasarskis, S. Wang, J. Eid, D. Rank, J. C. Redman, S. R. Steyert, J. Frimodt-Møller, C. Struve, A. M. Petersen, K. A. Krogfelt, J. P. Nataro, E. E. Schadt, and M. K. Waldor. 2011. Origins of the E. coli Strain Causing an Outbreak of Hemolytic–Uremic Syndrome in Germany. New England Journal of Medicine **365**:709-717.

Ribot, E. M., M. Fair, R. Gautom, D. Cameron, S. Hunter, B. Swaminathan, and T. J. Barrett. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157: H7, Salmonella, and Shigella for PulseNet. Foodbourne Pathogens & Disease **3**:59-67.

Ribot, E. M., R. K. Wierzba, F. J. Angulo, and T. J. Barrett. 2002. Salmonella enterica serotype typhimurium DT 104 Isolated from Humans, United States, 1985, 1990, and 1995. Emerging infectious diseases **8**:387-391.

Rwego, I. B., T. R. Gillespie, G. Isabirye-Basuta, and T. L. Goldberg. 2008a. High rates of Escherichia coli transmission between livestock and humans in rural Uganda. Journal of Clinical Microbiology **46**:3187-3191.

Rwego, I. B., G. Isabirye-Basuta, T. R. Gillespie, and T. L. Goldberg. 2008b. Gastrointestinal Bacterial Transmission among Humans, Mountain Gorillas, and Livestock in Bwindi Impenetrable National Park, Uganda. Conservation Biology **22**:1600-1607.

Salyer, S. J., T. R. Gillespie, I. B. Rwego, C. A. Chapman, and T. L. Goldberg. 2012. Epidemiology and Molecular Relationships of Cryptosporidium spp. in People, Primates, and Livestock from Western Uganda. Plos Neglected Tropical Diseases **6**.

Salzer, J. S., I. B. Rwego, T. L. Goldberg, M. S. Kuhlenschmidt, and T. R. Gillespie. 2007. Giardia sp and Cryptosporidium sp infections in primates in fragmented and undisturbed forest in western Uganda. Journal of Parasitology **93**:439-440.

Schultsz, C., G. J. Pool, R. Van Ketel, B. De Wever, P. Speelman, and J. Dankert. 1994. Detection of enterotoxigenic Escherichia coli in stool samples by using nonradioactively labeled oligonucleotide DNA probes and PCR. Journal of clinical microbiology **32**:2393-2397.

Sethabutr, O., M. Venkatesan, G. S. Murphy, B. Eampokalap, C. W. Hoge, and P. Echeverria. 1993. Detection of Shigellae and Enteroinvasive Escherichia coli by Amplification of the Invasion Plasmid Antigen H DNA Sequence in Patients with Dysentery. Journal of Infectious Diseases **167**:458-461.

Sheth, A. N., M. Hoekstra, N. Patel, G. Ewald, C. Lord, C. Clarke, E. Villamil, K. Niksich, C. Bopp, and T.-A. Nguyen. 2011. A national outbreak of Salmonella serotype Tennessee infections from contaminated peanut butter: a new food vehicle for salmonellosis in the United States. Clinical infectious diseases **53**:356-362.

Stokes, H. W., and M. R. Gillings. 2011. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiology Reviews **35**:790-819.

Tanzania Commission for AIDS (TACAIDS), Z. A. C. Z., National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), Macro International Inc. . 2008. Tanzania HIV/AIDS and Malaria Indicator Survey 2007-98, Dar Es Salaam.

Taylor, L. H., S. M. Latham, and E. Mark. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London B: Biological Sciences **356**:983-989.

Villa, L., and A. Carattoli. 2005. Integrons and transposons on the Salmonella enterica serovar Typhimurium virulence plasmid. Antimicrobial agents and chemotherapy **49**:1194-1197.

Walker, C. L. F., I. Rudan, L. Liu, H. Nair, E. Theodoratou, Z. A. Bhutta, K. L. O'Brien, H. Campbell, and R. E. Black. 2013. Global burden of childhood pneumonia and diarrhoea. The Lancet **381**:1405-1416.

Wallis, J., and D. R. Lee. 1999. Primate conservation: The prevention of disease transmission. International Journal of Primatology **20**:803-826.

WHO. 2008. The Global Burden of Disease: 2004 Update. World Health Organization.

Williams, J. M., E. V. Lonsdorf, M. L. Wilson, J. Schumacher-Stankey, J. Goodall, and A. E. Pusey. 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:766-777.

Wolfe, N. D., P. Daszak, A. M. Kilpatrick, and D. S. Burke. 2005. Bushmeat hunting, deforestation, and prediction of zoonotic disease. Emerging infectious diseases **11**:1822-1827.

Woolhouse, M. E., and S. Gowtage-Sequeria. 2006. Host range and emerging and reemerging pathogens. Page 192. Ending the War Metaphor:: The Changing Agenda for Unraveling the Host-Microbe Relationship-Workshop Summary. National Academies Press.

Ye, C., R. Lan, S. Xia, J. Zhang, Q. Sun, S. Zhang, H. Jing, L. Wang, Z. Li, and Z. Zhou. 2010. Emergence of a new multidrug-resistant serotype X variant in an epidemic clone of Shigella flexneri. Journal of clinical microbiology **48**:419-426.

Zankari, E., H. Hasman, S. Cosentino, M. Vestergaard, S. Rasmussen, O. Lund, F. M. Aarestrup, and M. V. Larsen. 2012. Identification of acquired antimicrobial resistance genes. Journal of antimicrobial chemotherapy **67**:2640-2644.

Zommers, Z., D. W. Macdonald, P. J. Johnson, and T. R. Gillespie. 2013. Impact of human activities on chimpanzee ground use and parasitism (Pan troglodytes). Conservation Letters **6**:264-273.

Chapter 6

Principle Findings, Limitations and Future Directions

Understanding and subsequently minimizing the ecological, demographic and economic factors fostering zoonotic disease emergence and transmission is a global challenge (King et al. 2006). As human population growth further encroaches on wildlife habitats, the impact of anthropogenic disturbance on wildlife populations is of continual concern. The chimpanzees in the Gombe stream national park rural western Tanzania are susceptible to these anthropogenic changes. Loss of habitat and increased human activity in the park has placed pressure on the chimpanzee communities. This increases inter-community conflict, stress and risk for infectious diseases that lead to population decline (Earnhardt JM 2003; Gillespie et al. 2010; Pusey et al. 2008; Williams et al. 2008).

The findings in this dissertation contribute to our understanding of the ecology and epidemiology of zoonotic enteric pathogen transmission among humans, domesticated animals and wildlife; the impact of anthropogenic disturbance on wildlife; and the risk for zoonotic pathogen exchange among these groups at the human-animal interface in western Tanzania. Enteric bacterial pathogen species, frequency and distribution data from natural wildlife studies are not readily available and findings are typically limited to the detection of genus or species specific markers from studied groups making it difficult to infer host-pathogen transmission dynamics in a natural system. My research deepens our understanding of host-pathogen transmission dynamics in an endangered wildlife population. I identified multiple pathogens (*Cryptosporidium*, *Salmonella* and *Shigella*) from wild primates. Using a combination of molecular diagnostics in a spatially explicit framework, we have biological evidence supporting a

complex epidemiology of enteric transmission occurring in Gombe National Park among humans, domesticated animals, NHPs and the environment in which they reside (Figure 6.1a-c). These results have broad implications. First, the relatedness of enteric pathogens/strains between species provides strong evidence for a shared ecological context between pathogens and theirs hosts among these groups. The results suggest that we must consider employing modeling approaches that account for multi-host, multi-pathogen systems in the environment to expand our understanding of disease dynamics in natural systems like Gombe (Holt et al. 2003; Woolhouse et al. 2001). Under future directions, a brief discussion of the value of metagenomics will be discussed to facilitate investigations into pathogen-host community interactions.

The results also have implications for wildlife conservation approaches. Conservation strategies have typically prioritized habitat size and quality as key regulators for the population success of endangered species, increased biodiversity and general ecosystem health (Myers et al. 2000; Noss et al. 1997; Struhsaker 1981). However, our results highlight the importance of considering the maintenance and spread of infectious diseases in conservation design plans. While Gombe National Park has established habitat for endangered chimpanzees and other wildlife (e.g. baboons, colobus monkeys), the park border is porous to humans and domesticated animals that have residence outside the park and researchers that work and live in the park. Evidence of local multi-pathogen-multi-host interactions in this community suggest that the human population in combination with wild primates can lead to increased host densities, or serve as natural reservoirs of disease of wildlife, potentially increasing disease transmission rates (Anderson & May 1991). Conservation efforts should include local

efforts to reduce the spread of enteric transmission in humans, such as water, sanitation, and hygiene (WASH) activities. Successful implementation of these measures will require multidisciplinary engagement from health, and water sectors to set policy, investment priorities and raise public awareness of the importance of the water and sanitation resource development to control infectious diseases and enhance socioeconomic development (Bartram et al.). Reducing the spread of infection and disease will likewise be an important determinant of the health and well-being of wildlife populations and should be considered in the design of conservation policy (Scott 1988). Despite study limitations, my dissertation offers new ideas and questions whose answers will require further scientific examination.

6.1 PRINCIPLE FINDINGS

Portable GPS technology can be used to identify fine-scale mobility patterns of domesticated animals and hot spots for pathogen spillover

I found that combining integrated GPS/GIS technology with laboratory diagnostics could clarify where along the Mwamgongo Village / Gombe Stream National Park border there were risks of zoonotic pathogen transmission from domesticated animals to the endangered chimpanzee population. Although there was evidence of animal mobility into the park, we found few animal movement points beyond the forest edge indicate limited intrusion into the park. Rather, the village area where wild chimpanzees are known to raid agricultural crops was a potential hot spot for pathogen spillover due to high visitation rates by domesticated animals and the dispersion of their potentially infected feces (Parsons MB 2014). Domesticated animals are an important

source of pathogens to endangered wildlife populations, especially when anthropogenic activities increase their overlap with humans (Cleaveland et al. 2001; Daszak et al. 2000). Application of animal feces to crops can perpetuate the transmission of foodborne pathogens (Newell et al. 2010). While the focus of conservation strategies traditionally has been on wildlife corridors to minimize habitat fragmentation and risk for population isolation, my data suggest a need to monitor borders and edges, in wildlife management and park design.

The zoonotic pathogen Cryptosporidium has a complex ecology in the Greater Gombe Ecosystem

My research uncovered a complex cycle of *Cryptosporidium* occurring in Gombe National Park that was previously undescribed in this system and also provides empirical evidence that subtypes of *Cryptosporidium* vary in zoonotic potential (Xiao and Feng 2008). Humans, baboons and a subset of chimpanzees were infected with an anthropogenic strain of *C. hominis* that suggests cross-species transmission occurs regularly. This finding has also been observed in other study systems with frequent human-primate contact (Wang et al. 2013; Xiao & Feng 2008). Despite a high degree of habitat overlap between village people and livestock, our results suggest that there are distinct *Cryptosporidium* transmission dynamics for humans and livestock in this system as all positive goats and sheep were infected with *C. xiaoi*. A subset of chimpanzees from the Kasekela community, were infected with *C. suis*, a pig subtype. This subtype was previously detected in an HIV positive person in Lima, Peru and recently in a human in Madagascar. (Bodager et al. 2015; Cama et al. 2007). We hypothesize that there is cross-species transmission from bush pigs (*Potaochoerus larvatus*) to chimpanzees in

Gombe forest, since domesticated pigs are regionally absent.

Empirical evidence for the spillover of antimicrobial resistance genes between humans and NHPs: a proxy for pathogen spillover.

My research suggests that humans are the reservoir for antimicrobial resistance genes that spread to NHPs, regardless of human density. Fecal prevalence of antimicrobial resistance genes to the frequently administered sulfonamides was highest among humans followed by NHPs, then domesticated animals. Genes that encode tetracycline resistance, the less commonly administered antimicrobial, were detected at much lower frequencies in all groups. The prevalence of resistance genes in the chimpanzees is concerning; both as an indicator for pathogen spillover and in extenuating circumstances where antimicrobial treatment may be administered. Sustainable interventions, including the promotion of safe water and hygiene activities, drug resistance monitoring and prudent antimicrobial use are needed to minimize the spread of antimicrobial resistance in this community (Levy & Marshall 2004; Wise 2002).

Characterization of enteric bacterial pathogens infecting humans, domesticated animals and NHPs in the Greater Gombe Ecosystem

My research detected Enterotoxigenic *E. coli* and recovered *Salmonella* and *Shigella* isolates from humans, domesticated animals and wild NHPs in and around Gombe Stream National Park. Prior to this research, enteric pathogenic bacteria had not been characterized for these groups. These pathogens are leading contributors to the

morbidity and mortality of children in developing countries and captive primates (Kotloff et al. ; Rewell & Bridges 1948).

Humans are the likely source for enteric pathogens to NHPs

I found that NHPs have resistant enteric bacterial strains that are similar in genotype and resistance pattern to strains circulating in humans. As NHPs are not routinely administered antimicrobials, this suggests spillover of resistance genes and associated pathogens from humans to NHPs. The *Salmonella* Typhimurium strains observed in domesticated animals in this study were part of a different genotype cluster than those in humans or NHPs and were not resistant to as many drug classes suggesting different transmission patterns or host susceptibility.

Human and NHP bacterial strains recovered from Gombe National Park were resistant to antimicrobials administered locally to humans.

My research determined that the majority of the *Salmonella* and *Shigella* strains recovered from the human and NHP populations were resistant to ampicillin, streptomycin, sulfisoxazole, and trimethoprim-sulfamethoxazole (ASSuTmp-Su) and contained genetic determinants associated with these drug classes. A much smaller subset of strains from both genera were also resistance to tetracycline and chloramphenicol. Many human Gombe residents reported using antibiotics from one of these resistant drug classes when they had a diarrheal illness. This is concerning since drug resistance would complicate treatment of human infections. In addition, although NHPs are not routinely treated with antimicrobials, the spillover of pathogenic bacterial

strains from humans suggests limited antimicrobial options for extenuating circumstances when treatment may be desired for NHPs.

Findings build upon historical research

Historical studies of parasitism of Gombe wild chimpanzees began in the 1970s (File et al. 1976) and continue today with a comprehensive health monitoring system in place in combination with laboratory diagnostics to assess threats to chimpanzee health and wellness (Lonsdorf et al. 2011; Lonsdorf et al. 2006; Travis et al. 2008). A recent assessment of patterns of parasitism among the Gombe chimpanzee communities found increased prevalence of *Ascaris* and *Balantidium coli*, two human-associated zoonotic pathogens in this community, which could be the result of increased human-wildlife habitat sharing (Gillespie et al. 2010). The data from my research complements a continuous syndromic (e.g. behavioral, gastrointestinal, and respiratory) and diagnostic (e.g. fecal testing, necropsy, SIV testing, stress monitoring) health monitoring program to comprehensively examine the relationship among wildlife population dynamics, habitat disturbance, zoonotic pathogen transmission risk, and wildlife disease emergence (Chapman et al. 2005; Pusey et al. 2008).

6.2 STUDY LIMITATIONS

Despite the strengths and findings within my dissertation, my data and analyses had notable limitations. Many of these limitations are due to inherent shortcomings of field collection. Both sample size and sample replication opportunities were limited by the size of the populations and the duration of the study. Increasing the number of individuals sampled within each group, and expanding sampling over multiple years

would have provided more data. The water sampling methods and the bacterial enteric panel selected for this study were limited due to the logistics of field sampling, transport and storage. Since little was known regarding the bacterial etiology and sources of diarrheal disease among the chimpanzees, humans and domesticated animals in and around Gombe Stream National Park in western Tanzania prior to this study, I focused on collecting a representative number of samples in a short time and extensively investigated each group, fecal specimen, and diarrheal agent recovered to inform future efforts.

Lack of temporal variation in sampling

Population dynamics and chimpanzee sightings vary in Gombe Stream National Park (Goodall 1983, 1986). The time available for specimen collection and behavioral health monitoring for this research was limited to a single dry and wet season in 2010. It has been shown previously in Gombe and at other sites that patterns of parasitism can vary between years (Gillespie et al. 2010). Therefore, caution is required when extrapolating these findings to other seasons or years. While it is likely that similar pathogens and parasites would circulate at comparable frequency among these groups, additional work across years would be ideal to investigate fluctuations in the population dynamics of host groups and pathogens.

Lack of rigorous water sampling methods

Due to the logistics of field sampling, water sampling was opportunistic using smaller volumes of water than advocated by standard screening protocols (Graczyk et al. 1997) and provided only limited inter-seasonal sampling. Therefore, our negative results do not assure that waterborne transmission is not important in this system. Future studies

using more comprehensive watershed sampling would help to expand this aspect of *Cryptosporidium* and other enteric pathogen transmission.

Small samples sizes for some risk factor analyses

An analysis of the risk factors for diarrheal disease was difficult for ETEC, *Salmonella* and *Shigella* due to a small number of positive samples. This shortcoming may be resolved in future studies by increased sampling effort among all groups across multiple seasons to strengthen associations between host clinical manifestation and pathogen both within and between groups. Despite this shortcoming, my research was able to characterize recovered strains of *Salmonella* and *Shigella* in detail and show associations with potential sources of infection in each group.

Limited detection of some zoonotic enteric pathogens

A large number of zoonotic enteric pathogens, including bacteria, viruses and parasites, can cause disease in humans, domesticated animals and wildlife. It was not feasible to look for all enteric pathogens. Previous studies in Gombe have evaluated parasitism in wild chimpanzees and baboons (Bakuza & Nkwengulila 2009; Bakuza 2012; Gillespie et al. 2010). Bacterial causes of diarrheal illness had not been looked at extensively in this system. Based on the field system and study constrains for transport and storage, I chose to look for zoonotic pathogens *Cryptosporidium,* ETEC, *Salmonella*, *Shigella* and *Vibrio* based on previous human and wildlife studies in Africa (Cegielski et al. 1999; Crump et al. 2011; Kotloff et al. ; Nizeyi et al. 2001; Nizeyi et al. 1999). Many field studies have been limited to storing a fecal specimen in a preservative medium, then

subsequently detect genus-specific genes by molecular typing, usually PCR. This approach will detect the presence of the bacterial pathogen but would not allow for the characterization of antimicrobial resistance patterns or strain comparisons across groups, which was a specific aim for this study. In our approach, we were able to store specimens short-term in liquid nitrogen which allows recovery of the pathogens we selected. However this approach made it difficult to recover *Campylobacter* spp., that has special atmospheric conditions for growth and does not recover well from extreme cold temperatures and delayed transport (Stern & Kazmi 1989). Enteric viruses, such as rotavirus and norovirus were also not evaluated in this study. However, my research into the selected pathogens provides a baseline of patterns of infection in this community and highlight important findings on the source and dissemination of enteric pathogens recovered in this system.

6.3 FUTURE DIRECTIONS

My work has answered many questions and created opportunities for additional research in and around Gombe Stream National Park (GSNP) to advance our understanding of the risk factors for pathogen exchange at the human-animal-wildlife interface and the infection patterns that may emerge in NHPs. My research suggests that humans and their activities increase the risk for enteric pathogen exchange into NHP populations. In addition, there may be other sylvatic cycles (e.g. *C. suis* suspected to come from pigs) that serve as reservoirs for pathogen spillover to NHP populations. Future research is needed to understand how this interspecies transmission occurs. The results of this work can in turn be used to develop strategies to reduce transmission risk. Characterizing the ecology and epidemiology of infectious disease transmission in

tropical systems like GSNP is challenging. While disease modeling has helped us understand the complexity of disease in ecosystems, most approaches have targeted single pathogen systems, ignoring the reality that disease often results from multiple infections within a host (Kosoy 2013; Sterling et al. 2013). A true understanding of the disease dynamics occurring in a natural system, like Gombe will require an analysis of the interaction of multiple co-existing and sometimes confounding factors in a changing environment (Kosoy 2013). In the Gombe study system, one has the capability to combine non-invasive field collection techniques, health monitoring and disease modeling to improve our understanding of complex disease transmission dynamics in wildlife.

Historically, new pathogen discoveries occurred following investigation of atypical symptoms in an affected population with microbiological detection limited to what could be cultured from a biological specimen. This approach has skewed our view of microbial diversity and appreciation for the complex microbiological interactions that manifest as clinical disease (Hugenholtz & Tyson 2008). However, recent advances in molecular diagnostics, including metagenomics techniques make it possible to detect and characterize unculturable pathogens and investigate the presence of genes and genomes from a mixed community of organisms rather than an individual (Schloss & Handelsman 2005). Metagenomics provides an unbiased view of the community structure and metabolic potential of a community (Hugenholtz & Tyson 2008). These types of studies are likely to identify novel sequences from clinical specimens and in combination with health monitoring of humans, primates and domesticated animals correlate clinical illness with imbalances in commensal species and/or the acquisition of specific pathogenic gene

sequences (Finkbeiner et al. 2008; Wolfe et al. 2007; Zhang et al. 2005). Metagenomic approaches do not require *a priori* assumptions to define a community of pathogens and their host interactions (Vourc'h et al. 2012). In a spatially explicit framework, a molecular scheme with sufficient discrimination can be employed to analyze the genetic variability of complex pathogen-host interactions to identify transmission routes and the genes involved in important pathogen life history traits such as virulence (Frank & Pace 2008; Vourc'h et al. 2012).

The complexity of factors that influence zoonotic pathogen spillover and disease emergence in multiple-host systems, underscores the importance of engaging relevant partners and stakeholders among human, domesticated animal and wildlife sectors. Another study achievement was the scientific collaboration of experts from diverse fields of spatial ecology, microbiology, epidemiology, behavioral science, veterinary science, environmental health, and public health. Historically, these fields developed independently with human medicine integrated into the European university system and veterinary medicine the responsibility of equerries (Rüegg 2004). The basic framework of One Health begins with education and collaborative research (Dhama et al. 2013; Gibbs 2014; Osburn et al. 2009). Although there have been advancements in promoting a One Health agenda globally, substantially more can be done. A recent literature review of zoonoses and One Health found that the distribution of One Health initiatives have been predominant in developed countries even though the majority of emerging zoonoses have been detected in developing nations (Bidaisee & Macpherson 2014). One reason may be the lack of comprehensive prevalence data in many of these countries that would allow human, veterinary and wildlife sectors to prioritize zoonoses to monitor. A semi-

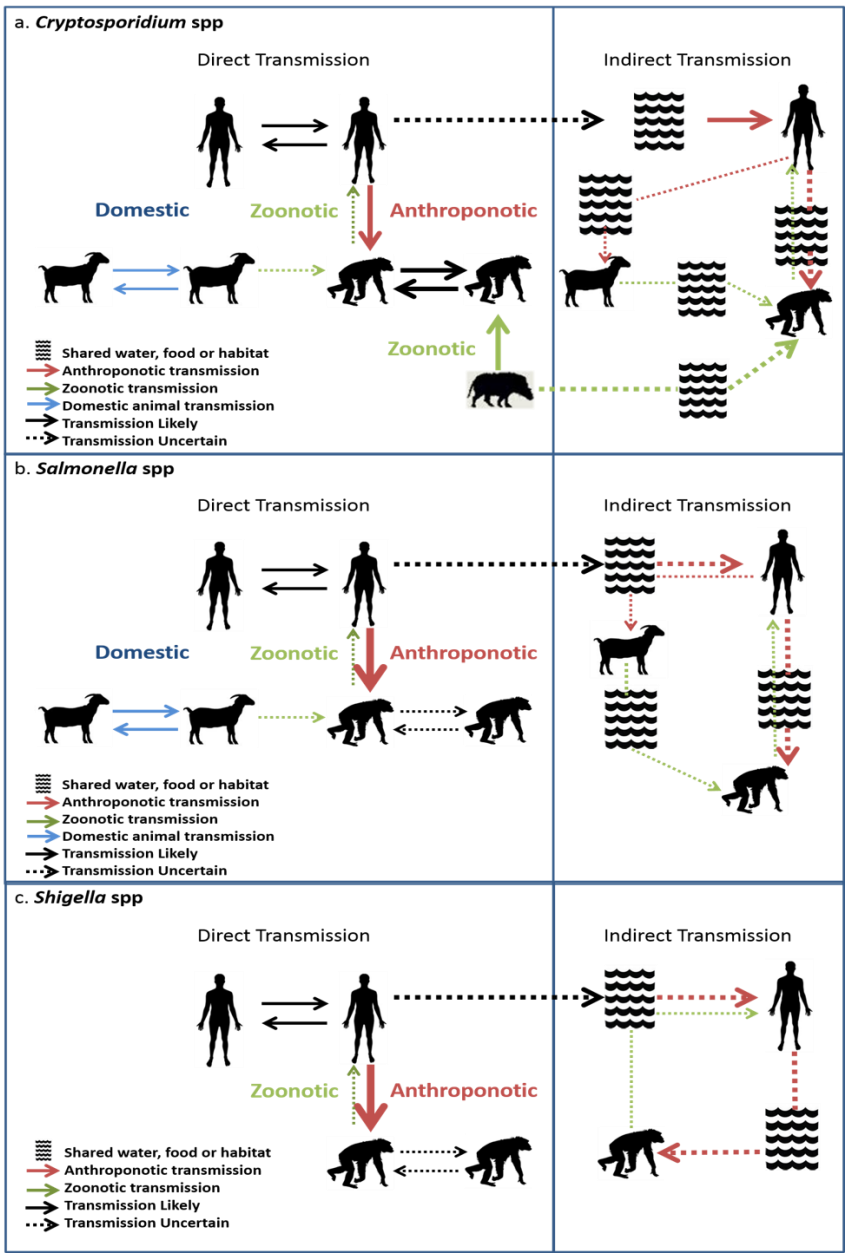
quantitative tool was recently published to attempt to prioritize zoonoses in the absence of comprehensive surveillance data (Rist et al. 2014). For the zoonotic disease prioritization tool to be fully successful, agency representatives and stakeholders from the human, animal and wildlife sectors must jointly identify criteria for defining a concerning zoonotic disease or pathogen (Rist et al. 2014). This coordinated effort and consensus among the parties on the zoonotic pathogens or diseases of greatest concern allows for a directed use of limited financial resources and personnel. In addition, this strategic framework creates opportunities to then discuss ways in which data will be collected, managed and shared and laboratory testing conducted to make the results comparable across groups (Plowright et al. 2008).

In conclusion, zoonotic diseases related to tropical rainforests have been affecting the health and history of humans for centuries, from sylvatic yellow fever to most recently the large epidemic of Ebola in West Africa (Soper 1937; Team 2014). Disease emergence is driven by a number of factors with anthropogenic activities attributed to recent increases (Cleaveland et al. 2001; Daszak et al. 2001; Taylor et al. 2001), particularly in tropical areas which have seen the greatest increase in human populations that expand into natural forested areas, placing humans and wildlife in closer contact with one another (Chapman et al. 2005; Chapman et al. 2006). There has been recent evidence of pathogen transmission to humans from wild primates (Calvignac-Spencer et al. 2012) and increased recognition of the risk of human pathogen spillover to wild primates (Köndgen et al. 2008). My research findings from Gombe National Park in conjunction with future research undertaken by future generations of scientists will be used to

implement prevention measures to reduce transmission for the health and conservation of all primates.

6. 4 FIGURES

Figure 6.1a-c. Summary of transmission dynamics based on patterns of infection from enteric pathogens recovered among humans, wild primates and domesticated animals in Gombe National Park. Arrow width denotes estimate for intensity of infection.



6.5 REFERENCES

Anderson, R. M., and R. M. May 1991. Infectious diseases of humans. Oxford university press Oxford.

Bakuza, J., and G. Nkwengulila. 2009. Variation over Time in Parasite Prevalence Among Free-ranging Chimpanzees at Gombe National Park, Tanzania. International Journal of Primatology **30**:43-53.

Bakuza, J. S. 2012. Epidemiology of Schistosoma mansoni infection in sympatric humans and non-human primates in the Gombe ecosystem Tanzania. University of Glasgow.

Bartram, J., K. Lewis, R. Lenton, and A. Wright. Focusing on improved water and sanitation for health. The Lancet **365**:810-812.

Bidaisee, S., and C. N. L. Macpherson. 2014. Zoonoses and One Health: A Review of the Literature. Journal of Parasitology Research **2014**:8.

Bodager, J. R., M. B. Parsons, P. C. Wright, F. Rasambainarivo, D. Roellig, L. Xiao, and T. R. Gillespie. 2015. Complex epidemiology and zoonotic potential for Cryptosporidium suis in rural Madagascar. Veterinary parasitology **207**:140-143.

Calvignac-Spencer, S., S. Leendertz, T. Gillespie, and F. Leendertz. 2012. Wild great apes as sentinels and sources of infectious disease. Clinical Microbiology and Infection **18**:521-527.

Cama, V. A., J. M. Ross, S. Crawford, V. Kawai, R. Chavez-Valdez, D. Vargas, A. Vivar, E. Ticona, M. Navincopa, J. Williamson, Y. Ortega, R. H. Gilman, C. Bern, and L. Xiao. 2007. Differences in clinical manifestations among Cryptosporidium species and subtypes in HIV-infected persons. J Infect Dis **196**:684-691.

Cegielski, J. P., Y. R. Ortega, S. McKee, J. F. Madden, L. Gaido, D. A. Schwartz, K. Manji, A. F. Jorgensen, S. E. Miller, U. P. Pulipaka, A. E. Msengi, D. H. Mwakyusa, C. R. Sterling, and L. B. Reller. 1999. Cryptosporidium, Enterocytozoon, and Cyclospora Infections in Pediatric and Adult Patients with Diarrhea in Tanzania. Clinical Infectious Diseases **28**:314-321.

Chapman, C. A., T. R. Gillespie, and T. L. Goldberg. 2005. Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? Evolutionary Anthropology **14**:134-144.

Chapman, C. A., M. L. Speirs, T. R. Gillespie, T. Holland, and K. M. Austad. 2006. Life on the edge: Gastrointestinal parasites from the forest edge and interior primate groups. American Journal of Primatology **68**:397-409.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **356**:991-999.

Crump, J. A., H. O. Ramadhani, A. B. Morrissey, W. Saganda, M. S. Mwako, L.-Y. Yang, S.-C. Chow, S. C. Morpeth, H. Reyburn, B. N. Njau, A. V. Shaw, H. C. Diefenthal, J. F. Shao, J. A. Bartlett, and V. P. Maro. 2011. Invasive Bacterial and Fungal Infections Among Hospitalized HIV-Infected and HIV-Uninfected Adults and Adolescents in Northern Tanzania. Clinical Infectious Diseases **52**:341-348.

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

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

Dhama, K., S. Chakraborty, S. Kapoor, R. Tiwari, A. Kumar, R. Deb, S. Rajagunalan, R. Singh, K. Vora, and S. Natesan. 2013. One world, one health-veterinary perspectives. Adv. Anim. Vet. Sci **1**:5-13.

Earnhardt JM, R. S., Lonsdorf EV, Pusey AE. 2003. A demographic comparison of wild chimpanzees from Gombe and a managed population from North American zoos. American Journal of Primatology **60**:2.

File, S. K., W. McGrew, and C. E. Tutin. 1976. The intestinal parasites of a community of feral chimpanzees, Pan troglodytes schweinfurthii. The Journal of parasitology:259-261.

Finkbeiner, S. R., A. F. Allred, P. I. Tarr, E. J. Klein, C. D. Kirkwood, and D. Wang. 2008. Metagenomic analysis of human diarrhea: viral detection and discovery. PLoS pathogens **4**:e1000011.

Frank, D. N., and N. R. Pace. 2008. Gastrointestinal microbiology enters the metagenomics era. Current opinion in gastroenterology **24**:4-10.

Gibbs, E. P. J. 2014. The evolution of One Health: a decade of progress and challenges for the future. Veterinary Record **174**:85-91.

Gillespie, T. R., E. V. Lonsdorf, E. P. Canfield, D. J. Meyer, Y. Nadler, J. Raphael, A. E. Pusey, J. Pond, J. Pauley, T. Mlengeya, and D. A. Travis. 2010. Demographic and Ecological Effects on Patterns of Parasitism in Eastern Chimpanzees (Pan troglodytes schweinfurthii) in Gombe National Park, Tanzania. American Journal of Physical Anthropology **143**:534-544.

Goodall, J. 1983. Population Dynamics during a 15 Year Period in one Community of Free-living Chimpanzees in the Gombe National Park, Tanzania. Zeitschrift für Tierpsychologie **61**:1-60.

Goodall, J. 1986. The Chimpanzees of Gombe: Patterns of Behavior Bellknap Press of the Harvard University Press Boston.

Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1997. Recovery of waterborne oocysts of Cryptosporidium from water samples by the membrane-filter dissolution method. Parasitology Research **83**:121-125.

Holt, R. D., A. P. Dobson, M. Begon, R. G. Bowers, and E. M. Schauber. 2003. Parasite establishment in host communities. Ecology Letters **6**:837-842.

Hugenholtz, P., and G. W. Tyson. 2008. Microbiology: metagenomics. Nature **455**:481-483.

King, D., C. Peckham, J. Waage, J. Brownlie, and M. E. Woolhouse. 2006. Infectious diseases: preparing for the future. SCIENCE-NEW YORK THEN WASHINGTON- **313**:1392.

Köndgen, S., H. Kühl, P. K. N'Goran, P. D. Walsh, S. Schenk, N. Ernst, R. Biek, P. Formenty, K. Mätz-Rensing, and B. Schweiger. 2008. Pandemic human viruses cause decline of endangered great apes. Current Biology **18**:260-264.

Kosoy, M. 2013. Deepening the conception of functional information in the description of zoonotic infectious diseases. Entropy **15**:1929-1962.

Kotloff, K. L., J. P. Nataro, W. C. Blackwelder, D. Nasrin, T. H. Farag, S. Panchalingam, Y. Wu, S. O. Sow, D. Sur, R. F. Breiman, A. S. G. Faruque, A. K. M. Zaidi, D. Saha, P. L. Alonso, B. Tamboura, D. Sanogo, U. Onwuchekwa, B. Manna, T. Ramamurthy, S. Kanungo, J. B. Ochieng, R. Omore, J. O. Oundo, A. Hossain, S. K. Das, S. Ahmed, S. Qureshi, F. Quadri, R. A. Adegbola, M. Antonio, M. J. Hossain, A. Akinsola, I. Mandomando, T. Nhampossa, S. Acácio, K. Biswas, C. E. O'Reilly, E. D. Mintz, L. Y. Berkeley, K. Muhsen, H. Sommerfelt, R. M. Robins-Browne, and M. M. Levine. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet **382**:209-222.

Levy, S., and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med **10**:S122 - S129.

Lonsdorf, E., C. Murray, E. Lonsdorf, D. Travis, I. Gilby, J. Chosy, J. Goodall, and A. Pusey. 2011. A Retrospective Analysis of Factors Correlated to Chimpanzee (Pan troglodytes schweinfurthii) Respiratory Health at Gombe National Park, Tanzania. EcoHealth **8**:26-35.

Lonsdorf, E. V., D. Travis, A. E. Pusey, and J. Goodall. 2006. Using retrospective health data from the Gombe chimpanzee study to inform future monitoring efforts. American Journal of Primatology **68**:897-908.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. van der Giessen, and H. Kruse. 2010. Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge. International Journal of Food Microbiology **139**:S3-S15.

Nizeyi, J. B., R. B. Innocent, J. Erume, G. Kalema, M. R. Cranfield, and T. K. Graczyk. 2001. Campylobacteriosis, salmonellosis, and shigellosis in free-ranging human-habituated mountain gorillas of Uganda. Journal of Wildlife Diseases **37**:239-244.

Nizeyi, J. B., R. Mwebe, A. Nanteza, M. R. Cranfield, G. Kalema, and T. K. Graczyk. 1999. Cryptosporidium sp and Giardia sp infections in mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. Journal of Parasitology **85**:1084-1088.

Noss, R. F., M. O'Connell, and D. D. Murphy 1997. The science of conservation planning: habitat conservation under the Endangered Species Act. Island Press.

Osburn, B., C. Scott, and P. Gibbs. 2009. One world—one medicine—one health: Emerging veterinary challenges and opportunities. Revue scientifique et technique **28**:481.

Parsons MB, G. T. L., Travis D, Lipende L, Gilagiza B, Kamenya S, Pintea L, Vazquez-Prokopec GM. 2014. Global Positioning System Data-Loggers: A Tool to Quantify Fine-Scale Movement of Domestic Animals to Evaluate Potential for Zoonotic Transmission to an Endangered Wildlife Population. Plos ONE.

Plowright, R. K., S. H. Sokolow, M. E. Gorman, P. Daszak, and J. E. Foley. 2008. Causal inference in disease ecology: investigating ecological drivers of disease emergence. Frontiers in Ecology and the Environment **6**:420-429.

Pusey, A. E., M. L. Wilson, and D. A. Collins. 2008. Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:738-744.

Rewell, R., and R. Bridges. 1948. An outbreak of Shigella flexneri infection among rheus monkeys. Monthly bulletin of the Ministry of Health and the Public Health Laboratory Service **7**:25-29.

Rist, C. L., C. S. Arriola, and C. Rubin. 2014. Prioritizing Zoonoses: A Proposed One Health Tool for Collaborative Decision-Making. PLoS ONE **9**:e109986.

Rüegg, W. 2004. A history of the university in Europe: Volume 3, universities in the nineteenth and early twentieth centuries (1800–1945). Cambridge University Press.

Schloss, P. D., and J. Handelsman. 2005. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. Genome biology **6**:229.

Scott, M. E. 1988. The Impact of Infection and Disease on Animal Populations: Implications for Conservation Biology. Conservation Biology **2**:40-56.

Soper, F. L. 1937. The Newer Epidemiology of Yellow Fever. American Journal of Public Health and the Nations Health **27**:1-14.

Sterling, E., N. Bynum, and M. Blair 2013. Primate Ecology and Conservation. Oxford University Press.

Stern, N. J., and S. Kazmi. 1989. Campylobacter jejuni. Foodborne bacterial pathogens **3**:71-110.

Struhsaker, T. T. 1981. Forest and primate conservation in East Africa. African Journal of Ecology **19**:99-114.

Taylor, L. H., S. M. Latham, and E. Mark. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London B: Biological Sciences **356**:983-989.

Team, W. E. R. 2014. Ebola virus disease in West Africa—the first 9 months of the epidemic and forward projections. N Engl J Med **371**:1481-1495.

Travis, D. A., E. V. Lonsdorf, T. Mlengeya, and J. Raphael. 2008. A science-based approach to managing disease risks for ape conservation. American Journal of Primatology **70**:745-750.

Vourc'h, G., O. Plantard, and S. Morand. 2012. How does biodiversity influence the ecology of infectious disease? Pages 291-309. New frontiers of molecular epidemiology of infectious diseases. Springer.

Wang, L., H. Zhang, X. Zhao, L. Zhang, G. Zhang, M. Guo, L. Liu, Y. Feng, and L. Xiao. 2013. Zoonotic Cryptosporidium Species and Enterocytozoon bieneusi Genotypes in HIV-Positive Patients on Antiretroviral Therapy. Journal of Clinical Microbiology **51**:557-563.

Williams, J. M., E. V. Lonsdorf, M. L. Wilson, J. Schumacher-Stankey, J. Goodall, and A. E. Pusey. 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**:766-777.

Wise, R. 2002. Antimicrobial resistance: priorities for action. Journal of Antimicrobial Chemotherapy **49**:585-586.

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

Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science **292**:1109-1112.

Xiao, L. H., and Y. Y. Feng. 2008. Zoonotic cryptosporidiosis. Fems Immunology and Medical Microbiology **52**:309-323.

Zhang, T., M. Breitbart, W. H. Lee, J.-Q. Run, C. L. Wei, S. W. L. Soh, M. L. Hibberd, E. T. Liu, F. Rohwer, and Y. Ruan. 2005. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS biology **4**:e3