Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

______________________________ _______________

Emily Strahan Date

A cross-comparison of enteric pathogen prevalence and patterns of infection within and between wild-living Cross River gorillas (*Gorilla gorilla diehli*) and eastern chimpanzees (*Pan troglodytes schweinfurthii*)

By

Emily Strahan
Master of Science

Environmental Science

____
Thomas Gillespie, Ph.D.
Advisor

____
Lance Gunderson, Ph.D.
Committee Member

____
Uriel Kitron, Ph.D.
Committee Member

Accepted:

____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

____
Date

A cross-comparison of enteric pathogen prevalence and patterns of infection within and between wild-living Cross River gorillas (*Gorilla gorilla diehli*) and eastern chimpanzees (*Pan troglodytes schweinfurthii*)

By

Emily Strahan
B.S.F.R., University of Georgia, 2017

Advisor: Thomas Gillespie, Ph.D.

An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Environmental Science
2020

Abstract

A cross-comparison of enteric pathogen prevalence and patterns of infection within and between wild-living Cross River gorillas (*Gorilla gorilla diehli*) and eastern chimpanzees (*Pan troglodytes schweinfurthii*)
By: Emily Strahan

Emerging infectious diseases represent a serious threat to biodiversity conservation and global health. This is of particular concern for the great ape species, whose close evolutionary relatedness to humans puts them at high risk for cross-species transmission events. As humans and great apes increasingly come into contact with one another, the potential for pathogen exchange and disease emergence is heightened—threatening both great ape conservation and human health. Given what we know about shared susceptibility and pathogen transmission between humans and great apes, it is vital to understand baseline information on what pathogens exist in wild ape populations. Few studies have broadly investigated the pathogen communities of great apes, and those that have often tend to be limited in scope. The goals of this study were therefore to survey wild Cross River gorillas (*Gorilla gorilla diehli*, critically endangered) and eastern chimpanzees (*Pan troglodytes schweinfurthii,* endangered) for an array of viral, parasitic, and bacterial enteric pathogens. A novel real-time PCR diagnostic platform, The TaqMan Array Card, was used to noninvasively screen fecal samples for 39 enteric pathogen-specific gene targets in these two great ape subspecies. Pathogen prevalence, as well as patterns of infection and coinfection, were compared between species and within species among gorilla and chimpanzee populations. All gorilla individuals and approximately 70% of chimpanzee individuals were infected with at least one enteric pathogen. Of the infected gorillas, 44% showed single infections and 56% showed coinfections. Of the infected chimpanzees, 51% showed single infections and 49% showed coinfections. Adenovirus and *Cryptosporidium parvum* were the most common pathogens detected in both species. Proportionally, gorillas harbored more parasitic and bacterial infections than chimpanzees. These findings offer a comparative look into the pathogen profiles of a highly elusive and understudied great ape (the Cross River gorilla) and a habituated and extremely well-known great ape (the eastern chimpanzee). This study highlights the need for further research in order to better define health risks, monitor populations, and guide management actions to protect human and great ape health.

A cross-comparison of enteric pathogen prevalence and patterns of infection within and between wild-living Cross River gorillas (*Gorilla gorilla diehli*) and eastern chimpanzees (*Pan troglodytes schweinfurthii*)

By

Emily Strahan
B.S.F.R., University of Georgia, 2017

Advisor: Thomas Gillespie, Ph.D.

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Environmental Science
2020

Acknowledgements

I would like to sincerely thank my advisor, Dr. Thomas R. Gillespie, for providing guidance and support during this research. This project would not have been possible without his constant motivation, knowledge and expertise.

I would also like to thank Dr. Lance Gunderson and Dr. Uriel Kitron for serving as my thesis committee members. They were always available for questions, feedback and support, and largely contributed to a well-rounded and dependable thesis committee.

I wish to express my gratitude and appreciation to the CHAMPS Team at the Centers for Disease Control and Prevention (CDC) for allowing me to utilize their molecular lab and novel TaqMan Array Card testing platform. I would like to give a special thanks to Jacob Witherbee for devoting his time and effort to assist me in running and analyzing samples. He provided invaluable training for use of the TAC platform and assisted with data analysis.

A special thanks to Dr. Julie Clennon in the Rollins School of Public Health at Emory University (Atlanta, Georgia) for providing her time and assistance in the statistical analyses of my data. I would also like to thank Michele Parsons at the CDC for her support and providing insight to the findings from my study.

I thank the Gombe Ecosystem Health Project (Thomas Gillespie and Elizabeth Lonsdorf, co-directors) for coordinating the fieldwork that produced the chimpanzee diagnostic samples used in this study. I also thank Beatrice Hahn for biobanking and coordinating shipment of samples. I thank Jane Goodall and the Jane Goodall Institute for permitting the use of long-term supplementary data. I am also grateful to the Tanzanian agencies TANAPA, TAWIRI, and COSTECH for allowing this research to be conducted in Gombe National Park, and to the Gombe Stream Research Center staff for their hard work and efforts to collect fecal samples and daily observational data.

I thank Richard Bergl and Wildlife Conservation Society Cameroon (especially Romanus Ikfuingei and Christopher Jameson) for coordinating the fieldwork that produced the gorilla diagnostic samples used in this study, I also thank Mimi Arandjelovic for biobanking and coordinating shipment of samples. I am also grateful to the Government of Cameroon and the Ministry of Forests and Wildlife for granting permission to conduct research at Kagwene Gorilla Sanctuary and Mone River Forest Reserve. A special thanks goes to the dog handlers Aimee Hurt and Ngaio Richards, and the Working Dogs for Conservation (Lily, Orbee and Wicket). who conducted a majority of the fieldwork. I also thank the many WCS and local field assistants who facilitated the surveys.

Lastly, I am extremely appreciative of the Department of Environmental Sciences at Emory University for awarding me the James G. Lester Research Scholarship. Further funding for this work was provided by the US Fish and Wildlife Great Ape Conservation Fund, the Arcus Foundation, the North Carolina Zoo, the Wildlife Conservation Society, the Leo S. Guthman Foundation, a Robert W. Woodruff Foundation Synergy II Award, and the National Institutes of Health (grant numbers: R01 AI58715, R00 HD057992).

Table of Contents

1. Introduction 1-7
    1.1. Emerging Infectious Diseases 1-2
    1.2. Disease in Great Ape Conservation 2
    1.3. Great Ape Pathogen Surveillance 3-5
    1.4. Cross River Gorilla 5-6
    1.5. Eastern Chimpanzee 6-7

2. Goals and Hypothesis 7

3. Materials and Methods 7-16
    3.1. Cross River Gorilla Study Site 7-9
    3.2. Eastern Chimpanzee Study Site 9-10
    3.3. Sample Collection 11-12
    3.4. TaqMan Array Card Diagnostic Platform 12-13
    3.5. Molecular Analysis 13-14
    3.6. Analysis 14-16

4. Results 16-19
    4.1. Infection and Pathogen Prevalence 16-17
    4.2. Patterns of Coinfection 17-19

5. Discussion 19-25

6. References 26-39

7. Tables 40-47
    7.1. Table 1 40
    7.2. Table 2 41
    7.3. Table 3 41
    7.4. Table 4 42
    7.5. Table 5 42
    7.6. Table 6 42
    7.7. Table 7 43
    7.8. Table 8 44
    7.9. Table 9 45
    7.10. Table 10 45
    7.11. Table 11 46
    7.12. Table 12 47

1. Introduction

1.1. Emerging Infectious Diseases

Emerging infectious diseases are a growing and serious concern for global and ecosystem health. Over 75% of emerging infectious diseases are zoonotic—that is, transmissible from animals to humans (Jones et al., 2008; Taylor et al., 2001). Their emergence frequently entails complex interactions among people, wildlife, and livestock in fast-changing environments (Jones et al., 2013; Karesh et al., 2012; Wolfe et al., 2007). As the human population continues to expand, so too does the human-animal interface, further increasing the probability of cross-species transmission and spillover events (Parrish et al., 2008; Streicker et al., 2010). The risk of zoonotic disease emergence is especially high in tropical regions (Allen et al., 2017). Other risk factors include areas of high human population density and wildlife biodiversity (Jones et al., 2008; Keesing et al., 2010; Morse, 1995) and those experiencing human-induced land use changes related to agricultural or logging practices (Allen et al., 2017; Faust et al., 2018; McFarlane et al., 2013; Patz et al., 2004; Weiss & McMichael, 2004).

The nature and frequency of human-wildlife interaction in tropical forest communities have changed drastically over the last few decades as human encroachment on wildlife habitats, research, ecotourism, and other activities have brought people and animals into close proximity or direct contact (Adams et al., 2001). These dynamics are especially prevalent between human and wild nonhuman primate populations, as rampant deforestation and fragmentation have forced primates out of their natural and often protected habitat into isolated patches of farmland, pastures, and human settlements (Chapman & Onderdonk, 1998; Marsh, 2003). Human-primate contact can also occur as local people engaged in subsistence living venture into primate habitat to harvest and extract natural resources (Gillespie & Chapman, 2006; Parsons et al., 2015). As

land becomes increasingly shared by humans, primates, and also domestic animals, opportunities arise for both anthroponotic and zoonotic pathogen exchange and exposure. Further exacerbating the potential for cross-species disease transmission is the close phylogenetic relatedness between humans and primates—especially the African great ape species (Calvignac-Spencer et al., 2012; Gilardi et al., 2015; Gillespie et al., 2008).

1.2. Disease in Great Ape Conservation

A rapidly expanding human-primate interface has made it increasingly apparent that disease plays an important role in great ape health and conservation (Gilardi et al., 2015; Gillespie et al., 2008). In fact, infectious disease is now listed among the top three threats to some great ape taxa, alongside poaching and habitat loss. Evidence suggests that pathogens such as Ebola virus (Leendertz et al., 2017; Leroy et al., 2004)—known to have decimated gorilla and chimpanzee populations—Anthrax (Hoffmann et al., 2017; Leendertz et al., 2006a), Simian immunodeficiency virus (SIV) (Keele et al., 2009), and a variety of human respiratory viruses (Kondgen et al., 2008; Palacios et al., 2011) pose potential risks to great ape individuals and populations. Studies have also shown the ease of wild ape exposure to enteric bacterial and parasitic pathogens from human populations in close proximity to parks and from tourism and research activities (Deere, 2019; Parsons et al., 2015, Rwego et al., 2008). It is important to note, however, that these pathogens and several others are transmitted through various routes and directions of exchange (Wolfe et al., 1998). Just as pathogens spreading from humans and domesticated animals to primates is a conservation concern (Chi et al., 2007; Gillespie & Chapman, 2006, 2008; Nizeyi et al., 2002; Sapolsky & Else, 1987), the reverse transmission path is also a serious threat to human health (Gao et al., 1999; Calvignac-Spencer et al., 2012).

1.3. Great Ape Pathogen Surveillance

Although there has been an increase in awareness surrounding the threat of zoonoses on human and great ape populations, our current understanding of wild ape health and the pathogens they routinely carry is minimal (Gillespie et al., 2008; Travis et al., 2018). Further complicating this matter are the few available diagnostic tools and validated noninvasive techniques for health research and surveillance in remote, resource-scarce environments (Gillespie et al., 2008; Travis et al., 2018). Efforts to overcome these obstacles have primarily focused on primate populations that are closely monitored for long-term research (e.g. chimpanzees of Gombe National Park) and tourism—skewing most of what we know about wild primate pathogens towards a single species and/or specific pathogen or pathogen group. Respiratory pathogens for instance, have long been recognized as a significant cause of morbidity and mortality among great apes, most notably in chimpanzees (Goodall, 1983, 1986; Sugiyama, 2004; Williams et al., 2008). Enteric pathogens on the other hand, are less understood both in terms of potential exposure and typical baseline profiles in wild great ape populations (Gillespie et al., 2010; McLennan et al., 2018).

Growing evidence suggests a link between anthropogenic activity and human-primate pathogen transmission, but gaps still exist in our knowledge of baseline pathogen diversity and distribution in great ape populations (Bublitz et al., 2015; Gillespie et al., 2010; Goldberg et al., 2007, 2008; McLennan et al., 2018; Rwego et al., 2008; Zhody et al., 2015). One particular study found that even in the most well-studied primate species, only about half of all micro- and macro-organisms are documented (Cooper & Nunn, 2013). Establishing baseline data of primate pathogens can be critical in providing site-specific indices of population health and assessing and managing disease threats to great apes and humans (Leendertz et al., 2006a, b; Travis et al., 2018). As human-great ape contact continues to increase, it is vital that we gain a better

understanding of which pathogens are naturally occurring or human-induced in great ape populations, what conditions promote pathogen exposure and transmission, and how certain pathogens might impact conservation and disease-monitoring efforts on a broader scale.

In order to improve our understanding of what enteric pathogens great apes are typically exposed to, I noninvasively examined two wild African great ape subspecies: 1) the Cross River gorilla (*Gorilla gorilla diehli*) and 2) the eastern chimpanzee (*Pan troglodytes schweinfurthii*). These two species—also our closest living relatives—share a higher proportion of pathogens with humans and are promising candidates for zoonotic pathogen screening (Calvignac-Spencer et al., 2012). Gorillas and chimpanzees also happen to be some of the slowest reproducing animals on earth, which makes them especially vulnerable to population declines (Genton et al., 2012) and of critical importance for future conservation and disease research. By cross-comparing pathogen profiles of one of the most endangered and elusive great apes—the Cross River gorilla—with one of the best understood and highly studied great apes—the eastern chimpanzee—I am able to investigate the diversity of pathogens among individual primates and variation of pathogens across species.

In this study, I present the first enteric pathogen survey of Cross River gorillas and aim to better understand the similarities and differences of pathogen communities found in two distinct, yet closely related species. I will also investigate differences in infection prevalence and coinfection between and within species. This comparative approach can provide insight into how patterns of infection might be influenced by human habituation, as well as by variation in host traits across species (e.g. diet, terrestrial use and body mass) rather than within species (Gillespie et al., 2008). I also utilize the diagnostic platform, The TaqMan® Array Card (TAC, Life Technologies, Carlsbad, CA), to screen fecal samples from individuals for 39 unique enteric

pathogen targets (Table 1). These targets are pathogen-specific genes typically associated with either virulence or biology (i.e. specific outer membrane protein genes or housekeeping genes). To my knowledge, this is the first study to test wildlife specimens on this platform.

1.4. Cross River Gorilla

The Cross River gorilla, one of two subspecies of the Western Gorilla (*Gorilla gorilla*), is the least studied and most threatened of the African great apes and listed as critically endangered by the International Union for Conservation of Nature (Etiendem et al., 2013; IUCN, 2015; Robbins & Robbins, 2018). Having only been discovered in the early 20th century, a majority of Cross River gorilla research has focused primarily on their abundance and distribution (Dunn et al., 2014). The less than 300 individuals that remain in the wild occupy approximately 600 km^2 of severely fragmented habitat along the Cameroon-Nigeria border (Arandjelovic et al., 2015; IUCN, 2015; Robbins & Robbins, 2018). Individuals are dispersed across 11-14 geographically distinct subpopulations, which typically range from 2-20 individuals (McFarland, 2007; Sunderland-Groves et al., 2009; Sawyer, 2012).

Groups mostly inhabit remote forested areas of high relief and dispersal is largely driven by avoidance of human activity (Dunn et al., 2014; Etiendem et al., 2013; Imong et al., 2014). Grouping and ranging behavior can also be attributed to the gorillas' markedly seasonal habitat (Dunn et al., 2014). Terrestrial herbs and tree bark are commonly consumed throughout the year, with diversified fruit consumption occurring during periods of increased availability (Etiendem & Tagg, 2013).

Despite legal protection in Nigeria and Cameroon, and designated protected area status across several of the localities where these gorillas occur, populations have continued to decline over the past decade (Etiendem et al., 2013; Thalmann et al., 2011). Some of the most imminent

threats populations face include poaching, habitat loss and fragmentation, and expanding human populations (Bergl et al., 2016; Dunn et al., 2014; Meder, 2015; Oates et al., 2003). Although cases of disease outbreaks have not yet been documented in this species, their close proximity to humans and domesticated animals, as well as their small and fragmented subpopulations, make them particularly vulnerable to the threat of emerging infectious diseases (Dunn et al., 2014).

1.5. Eastern Chimpanzee

The eastern chimpanzee, one of four recognized subspecies of the common chimpanzee (*Pan troglodytes),* is listed as Endangered by the IUCN and the most well-known and highly studied of any chimpanzee subspecies (Chapman et al., 1995; Goodall, 1986; Nishida, 1990; Reynolds, 2005; Wrangham et al., 1986, 1994). The vast majority of this subspecies reside in the Democratic Republic of Congo (Plumptre et al., 2010), with smaller numbers occurring mostly in western Uganda and Tanzania (Plumptre et al., 2003). They typically occupy lowland and submontane tropical forests, as well as savanna woodland habitat (IUCN, 2020). Eastern chimpanzees are omnivorous, with fruit constituting about half of their diet (IUCN, 2020). They also eat other plant parts, insects, and on occasion, small mammals (including other non-human primates) (IUCN, 2020; Nishida et al., 1983).

While the total population size of this subspecies is unknown due to a lack of surveying across large parts of their potential range, significant declines in numbers have occurred in the past 20-30 years (IUCN, 2015). Recent estimates suggest there may be about 200,000-250,000 individuals in the wild (Plumptre et al., 2010; Pusey et al., 2007). Chimpanzees are legally protected in their range countries, but often travel and live outside protected areas (IUCN, 2020). In Tanzania, for example, an estimated 60% of chimpanzees live outside protected areas (Moyer et al., 2006). Common threats populations face include hunting, habitat loss and fragmentation,

and disease (Plumptre et al., 2010). Though more numerous than the Cross River gorilla, chimpanzees occur in isolated, low density populations (20-150 individuals) across much of their range (Mitani & Watts, 2005). They also require large home ranges to sustain viable populations, which make them equally vulnerable to declines and mortality events (Plumptre et al., 2010).

2. Goals and Hypothesis

In this study, I aim to: 1) Provide a first-look into the enteric pathogen profiles of Cross River gorillas, 2) Broadly screen gorilla and chimpanzee fecal samples for enteric pathogens using a novel, noninvasive diagnostic platform, and 3) Compare patterns of pathogen prevalence, infection and coinfection between and within species.

I hypothesize the following: a) *between species*: Cross River gorillas are reclusive and unhabituated to human presence, whereas eastern chimpanzees at Gombe National Park have been habituated to researchers for about 60 years. Given these differences in the levels of human interaction, I hypothesize that chimpanzees will exhibit higher infection prevalence and levels of coinfection than gorillas. b) *within species*: Between the two populations of each species, I expect individuals living in populations characterized by greater accessibility and higher levels of human presence will exhibit higher infection prevalence and greater levels of coinfection.

3. Materials and Methods

3.1. Cross River Gorilla Study Site

Kagwene Gorilla Sanctuary (KGS) and Mone River Forest Reserve (MRFR) are gorilla conservation sites located in Cameroon (figure 1). KGS, established in 2008, is the longest running Cross River gorilla research site and has been continuously monitored by the Wildlife Conservation Society since 2002 (Dunn et al., 2014). It is located at the far eastern edge of the Mbulu Forest between 06° 05' 55" and 06° 08' 25" North and 09° 43' 35" and 09° 46' 35" East

(De Vere et al., 2011). The sanctuary covers approximately 19 km^2 of submontane and montane forest, reaching elevations over 2000 m—the highest altitude site at which this subspecies is found. A population of about 20-25 gorillas occupy this area (Arandjelovic et al., 2015; Ikfuingei, 2012). While a traditional ban on hunting and consumption of gorillas has protected the population from poaching, surrounding human settlements have significantly reduced viable habitat for these gorillas, threating their future status within the Sanctuary (De Vere et al., 2011; Dunn et al., 2014).

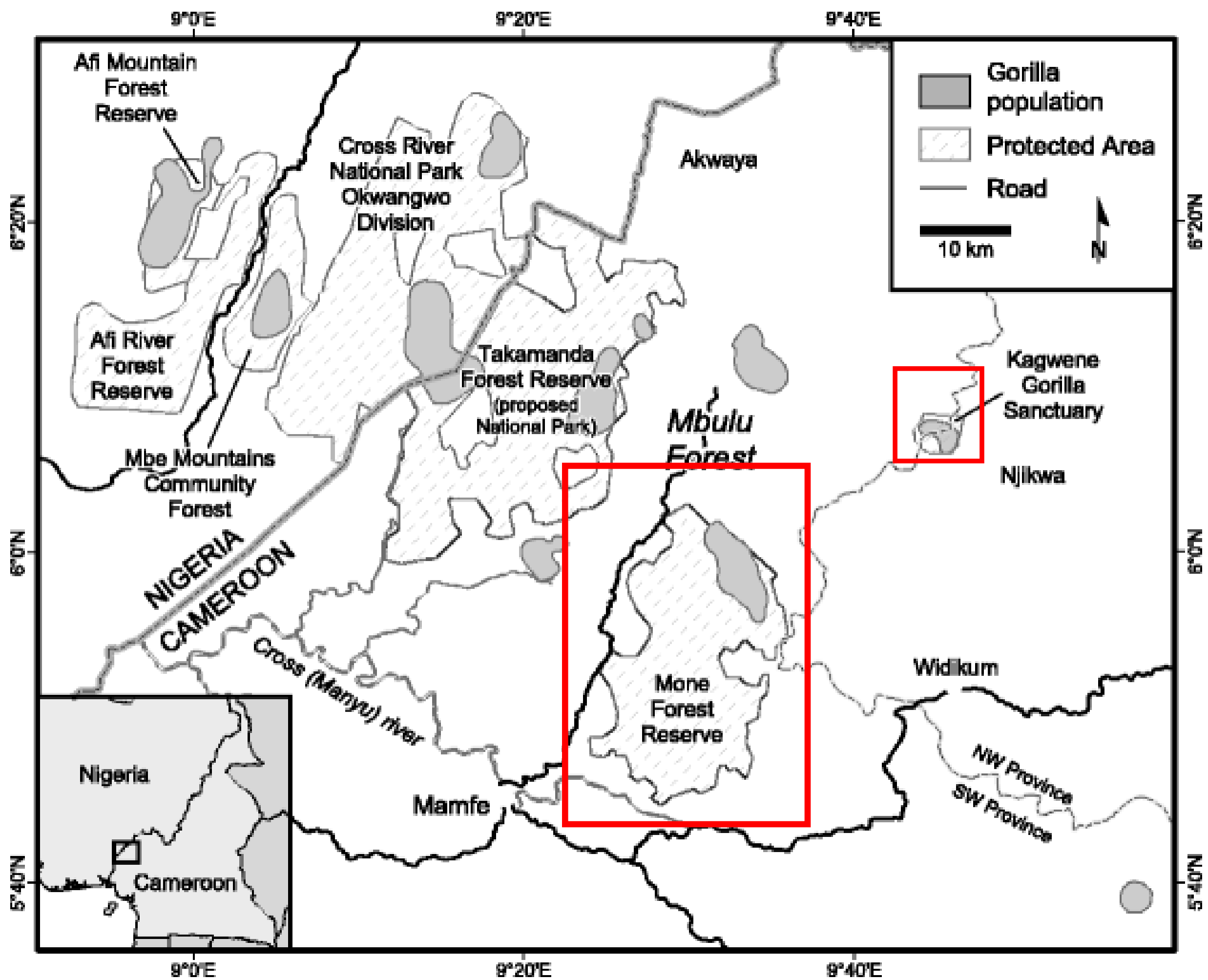

Figure 1. Location of Kagwene Gorilla Sanctuary and Mone Forest Reserve, Cameroon within the geographical range of Cross River gorillas (*Gorilla gorilla diehli*) (slightly modified from Sunderland-Groves et al., 2009).

The Mone River Forest Reserve is located southeast of the Takamanda Forest Reserve and covers approximately 538 km^2 of topographically diverse landscape (Arandjelovic et al., 2015; Dunn et al., 2014). The reserve, a site more characteristic of a typical gorilla locality, consists mostly of large contiguous forested areas across hilly terrain with elevations ranging from 350-1200 m (Arandjelovic et al., 2015; Oates et al., 2004). Having only confirmed the existence of gorillas at MRFR in 2000, population numbers and ranging behavior patterns are much less understood than those at KGS (Arandjelovic et al., 2015; Dunn et al., 2014). One estimate suggests the presence of about 20-30 individuals at this site (Dunn et al., 2014). While there are no human settlements within the boundaries of the reserve, no formal protection currently exists for hunting, timber exploitation, and resource extraction that occurs throughout the site.

3.2. Eastern Chimpanzee Study Site

Gombe National Park is located on the eastern shore of Lake Tanganyika in northwestern Tanzania (4°53′S, 29°38′E) (figure 2) (Gillespie et al., 2010). The park spans approximately 35 km^2 of mountainous landscape and is the site of the longest continuous field study—60 years of behavioral and ecological data collection—of wild chimpanzees in the world (Gillespie et al., 2010; Plumptre et al., 2010). Most of the park contains a series of steep-sided valleys that fall from a rift escarpment approximately 1,500 m above sea level. The valleys' lower slopes consist of evergreen and semideciduous forest, while the upper slopes contain a mosaic of thicket, woodland, and grassland vegetation (Goodall, 1986).

An estimated 100-115 chimpanzees reside in the park and are further divided among three recognized social communities that inhabit separate, but overlapping territories: the Mitumba Community, the Kasakela Community, and the Kalande Community (Gillespie et al.,

2010; Moyer et al., 2006; Wroblewski et al., 2015). Kasakela and Mitumba are both habituated and overlap slightly in habitat range (Pusey et al. 2007; Parsons et al., 2015). They differ, however, in the level of human encroachment (Pusey et al. 2008). The Kasakela community inhabits the central portion of the park in less disturbed forest, whereas the Mitumba community resides the park's northern range in close proximity to the village of Mwamgongo (4°40′S, 29°34'60′ E), home to ∼5000 inhabitants and their livestock (Parsons et al., 2015). Another large village occurs at the park's southern border. The park is managed by Tanzania National Parks and access is mostly restricted to researchers, ecotourists, park staff, and local field assistants (Gillespie et al., 2010; Plumptre et al., 2010).

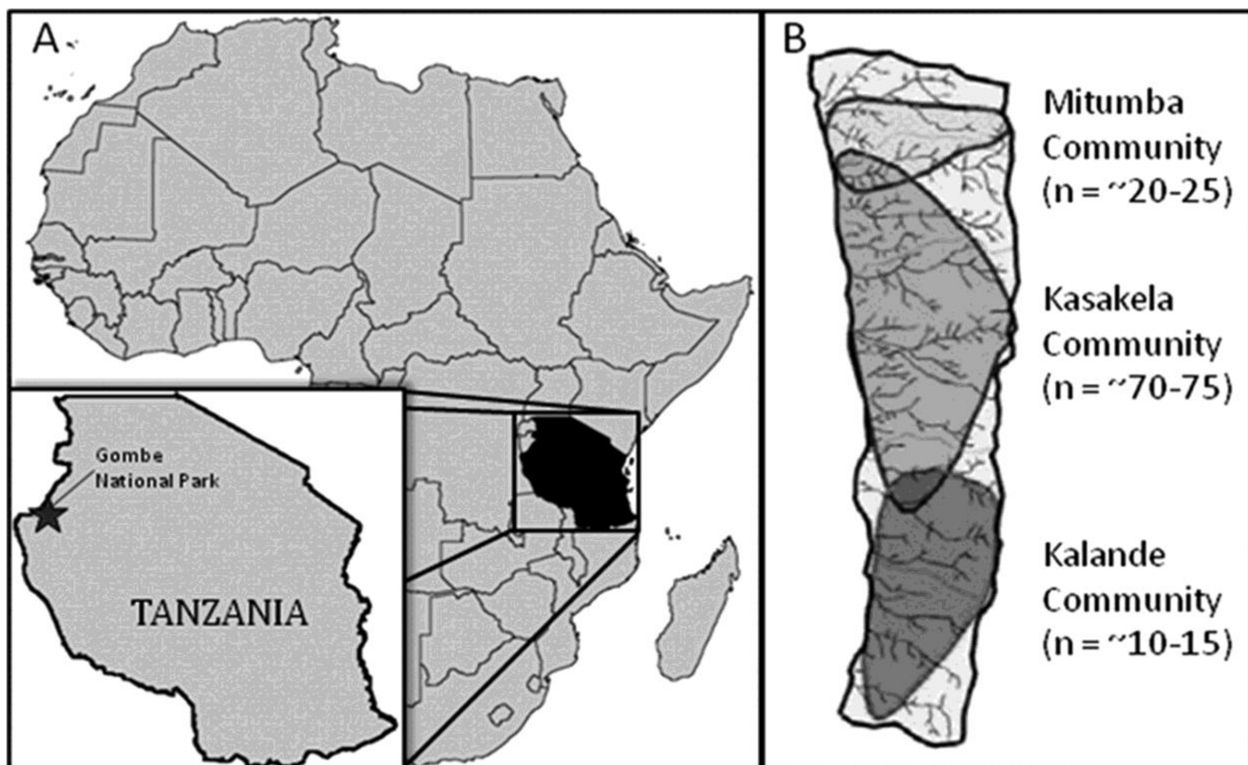

Figure 2. The eastern chimpanzee (*(Pan troglodytes schweinfurthii*) study site in Gombe National Park, Tanzania. A: Relative location of Gombe National Park within Africa and Tanzania. B: Ranges of the three chimpanzee groups of Gombe; Mitumba, Kasekela, and Kalande (n = chimpanzee community size) (Gillespie et al., 2010).

3.3. Sample Collection

Between December 2011 and January 2012, 58 fresh fecal samples (1-3 days old) were noninvasively collected from 18 individual gorillas across both study sites (KGS and MRFR) following the protocol outlined in Arandjelovic et al. (2015). The samples screened in this study are based on repeated sampling of these individuals: 10 individuals from KGS (i.e. ~50% of the population) and 8 individuals from MRFR (i.e. ~ 35-40% of the population). Samples were collected at nest sites or along trails by Working Dogs for Conservation and the WCS monitoring team. Multiple aliquots were prepared from each sample collection. One used a two-step ethanol-silica procedure outlined by Nsubuga et al. (2004), which were used to identify individual gorilla sample donors via fecal DNA extract and microsatellite genotyping as reported in Arandjelovic et al. (2015). An additional aliquot was added to an equivalent volume (roughly 20-25 mL) of RNA*later*® (Ambion, Austin, TX), and stored at ambient temperature until shipment to the United States (stored at−80°C thereafter).

Between September 2016 and February 2018, fresh fecal samples were noninvasively collected from 56 individual chimpanzees following the protocol outlined in Wroblewski et al. (2015). The samples screened in this study are based on single samples from those 56 individuals (i.e. ~ 50% of the population). Samples were collected from each of the three communities that reside within the park: 38 samples from the Kasakela Community, 15 samples from the Mitumba Community, and 3 samples from the Kalande Community. Given the fission-fusion dynamics of chimpanzee communities, individuals could not be followed only a daily basis. To account for this, the most recent sample from each individual was provided for use in this study. Samples were collected as soon as possible following defecation, placed in an equivalent volume (roughly 20-25 mL) of RNA*later*® (Ambion, Austin, TX), and frozen and stored in the Gombe National

Park field lab until shipment to the United States (stored at−80°C thereafter). Identification of individual chimpanzee sample donors was confirmed via fecal DNA extract using a variety of methods such as PCR-based sex determination (Sullivan et al., 1993; Wroblewski et al., 2009) and microsatellite genotyping (Constable et al., 2001; Liu et al., 2008; Rudicell et al., 2010; Wroblewski et al., 2009) (procedures further described in the respective references).

3.4. TaqMan Array Card Diagnostic Platform

The TaqMan Array Card is a novel testing platform that uses real-time PCR to simultaneously screen multiple specimens for multiple pathogens (Diaz et al., 2013). These microfluidic cards consist of 384 wells, each with primers and probes for a specific pathogen. The Centers for Disease Control and Prevention (CDC, Atlanta, Georgia)—the site of technical and expert support for this technology—has validated 125 targets that can be customized to create a multipathogen panel, such as those for respiratory pathogens (Kodani et al., 2011), enteropathogens (Liu et al., 2013), or biothreat agents (Rachwal et al., 2012; Weller et al., 2012). TAC can be used to rapidly determine etiologies of disease outbreaks and also for large-scale, population-based disease surveillance (Liu et al. 2013; Vernet et al., 2011).

The TAC platform is ideal for multiple-pathogen detection and offers several advantages over traditional PCR assays: (1) Specimens can be tested for multiple pathogens (up to 40 on one card) within 3 hours, about a sixth of the time required if using traditional methods, (2) The closed-system design is simple to use and requires fewer steps, which minimizes potential for contamination or other operator error that may lead to inaccurate results, (3) The platform requires relatively small amounts of specimen for testing, and (4) Target tests are replicated, increasing confidence in data interpretation and validity of results (CDC, 2017; Diaz et al., 2019). This technology can provide a snapshot in time of what pathogens and/or disease

etiologies might be circulating in a given population, allowing labs to customize future surveillance efforts based on presence /absence, rather than continuously screen for large numbers of pathogens.

Despite these advantages, however, there are also some limitations that come with using TAC. One in particular is that pathogen targets are chosen a priori, which creates a potential bias toward detections. Another challenge is the lack of commercial availability of complete TAC configurations; up-front production and quality control testing are often labor-intensive and expensive for the buyer (Diaz et al., 2013). Lastly, the ability to screen for multiple pathogens and use small reaction volumes might come at the expense of overall sensitivity and detection rates (although this can be mitigated by running replicate reactions) (Rachwal et al., 2012; Diaz et al., 2019).

3.5. Molecular Analysis

DNA and RNA were extracted from each fecal specimen and analyzed in a molecular testing laboratory at the CDC. Samples were prepared for total nucleic acid extraction using the Child Health and Mortality Prevention Surveillance (CHAMPS) Rectal Swab/Stool Specimens Extraction Standard Operating Procedure (SOP 7.1.2.3; Version 1.1; May 2019). In brief, 400 µL of prepared fecal specimen (1mL Phosphate-buffered saline solution: 100mg feces) was added to VWR® 2 mL Pre-Filled Bead Tubes, each containing reagents of specified amounts (e.g. TE buffer, Roche Bacterial Lysis Buffer, MS2 phage dilution, Proteinase K). Bead-beating tubes were incubated at 56°C for 15 minutes, then homogenized at 5,000 rpm (2x1 min), and centrifuged at 10,000xg for 1 min. 700 µL of supernatant was extracted and eluted in 100 µL using the Roche MagNA Pure Compact Extractor (Roche Diagnostics, Indianapolis, Indiana)

with Nucleic Acid Isolation Kit I and Total NA Plasma External Lysis protocol (Diaz et al., 2013).

Following the extractions, TAC assays were performed using the CHAMPS TaqMan Array Card Testing Standard Operating Procedure (SOP 7.1.1). In brief, cards were loaded by mixing 50 µL TNA extract with 50 µL enzyme mix (qScript XLT 1-step RT-qPCR ToughMix). The reaction mix was then loaded into the fill port of each channel. Each card consisted of a no template control, positive control, and six samples. Cards were centrifuged at 1,200 rpm (2x1 min) sealed, and run on the Applied Biosystems ViiA7 Real-Time PCR system (Life Technologies, Foster City, CA, USA) with the following cycling conditions: 45°C for 10 minutes, 94°C for 10 minutes, 45 cycles of 94°C for 30 seconds, and 60°C for 60 seconds (Diaz et al., 2019).

3.5. Analysis

QuantStudio 7 Flex Real-Time PCR Systems Software and the TaqMan Array Card Supplemental Analysis Standard Operating Procedure (SOP 7.1.2; Version 1.0; May 2019) were used to analyze each card's results; assign positive, negative, or indeterminate comments for each target replicate, internal control, positive control and no-template control on the card; and export results for each replicate, associated cycle threshold (Ct) values, and other data associated with the run (Diaz et al., 2019).

Overall infection prevalence was calculated as the proportion of individuals carrying any pathogen divided by the total number of individuals sampled for that species or population. Pathogen prevalence was calculated as the proportion of individuals carrying pathogen *x* divided by the total number of individuals sampled for that species or population. Coinfection was calculated as the proportion of individuals infected with two or more pathogens divided by the

total number of individuals sampled for that species or population. Coinfection by number of pathogens was determined by dividing the number of infected individuals with *x* number of pathogens by the total number of coinfected individuals for that species or population.

Statistical analyses were performed in IBM SPSS Statistics 26.0 (SPSS Inc., Armonk, NY). Analyses for between-species comparisons assessed all individuals (n = 18 for Cross river gorillas; n = 56 for eastern chimpanzees). Analyses for within-species comparisons assessed all gorilla individuals and only those from the two habituated chimpanzee communities of Kasakela (n = 38) and Mitumba (n = 15).

All *E. coli* pathotypes (enterotoxigenic (heat-stable (ST) and -labile (LT) enterotoxins; enteropathogenic (*bfpA* and *eae* genes); and enteroaggregative (*aaiC* and *aatA* genes)), though distinct targets, were grouped together as "diarrheagenic *E. coli*" for statistical analysis (M. Parsons, personal communication, March 11, 2020). The *E. coli*/*Shigella* (*ipaH* gene) target was not included in this grouping given the inability to distinguish between these highly-related species. While they can be distinguished by physiological and biochemical characteristics, screening for the presence of the *ipaH* gene alone cannot separate the two (van den Beld & Reubsaet, 2012). For infection and prevalence parameters, it was not necessary to differentiate between the diarrheagenic categories. Pathotypes were treated as distinct pathogens, however, when comparing coinfection by number of pathogens between and within species.

By constructing 2x2 contingency tables, Fisher's exact test of independence (two-tailed) was used to test for differences in percent infection prevalence and pathogen prevalence between and within species. I also tested for differences in the percentage of parasitic, viral, and bacterial infections between and within species. I evaluated whether coinfections of pathogen pairs within species were more frequent than would be expected by chance based on the assumption of

independent transmission (Table 2). In chimpanzees specifically, I looked at SIV status (provided by Gombe Stream Research Center staff) in relation to coinfection with *Cryptosporidium parvum* and adenovirus, common opportunistic pathogens in HIV-infected humans and SIV-infected primates (Wachtman & Mansfield, 2008). Values less than 0.05 were considered statistically significant.

4. Results

4.1. Infection and Pathogen Prevalence

All (100%) 18 gorillas tested positive for at least one enteric pathogen (Table 3), whereas 39 (70%) of the 56 chimpanzees tested positive for at least one pathogen (Table 8). In terms of overall infection prevalence, gorillas were more likely to be infected with any pathogen than chimpanzees ($p < 0.01$). Overall prevalence of infection between populations of both species did not differ ($p > 0.05$ for both comparisons) (Table 4 & 9). Adenovirus and *C. parvum* were the most common pathogens detected in both gorillas and chimpanzees, occurring in 39% and 67% of gorillas and 52% and 13% of chimpanzees, respectively (Table 3 & 8). Gorillas were more likely to be infected with *C. parvum* ($p < 0.01$) than chimpanzees and there was no difference in adenovirus infections between species ($p > 0.05$). There was also no difference in *C. parvum* infection or adenovirus infection between populations of both gorillas and chimpanzees ($p > 0.05$ for all comparisons). Among the other pathogens detected, gorillas were more likely to be infected with *Enterococcus faecalis* ($p < 0.01$) than chimpanzees. There was no difference between or within species for any other pathogens detected ($p > 0.05$ for all comparisons).

When pathogens were broadly grouped as parasitic, viral, or bacterial (Table 7 & 12), gorillas were more likely to be infected with parasitic ($p < 0.01$) and bacterial ($p < 0.05$) pathogens than chimpanzees. There was no difference between species in viral pathogen

infections ($p > 0.05$). There was also no difference within species for each group of pathogens ($p > 0.05$ for all comparisons).

4.2. Patterns of Coinfection

Of the 18 infected gorillas, eight (44%) showed single infections and ten (56%) showed coinfections (Table 5). *Cryptosporidium parvum* was detected in seven out of ten (70%) coinfected individuals (Table 7). Of the ten infected gorillas at Kagwene, six (60%) showed single infections and four (40%) showed coinfections (Table 6). Of the eight infected gorillas at Mone, two (25%) showed single infections and six (75%) showed coinfections (Table 6). Of the 39 infected chimpanzees, 20 (51%) showed single infections and 19 (49%) showed coinfections (Table 10). Adenovirus was detected in 17 of the 19 (90%) coinfected individuals (Table 12). Of the 27 infected chimpanzees in Kasakela, 12 (44%) showed single infections and 15 (56%) showed coinfections (Table 11). Of the ten infected chimpanzees in Mitumba, six (60%) showed single infections and four (40%) showed coinfections (Table 11).

Groups of pathogens and specific pathogens commonly associated with opportunistic infection and/or coinfection were compared within species. Coinfection with diarrheagenic *E.coli* (DEC) and other diarrhea-associated pathogens, such as adenovirus, *C. parvum*, and *E.coli*/*Shigella*, was assessed. Diarrheagenic *E.coli* was detected in five (28%) gorillas and nine (17%) chimpanzees. Adenovirus was detected in seven (39%) gorillas and 28 (53%) chimpanzees, *C. parvum* in 12 (67%) gorillas and seven (13%) chimpanzees, and *E.coli/Shigella* in two (11%) gorillas and two (4%) chimpanzees. Three (17%) gorillas tested positive for both DEC-adenovirus and DEC-*C. parvum* coinfections and one (6%) tested positive for DEC-*E.coli/Shigella* coinfection; expected frequencies of coinfection were 11% (~ 2 cases), 18% (~ 3 cases), and 3% (~ 1 case), respectively. Six (11%) chimpanzees tested positive for DEC-

adenovirus coinfection and one (2%) tested positive for both DEC-*C. parvum* and DEC-*E.coli/Shigella* coinfection; expected frequencies of coinfection were 9% (~ 5 cases), 2% (~1 case), and 0.6% (~ 0 cases), respectively. The coinfection frequencies that I detected in both species were not statistically different from the expected frequencies ($p > 0.05$ for all comparisons). This was also the case for all other pathogen-pathogen coinfection comparisons assessed within each species ($p > 0.05$ for all comparisons).

In chimpanzees, coinfection of SIV with *C. parvum* and adenovirus was compared. Eight (15%) individuals were positive for SIV, 7 (13%) for *C. parvum*, and 28 (53%) for adenovirus. No individuals showed SIV-*C. parvum* coinfection and four (8%) showed SIV-adenovirus coinfection. The expected frequency of SIV-*C. parvum* coinfection was 2% (~1 case) and 8% (~2 cases) for SIV-adenovirus coinfection, both of which did not differ from the observed frequencies ($p > 0.05$ for both comparisons).

Coinfections were also compared between viral, parasitic, and bacterial pathogens. Thirteen (25%) chimpanzees tested positive for parasitic infections, 32 (60%) for viral, and 12 (23%) for bacterial. Eleven (21%) individuals had parasite-virus coinfection, five (9%) had parasite-bacteria coinfection, and eight (15%) had virus-bacteria coinfection. The expected frequency of parasite-virus coinfection was 15% (~ 8 cases), 5% (~ 3 cases) for parasite-bacteria coinfection, and 14% (~7 cases) for virus-bacteria coinfection. The coinfection frequencies that I detected in chimpanzees was not statistically different from the expected frequencies ($p > 0.05$ for all comparisons). Twelve (67%) gorillas tested positive for parasitic infections, eight (44%) for viral, and nine (50%) for bacterial. Four (22%) individuals had parasite-virus coinfection, five (28%) had parasite-bacteria coinfection, and four (22%) had virus-bacteria coinfection. The expected frequency of parasite-virus coinfection was 29% (~ 5 cases), 33% (6 cases) for parasite-

bacteria coinfection, and 22% (4 cases) for virus-bacteria coinfection. The coinfection frequencies that I detected in gorillas was not statistically different from the expected frequencies ($p > 0.05$ for all comparisons).

5. Discussion

The results from this study demonstrate a higher prevalence of overall infection, as well as parasitic and bacterial infections, in gorillas. Although a variety of environmental, social, and genetic factors could have affected differences in infection patterns between species, one possible reason for the higher observed prevalence in gorillas, especially those at Mone River, may be attributed to the increasing pressure they face from anthropogenic activity. According to Dunn et al. (2014), there is little to no law enforcement throughout the reserve, which has led to uncontrolled hunting, timber exploitation, resource extraction and an increase in small-scale farming. Habitat loss and fragmentation have also increased within Cross River gorilla habitat over the past years (Dunn et al., 2014; Goldberg et al., 2008), which can promote both bacterial and parasitic transmission between primates (Gillespie & Chapman, 2006; Goldberg et al., 2008). Taken together, these anthropogenic pressures could elicit physiological stress responses that compromise immune function and consequently lead to increased infection susceptibility in gorilla individuals (Gillespie & Chapman, 2006; Hing et al., 2016). In contrast, measures specifically aimed at reducing anthropogenic disease risk to chimpanzees have been implemented at Gombe for years. The long-term success of ecosystem health approaches and methods to mitigate health risks at this site could further explain the lower infection prevalence observed in chimpanzees (Gillespie et al., 2010; Travis et al., 2018).

I also found that coinfections were relatively common in both species (10 of the 18 [56%] infected and studied gorillas and 19 of the 39 [49%] infected chimpanzees [34% of all

chimpanzees studied]). This finding was in line with several studies that document high prevalence of multiple pathogen infections in humans, especially with diarrhea-associated agents (Elfving et al., 2014; Kabayiza et al., 2014; Kotloff et al., 2013; Shrivastava et al., 2017; Zhang et al., 2016; Zohdy et al., 2014). It is thought that enteric pathogen coinfection plays an important role in acute diarrhea, a major cause of childhood morbidity and mortality in developing countries (Andersson et al., 2018; Kabayiza et al., 2014). Patterns of coinfection and the mechanisms between coinfecting enteric pathogens are much less understood in wild primate populations, despite their pathogenic and zoonotic potential (Klaus et al., 2017). The enteric pathogens and high rates of coinfection I detected in this study could have important implications for gorilla and chimpanzee health and conservation.

Groups of pathogens and pathogen pairs were evaluated based on commonly detected enteric pathogens and observed coinfection combinations in humans. For instance, diarrheagenic *E.coli* (Batabyal et al., 2013), *Cryptosporidium* (Kotloff et al., 2013; Sarkar et al., 2014), and *Shigella* (Livio et al., 2014), are major etiological agents of diarrheal disease, associated with coinfection, and linked to childhood morbidity and mortality events. Interestingly, coinfection with these specific pathogens were nonassociated in gorilla and chimp populations. I was also surprised to find that *C. parvum* and Adenovirus, both opportunistic pathogens, were not positively associated with SIV infection in chimpanzees. In humans, *C. parvum* is one of the most frequently identified *Cryptosporidium* spp. in AIDS patients (Fayer et al., 2000). Given that wild chimpanzees infected with SIV are known to experience AIDS-like immunopathology (Etienne et al., 2011; Keele et al., 2009), I expected coinfection between these pathogens to be dependent events. Butel et al. (2015) reported similar findings, however, suggesting that

Cryptosporidium spp. infection in wild chimpanzees was not significantly associated with an individual's respective SIV status.

Although the observed frequencies of all coinfections in gorilla and chimp populations did not differ from those expected, the synergistic effects could still result in more severe pathogenesis than infection with either pathogen alone (Shrivastava et al., 2017). Furthermore, these findings were based on statistical analyses and may not reflect the true behavior of these pathogens in the environment (Silva et al., 2019). Future research should focus on syndromic health data to further assess the pathogenic importance and severity of different pathogen pair coinfections.

Adenovirus and *Cryptosporidium parvum* were the two most common pathogens detected in gorillas and chimpanzees. Adenovirus prevalence in both species fell within comparable ranges reported in previous studies. Seimon et al. (2015) detected adenoviruses in 69.6% of fecal samples from free-ranging central chimpanzees (*Pan troglodytes troglodytes*) (n = 23) and 44.9% of samples from western lowland gorillas (*Gorilla gorilla gorilla*) (n = 136). Roy et al. (2009) detected adenovirus DNA in 40% of stool samples from wild chimpanzees in Central Africa (n = 67) and 50% of samples from mountain gorillas (*Gorilla beringei beringei*) (n = 6) in Rwanda. Though *C. parvum* infection in wild great ape populations is less understood, cases that have been documented also parallel the findings from this study. Nizeyi et al. (1999) reported 11% infection prevalence of *Cryptosporidium* in wild human-habituated mountain gorillas of Bwindi Impenetrable National Park in Uganda. These same gorillas were later found to be infected with *C. parvum* (Graczyk et al., 2001), as were humans (Nizeyi et al., 2001) and cattle (Nizeyi et al., 2002) in the surrounding community. Mynářová et al. (2016) also detected *C. parvum* in habituated captive and semi-wild orangutans, though in very low prevalence.

Adenoviruses are common in both humans and nonhuman primates (Chen et al., 2011; Wevers et al., 2011), can cause mild to severe disease, such as respiratory infections and gastroenteritis, and are also associated with high morbidity and mortality in immunosuppressed individuals, especially in developing countries (Kojaoghlanian et al., 2003; Kotloff et al., 2013; Lion, 2014; Tan et al., 2016; Wasimuddin et al., 2019). While pathogenesis of adenovirus is less understood in primate populations, a recent study on Malagasy mouse lemurs (*Microcebus griseorufus)* found that infection with this pathogen was linked to the disruption of the gut microbiome (Wasimuddin et al., 2019). If this association holds true, and homeostasis of the gastrointestinal tract is disturbed, individuals could become more susceptible to enteric pathogen infection and coinfection (Chen et al., 2017; Moeller et al., 2013; Wasimuddin et al., 2018; Zhao et al., 2017). Although additional studies are needed to corroborate findings by Wasimuddin et al. (2019), the promotion of coinfection by adenovirus infection could help explain the high prevalence of this pathogen in chimpanzee (89%) and gorilla (50%) coinfections.

It should also be noted that many of the observed Simian adenoviruses show high degrees of sequence relatedness to human strains, suggesting evidence of past cross-species transmission events and potential risk of such in the future (Pantó et al., 2015; Roy et al., 2009; Wevers et al., 2011). Though identifying specific adenovirus strains were beyond the scope of this study, the high detection rate of this virus, its zoonotic potential, and the sheer prevalence of immunosuppressed HIV individuals in Sub-Saharan Africa (Bennett et al., 2007) warrants the need for further characterization of this viral group and continual monitoring of these great ape populations.

Cryptosporidium parvum has been documented in over 150 mammalian hosts, including nonhuman primates (Fayer et al., 1997; Fayer, 2008; Xiao & Fayer, 2008), and has received

considerable attention given its zoonotic potential (Graczyk et al., 1997; Xiao & Fayer, 2008). Of the *Cryptosporidium* species, *C. parvum* appears to be the most widespread, have the broadest host range, and linked most often to human and livestock infections (Xiao et al., 2004). Studies that have detected *Cryptosporidium* spp. in nonhuman primates suggest a possible link between human-habituated great apes and enhanced zoonotic transmission (Fayer et al., 1997; Muriuki et al., 1997; Nizeyi et al. 1999; Gillespie et al., 2009; Gomez et al., 2000; van Zijll Langhout et al., 2010). This association was not supported by my results, however, as the (habituated) chimpanzees displayed lower infection prevalence of *C. parvum* (13%) than the (non-habituated) gorillas (67%).

Interestingly, Parsons et al. (2015) examined Gombe chimpanzees from the same communities and found no infection of *C. parvum*. The authors detected three other *Cryptosporidium* species, *C. hominis*, *C. suis* and *C. xiaoi*, which were not screened for in this study. The contrast between these findings and my results may be explained by differences in interannual or interseasonal variation (e.g. rainfall or temperature patterns), or more likely, by the use of diagnostic approaches to determine presence/absence of *C. parvum*. Parsons et al. (2015) utilized PCR and restriction fragment length polymorphism (PCR-RFLP) to target the *Cryptosporidium* SSU rRNA gene. While it is unclear which specific *C. parvum* gene was targeted on the TAC assay, Graczyk et al. (1996) noted that some tests highly sensitive to *C. parvum* showed cross-reactivity for non-*parvum Cryptosporidium* oocysts, which could lead to misidentification of species when using environmental samples.

Despite the many advantages of using the TAC platform, there were limitations to using this approach. The ability to broadly screen for enteric pathogens inherently reduces the opportunity to distinguish between specific strains and/or species. For instance, the assay for

detecting *Salmonella* spp. detects *Salmonella bongori* and all subspecies of *Salmonella enterica*. Though this platform proved extremely applicable for the scope of this study, the lack of molecular information provided could be seen as a potential drawback. My results are also based on small sample sizes, which should be considered when interpreting results. It is also possible that fecal degradation occurred from the time of sample collection to the time of analyses, which could result in underreporting of pathogen presence and diversity. Regardless of these limitations, findings from this study highlight the imperative need to continue efforts in defining health-related conservation threats and helping to guide managements actions towards protecting these great ape species.

Our understanding of pathogens and the diseases they can cause in nonhuman primates is growing, yet there still remains a gap in what we know regarding the breadth of pathogens that routinely infect wild populations. Understanding what constitutes "normal," in terms of which pathogens to expect, as well as their expected prevalence and severity, is a necessary step in managing disease risk (Travis et al., 2018). This study aimed to partially close that knowledge gap by establishing a baseline of enteric pathogen diversity in gorillas and chimpanzees. These results are especially pertinent for monitoring populations of these subspecies given the previously documented cases of disease and epidemics (e.g. respiratory, polio, mange) in Gombe (Williams et al., 2008), and the lack of such in Cross River gorilla populations. Furthermore, the threat of forest encroachment by humans and livestock near Kagwene and Mone River puts gorillas increasingly at risk of novel pathogen exposure, which could have catastrophic impacts on the population (Dunn et al., 2014). Findings from this study warrant the need for continuous monitoring of these subspecies but have laid a promising foundation towards a better understanding of health and disease patterns, potential health risks, and the diversity of

pathogens to which these species are typically exposed. This knowledge can help further guide conservation measures aimed at optimizing disease surveillance systems and measures for risk-management across a variety of great ape populations.

References

Adams, H., Sleeman, J., Rwego, I., & New, J. (2001). Self-reported medical history survey of humans as a measure of health risk to the chimpanzees (*Pan troglodytes schweinfurthii*) of Kibale National Park, Uganda. *Oryx, 35*(4), 308-312. DOI:10.1046/j.1365-3008.2001.00194.x

Allen, T., Murray, K. A., Zambrana-Torrelio, C., Morse, S. S., Rondinini, C., Di Marco, M., … Daszak, P. (2017). Global hotspots and correlates of emerging zoonotic diseases. *Nature Communications*, *8*(1), 1124. DOI: 10.1038/s41467-017-00923-8

Andersson, M., Kabayiza, J., Elfving, K., Nilsson, S., Msellem, M. I., Mårtensson, A., … Lindh, M. (2018). Coinfection with enteric pathogens in East African children with acute gastroenteritis-associations and interpretations. *The American Journal of Tropical Medicine and Hygiene*, *98*(6), 1566–1570. DOI: 10.4269/ajtmh.17-0473

Arandjelovic, M., Bergl, R. A., Ikfuingei, R., Jameson, C., Parker, M., & Vigilant, L. (2015). Detection dog efficacy for collecting faecal samples from the critically endangered Cross River gorilla (*Gorilla gorilla diehli*) for genetic censusing. *Royal Society Open Science*, *2*(2), 140423. DOI:10.1098/rsos.140423

Batabyal, P., Mookerjee, S., Sur, D., & Palit, A. (2013). Diarrheogenic *Escherichia coli* in potable water sources of West Bengal, India. *Acta Tropica*, *127*(3), 153–7. DOI: 10.1016/j.actatropica.2013.04.015

Bennett, E. L., Blencowe, E., Brandon, K., Brown, D., Burn, R. W., Cowlishaw, G., … Wilkie, D. S. (2007). Hunting for consensus: Reconciling bushmeat harvest, conservation, and development policy in West and Central Africa. *Conservation Biology*, *21*(3), 884-887. DOI: 10.1111/j.1523-1739.2006.00595.x

Bergl, R. A., Dunn, A., Fowler, A., Imong, I., Ndeloh, D., Nicholas, A. & Oates, J. F. (2016). *Gorilla gorilla* ssp. *diehli* (errata version published in 2016). *The IUCN Red List of Threatened Species* 2016: e.T39998A102326240. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T39998A17989492.en. Downloaded on 14 January 2020.

Bublitz, D. C., Wright, P. C., Rasambainarivo, F. T., Arrigo-Nelson, S. J., Bodager, J. R., & Gillespie, T. R. (2015). Pathogenic enterobacteria in lemurs associated with anthropogenic disturbance. *American Journal of Primatology, 77*(3), 330-337. DOI: 10.1002/ajp.22348

Butel, C., Mundeke, S. A., Drakulovski, P., Krasteva, D., Ngole, E. M., Mallie, M., … Locatelli, S. (2015). Assessment of infections with Microsporidia and *Cryptosporidium* spp. in fecal samples from wild primate populations from Cameroon and Democratic Republic of Congo. *International Journal of Primatol*ogy, *36*, 227–243. DOI:10.1007/s10764-015-9820-x

Calvignac-Spencer, S., Leendertz, S. A. J., Gillespie, T. R., & Leendertz, F. H. (2012). Wild great apes as sentinels and sources of infectious disease. *Clinical Microbiology and Infection, 18*(6), 521–527. DOI: 10.1111/j.1469-0691.2012.03816.x

Centers for Disease Control and Prevention. (2017). TaqMan Array Card. National Center for Immunization and Respiratory Diseases, Division of Bacterial Diseases.

Chapman, C., Chapman, L., & Wrangham, R. (1995). Ecological constraints on group size: An analysis of spider monkey and chimpanzee subgroups. *Behavioral Ecology and Sociobiology, 36*, 59–70. DOI: 10.1007/BF00175729

Chapman, C. A., & Onderdonk, D. A. (1998). Forests without primates: Primate/plant codependency. *American Journal of Primatology, 45*(1), 127-141. DOI:10.1002/(SICI)1098-2345(1998)45:1<127::AID-AJP9>3.0.CO;2-Y

Chen, E. C., Yagi, S., Kelly, K. R., Mendoza, S. P., Maninger N., Rosenthal, A., … Chiu, C. Y. (2011). Cross-Species transmission of a novel Adenovirus associated with a fulminant pneumonia outbreak in a New World monkey colony. *PLoS Pathogens, 7*(7), 1-16. DOI:10.1371/journal.ppat.1002155

Chen, S., Tsai, C., Lee, Y., Lin, C. Huang, K., Chao, H., … Chiu, C. (2017). Intestinal microbiome in children with severe and complicated acute viral gastroenteritis. *Scientific Reports*, *7*(46130), 1-6. DOI:10.1038/srep46130

Chi, F., Leider, M., Leendertz, F., Bergmann, C., Boesch, C., Schenk, S., … Hakenbeck, R. (2007). New *Streptococcus pneumoniae* clones in deceased wild chimpanzees. *Journal of Bacteriology*, *189*(16), 6085–6088. DOI: 10.1128/JB.00468-07

Constable, J. L., Ashley, M. V., Goodall, J., & Pusey, A. E. (2001). Noninvasive paternity assignment in Gombe chimpanzees. *Molecular Ecology*, *10*(5), 1279–300. DOI:10.1046/j.1365-294x.2001.01262.x

Cooper, N., & Nunn, C. L. (2013). Identifying future zoonotic disease threats: Where are the gaps in our under- standing of primate infectious diseases? *Evolution, Medicine, and Public Health*, *2013*(1), 27–36. DOI: 10.1093/emph/eot001

Deere, J. R., Parsons, M. B., Lonsdorf, E. V., Lipende, I., Kamenya, S., Collins, D. A., … Gillespie, T. R. (2019). *Entamoeba histolytica* infection in humans, chimpanzees, and baboons in the Greater Gombe Ecosystem, Tanzania. *Parasitology*, *146*(9), 1116–1122. DOI: 10.1017/S0031182018001397

De Vere, R. A., Warren, Y., Nicholas, A., Mackenzie, M. E., & Higham, J. P. (2011). Nest site ecology of the Cross River gorilla at the Kagwene Gorilla Sanctuary, Cameroon, with special reference to anthropogenic influence. *American Journal of Primatology*, *73*(3), 253-261. DOI:10.1002/ajp.20886

Diaz, M. H., Waller, J. L., Napoliello, R. A., Islam, S., Wolff, B. J., Burken, D. J., … Saha, S. K. (2013). Optimization of multiple pathogen detection using the TaqMan Array Card: Application for a population-based study of neonatal infection. *PLoS One*, *8*(6). DOI: 10.1371/journal.pone.0066183

Diaz, M. H., Waller, J. L., Theodore, M. J., Patel, N., Wolff, B. J., Benitez, A. J., … Winchell, J. M. (2019). Development and implementation of multiplex TaqMan Array Cards for specimen testing at Child Health and Mortality Prevention Surveillance site laboratories. *Clinical Infectious Diseases*, *69*(Supplement_4), S311- S321. DOI: 10.1093/cid/ciz571

Dunn, A., Bergl, R., Byler, D., Eben-Ebai, S., Etiendem, D. N., Fotso, R., … Williamson, E. A. (2014). Revised regional action plan for the conservation of the Cross River gorilla (*Gorilla gorilla diehli*) 2014–2019. New York, NY. IUCN/SSC Primate Specialist Group and Wildlife Conservation.

Elfving, K., Andersson, M., Msellem, M. I., Welinder- Olsson, C. Petzold, M., Björkman, A., … Lindh, M. (2014). Real-time PCR threshold cycle cutoffs help to identify agents causing acute childhood diarrhea in Zanzibar. *Journal of Clinical Microbiology*, *52*(3), 916-923. DOI**:** 10.1128/JCM.02697-13

Etiendem, D.N., & Tagg, N. (2013). Feeding ecology of Cross River Gorillas (*Gorilla gorilla diehli*) at Mawambi Hills: The influence of resource seasonality. *International Journal of Primatology, 34*(6), 1261–1280. DOI: 10.1007/s10764-013-9739-z

Etiendem, D. N., Funwi-Gabga, N., Tagg, N., Hens, L., & Indah, E. K. (2013). The Cross River gorillas *(Gorilla gorilla diehli)* at Mawambi Hills, South-West Cameroon: Habitat suitability and vulnerability to anthropogenic disturbance. *Folia Primatologica, 84*(1), 18–31. DOI: 10.1159/000345853

Etienne, L., Nerrienet, E., LeBreton, M., Bibila, G. T., Foupouapouognigni, Y., Rousset, D., … Ayouba, A. (2011). Characterization of a new simian immunodeficiency virus strain in a naturally infected *Pan troglodytes troglodytes* chimpanzee with AIDS related symptoms. *Retrovirology, 8*(4), 1-13. DOI: 10.1186/1742-4690-8-4

Faust, C. L., McCallum, H. I., Bloomfield, L. S. P., Gottdenker, N. L., Gillespie, T. R., Torney, C. J., … Plowright, R. K. (2018). Pathogen spillover during land conversion. *Ecology Letters*, *21*(4), 471-483. DOI: 10.1111/ele.12904

Fayer, R., Spear, C. A., & Dubey, J. P. (1997). General biology of *Cryptosporidium* and cryptosporidiosis. R. Fayer (Ed.), In *Cryptosporidium* and cryptosporidiosis, (pp. 1-49). Boca Raton, FL: CRC Press and IWA Publishing.

Fayer, R., Morgan, U., & Upton, S. J. (2000). Epidemiology of *Cryptosporidium*: Transmission, detection and identification. *International Journal of Parasitology*, *30*(12-13), 1305–1322. DOI: 10.1016/S0020-7519(00)00135-1

Fayer, R. (2008). *Cryptosporidium* and Cryptosporidiosis (second ed.). R. Fayer & L. Xiao (Eds.), (pp. 1-42). Boca Raton, FL: CRC Press and IWA Publishing.

Gao, F., Bailes, E., Robertson, D. L., Chen, Y., Rodenburg, C.M., Michael, S. F., … Hahn, B. H. (1999). Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes. Nature*, *397*(6718), 436-441. DOI: 10.1038/17130

Genton, C., Cristescu, R., Gatti, S., Levrero, F., Bigot, E., Caillaud, D., … Menard, N. (2012). Recovery potential of a western lowland gorilla population following a major Ebola outbreak: Results from a ten year study. *PLoS ONE*, *7*(5), 1–10. DOI: 10.1371/journal.pone.0037106

Gilardi, K. V., Gillespie, T. R., Leendertz, F. H., Macfie, E. J., Travis, D. A., Whittier, C. A., & Williamson, E. A. (2015). Best practice guidelines for health monitoring and disease control in great ape populations. Gland, Switzerland: IUCN SSC Primate Specialist Group. DOI: 10.2305/IUCN.CH.2015.SSC-OP.56.en

Gillespie, T. R., & Chapman, C. A. (2006). Prediction of parasite infection dynamics in primate metapopulations based on attributes of forest fragmentation. *Conservation Biology, 20*(2), 441–448. DOI: 10.1111/j.1523-1739.2006.00290.x

Gillespie, T. R., Nunn, C. L., & Leendeertz, F. H. (2008). Integrative approaches to the study of primate infectious disease: Implications for biodiversity conservation and global health. *American Journal of Physical Anthropology*, *51*(S47), 53-69. DOI: 10.1002/ajpa.20949

Gillespie, T. R., & Chapman, C. A. (2008). Forest fragmentation, the decline of an endangered primate, and changes in host-parasite interactions relative to an unfragmented forest. *American Journal of Primatology*, *70*(3), 222–230. DOI: 10.1002/ajp.20475

Gillespie, T. R., Morgan, D., Deutsch, J. C., Kuhlenschmidt, M. S., Salzer, J. S., Cameron, K., … Sanz, C. (2009). A legacy of low-impact logging does not elevate prevalence of potentially pathogenic protozoa in free-ranging gorillas and chimpanzees in the Republic of Congo: Logging and parasitism in African apes. *Ecohealth, 6*(4), 557–564. DOI: 10.1007/s10393-010-0283-4

Gillespie, T. R., Lonsdorf, E. V., Canfield, E. P., Meyer, D. J., Nadler, Y., Raphael, J., … Travis, D.A. (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*(4), 534-544. DOI: 10.1002/ajpa.21348

Goldberg, T. L., Gillespie, T. R., Rwego, I. B., Wheeler, E., Estoff, E. L., & Chapman, C. A. (2007). Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation*, *135*(4), 511–517. DOI: 10.1016/j.biocon.2006.10.048

Goldberg, T. L., Gillespie, T. R., Rwego, I. B., Estoff, E. L., & Chapman, C. A. (2008). Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. *Emerging infectious diseases*, *14*(9), 1375–1382. DOI: 10.3201/eid1409.071196

Gomez, M. S., Torres, J., Gracenea, M., Fernandez-Moran, J., & Gonzalez- Moreno, O. (2000). Further report on *Cryptosporidium* in Barcelona Zoo mammals. *Parasitology Research*, *86*(4), 318-323. DOI: 10.1007/s004360050049

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), 1–60. DOI: 10.1111/j.1439-0310.1983.tb01324.x

Goodall J. (1986). The chimpanzees of Gombe: Patterns of behavior. Cambridge, MA: The Belknap Press of Harvard University Press.

Graczyk, T. K., Cranfield, M. R., & Fayer, R. (1996). Evaluation of commercial Enzyme Immunoassay (EIA) and Immunofluorescent Antibody (IFA) test kits for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum. The American Journal of Tropical Medicine and Hygiene*, *54*(3), 274 – 279. DOI: 10.4269/ajtmh.1996.54.274

Graczyk, T. K., Fayer, R., & Cranfield, M. R. (1997). Zoonotic potential of cross-transmission of *Cryptosporidium parvum*: Implications for waterborne cryptosporidiosis. *Parasitology Today*, *13*(9), 348-351. DOI: 10.1016/s0169-4758(97)01076-4

Graczyk, T. K., DaSilva, A. J., Cranfield, M. R., Nizeyi, J. B., Kalema, G.R., Pieniazek, N. J. (2001). *Cryptosporidium parvum* genotype 2 infections in free-ranging mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. *Parasitology Research*, *87*(5), 368–70. DOI: 10.1007/s004360000337

Hing, S., Narayan, E. J., Thompson, R. C. A., & Godfrey, S. S. (2016). The relationship between physiological stress and wildlife disease: Consequences for health and conservation. *Wildlife Research*, *43*(1), 51-60. DOI: 10.1071/WR15183

Hoffmann, C., Zimmermann, F., Biek, R., Kuehl, H., Nowak, K., Mundry, R., … Leendertz, F. H. (2017). Persistent anthrax as a major driver of wildlife mortality in a tropical rainforest. *Nature, 548*(7665), 82–86. DOI: 10.1038/nature23309

Ikfuingei, R. (2012). Annual report of Cross River gorilla ecological monitoring at the Kagwene Gorilla Sanctuary, Cameroon, progress report July 2011 to June 2012. Unpublished report. Wildlife Conservation Society (WCS) Cameroon, Limbe.

Imong I., Robbins, M. M., Mundry, R., Bergl, R., & Kuehl, H. S. (2014). Distinguishing ecological constraints from human activity in species range fragmentation: The case of Cross River gorillas. *Animal Conserv*ation, *17*(4**)**, 323–331. DOI: 10.1111/acv.12100

IUCN (2015). The IUCN Red List of Threatened Species. Version 2015.4. http://www.iucnredlist.org

IUCN (2020). The IUCN Red List of Threatened Species. Version 2019.3. https://www.iucnredlist.org

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman J. L., & Dasak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*, 990–993. DOI: 10.1038/nature06536

Jones, B. A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M. Y., … Pfeiffer, D. U. (2013). Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences USA*, *110*(21), 8399–8404. DOI: 10.1073/pnas.1208059110

Kabayiza, J., Andersson, M. E., Staffan, N., Bergström, T., Muhirwa, G., & Lindh, M. (2014). Real-time PCR identification of agents causing diarrhea in Rwandan children less than 5 years of age. *The Pediatric Infectious Disease Journal*, *33*(10), 1037-1042. DOI: 10.1097/INF.0000000000000448

Karesh, W. B., Dobson, A., Lloyd-Smith, J. O., Lubroth, J., Dixon, M. A., Bennett, M., … Heymann, D. L. (2012). Ecology of zoonoses: Natural and unnatural histories. *Lancet*, *380*(9857), 1936–1945. DOI: 1016/S0140-6736(12)61678-X

Keele, B. F., Jones, J. H., Terio, K. A., Estes, J. D., Rudicell, R. S., Wilson, M. L., …Hahn, B. H. (2009). Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. *Nature, 460*(7254), 515–519. DOI: 10.1038/nature08200

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., … Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, *468,* 647–652. DOI: 10.1038/nature09575

Klaus, A., Zimmermann, E., Röper, K. M., Radespiel, U., Nathan, S., Goossens, B., & Strube, C. (2017). Co-infection patterns of intestinal parasites in arboreal primates (proboscis monkeys, *Nasalis larvatus*) in Borneo. *International Journal for Parasitology: Parasites and Wildlife*, *6*(3), 320–329. DOI: 10.1016/j.ijppaw.2017.09.005

Kodani, M., Yang, G., Conklin, L. M., Travis, T. C., Whitney, C. G., Anderson, L. J., … Fields, B. S. (2011). Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. *Journal of Clinical Microbiology*, *49*(6), 2175-2182. DOI: 10.1128/JCM.02270-10

Kojaoghlanian, T., Flomenberg, P., & Horwitz, M. S. (2003). The impact of adenovirus infection on the immunocompromised host. *Reviews in Medical Virology*, *13*(3), 155–171. DOI: 10.1002/rmv.386

Kondgen S., Kuhl, H., N'Goran, P. K., Walsh, P. D., Schenk, S., Ernst, S., … Leendertz, F. H. (2008). Pandemic human viruses cause decline of endangered great apes. *Current Biology*, *18*(4), 260–264. DOI: 10.1016/j.cub.2008.01.012

Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., … Levine, M. M. (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*(9888), 209–22. DOI: 10.1016/S0140-6736(13)60844-2

Leendertz F., Lankester F., Guislain P., Ne´el C., Drori O., Dupain, J., … Leroy, E. M. (2006a). Anthrax in Western and Central African great apes. *American Journal of Primatology*, *68*(9), 928–933. DOI: 10.1002/ajp.20298

Leendertz, F. H., Pauli, G., Maetz-Rensing, K., Boardman, W., Nunn, C., Ellerbrok, H., … Boesch, C. (2006b). Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals. *Biological Conservation*, *131*(2), 325–337. DOI: 10.1016/j.biocon.2006.05.002

Leendertz, S. A. J., Wich, S. A., Ancrenaz, M., Bergl, R. A., Gonder, M. K., Humle, T. & Leendertz, F. H. (2017). Ebola in great apes – current knowledge, possibilities for vaccination, and implications for conservation and human health. *Mammal Review*, *47*(2), 98-111. DOI:10.1111/mam.12082

Leroy, E. M., Rouquet, P., Formenty, P., Souquiere, S., Kilbourne, A., Froment, J. M., … Rollin, P. E. (2004). Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science, 303*(5656), 387–390. DOI: 10.1126/science.1092528

Lion, T. (2014). Adenovirus infections in immunocompetent and immunocompromised patients. *Clinical Microbiology Reviews, 27*(3), 441–462. DOI**:** 10.1128/CMR.00116-13

Liu, W., Worobey, M., Li, Y., Keele, B. F., Bibollet-Ruche, F., Guo, Y., … Hahn, B. H. (2008). Molecular ecology and natural history of simian foamy virus infection in wild-living chimpanzees. *PLoS pathogens*, *4*(7), e1000097. DOI: 10.1371/journal.ppat.1000097

Liu, J., Gratz, J., Amour, C., Kibiki, G., Becker, S., Janaki, L., … Houpt, E. R. (2013). A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. *Journal of Clinical Microbiology*, *51*(2), 472–480. DOI: 10.1128/JCM.02658-12

Livio, S., Strockbine, N. A., Panchalingam, S., Tennant, S. M., Barry, E. M., Marohn, M. E, … Levine, M. M. (2014). *Shigella* isolates from the Global Enteric Multicenter Study inform vaccine development. *Clinical Infectious Diseases*, *59*(7), 933- 941. DOI: 10.1093/cid/ciu468

Marsh, L. K. (2003). Primates in fragments: ecology and conservation. New York, NY: Springer U.S.

McFarland, K. L. (2007). Ecology of Cross River Gorillas (*Gorilla gorilla diehli*) on Afi Mountain, Cross River State, Nigeria. Doctoral Dissertation, City University of New York, NY.

McFarlane, R. A., Sleigh, A. C., & McMichael, A. J. (2013). Land-use change and emerging infectious disease on an island continent. *International Journal of Environmental Research and Public Health*, *10*(7), 2699–2719. DOI: 10.3390/ijerph10072699

McLennan, M. R., Mori, H., Mahittikorn, A., Prasertbun, R., Hagiwara, K., & Huffman, M. A. (2018). Zoonotic enterobacterial pathogens detected in wild chimpanzees. *EcoHealth*, 15, 143–147. DOI: 10.1007/s10393-017-1303-4

Meder, A. (2015). Conserving gorilla diversity. In A. Behie. & M. Oxenham (Eds.), *Taxonomic Tapestries: The Threads of Evolutionary, Behavioural and Conservation Research* (pp. 361-372). Australia: ANU Press.

Mitani, J. C., & Watts, D. P. (2005). Correlates of territorial boundary patrol behaviour in wild chimpanzees. *Animal Behaviour, 70*(5), 1079–1086. DOI: 10.1016/j.anbehav.2005.02.012

Moeller, A. H., Shilts, M., Li, Y., Rudicell, R. S., Londsdorf, E. V., Pusey, A. E., … Ochman, H. (2013). SIV-induced instability of the chimpanzee gut microbiome. *Cell Host Microbe, 14*(3), 340–5. DOI: 10.1016/j.chom.2013.08.005

Morse, S. S. (1995). Factors in the emergence of infectious diseases. *Emerging Infectious Diseases*, *1*(1), 7-15. DOI: 10.3201/eid0101.950102

Moyer, D., Plumptre, A. J., Pintea, L., Hernandez-Aguilar, A., Moore, J., Stewart, F., … Mwangoka, M. (2006). *Surveys of Chimpanzees and Other Biodiversity in Western Tanzania*. Unpublished report. Arlington, VA: United States Fish and Wildlife Service (USFWS).

Muriuki, S. M., Farah, I. O., Kagwiria, R. M., Chai, D. C., Nja-munge, G., Suleman, M., & Olobo, J. O. (1997). The presence of *Cryptosporidium* oocysts in stools of clinically diarrhoeic and nor- mal nonhuman primates in Kenya. *Veterinary Parasitology*, *72*(2), 41-147. DOI:10.1016/S0304-4017(97)00021-6

Mynářová, A., Foitová, I., Kváč, M., Květoňová, D., Rost, M., Morrogh-Bernard, H., … Sak, B. (2016). Prevalence of *Cryptosporidium* spp., *Enterocytozoon bieneusi*, *Encephalitozoon* spp. and *Giardia intestinalis* in wild, semi-wild and captive orangutans (*Pongo abelii* and *Pongo pygmaeus*) on Sumatra and Borneo, Indonesia. *PLoS One*, *11*(3), e0152771. DOI: 10.1371/journal.pone.0152771

Nishida, T., Wrangham, R. W., Goodall, J., & Uehara, S. (1983). Local differences in plant feeding habits of chimpanzees between the Mahale Mountains and Gombe National Park, Tanzania. *Journal of Human Evolution*, *12*(5), 467–480. DOI: 10.1016/S0047-2484(83)80142-0

Nishida, T. (1990). *The Chimpanzees of the Mahale Mountains: Sexual and Life History Strategies*. Tokyo: University of Tokyo Press.

Nizeyi, J. B., Mwebe, R., Nanteza, A., Cranfield, M. R., Kalema, G. R. N. N., & Graczyk, T. K. (1999). *Cryptosporidium* sp. and *Giardia* sp. infections in mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. *The Journal of Parasitology*, *85*(6), 1084-1088. DOI:10.2307/3285672

Nizeyi, J. B., Sebunya, D., Dasilva, A. J., Cranfield, M. R., Pieniazek, N. J., & Graczyk, T. K. (2001). Cryptosporidiosis in people sharing habitats with free-ranging mountain gorillas (*Gorilla gorilla beringei*), Uganda. *American Journal of Tropical Hygiene*, *66*(4), 442–444. DOI: 10.4269/ajtmh.2002.66.442

Nizeyi, J., Cranfield, M., & Graczyk, T. (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*(4), 380–385. DOI: 10.1007/s00436-001-0543-x

Nsubuga, A. M., Robbins, M. M., Roeder, A. D., Morin, P. A., Boesch, C., & Vigilant, L. (2004). Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Molecular Ecology, 13*(7), 2089–2094. DOI:10.1111/j.1365-294X.2004.02207.x

Oates, J. F., McFarland, K. L., Groves, J. L., Bergl, R. A., Linder, J. M., & Disotell, T. R. Disotell. (2003). The Cross River gorilla: The natural history and status of a neglected and critically endangered subspecies. In A. Taylor & M. Goldsmith (Eds.), *Gorilla Biology: A Multidisciplinary Perspective* (pp. 472–497). Cambridge University Press, Cambridge.

Oates, J. F., Bergl, R. A., & Linder, J. M. (2004). Review of Existing Conservation Areas. In *Africa's Gulf of Guinea Forests: Biodiversity Patterns and Conservation Priorities* (pp. 65-78). Conservation International. DOI: 10.1896/1-881173-82-8

Palacios, G., Lowenstine, L. J., Cranfield, M. R., Gilardi, K. V., Spelman, L., Lukasik-Braum, M., … Lipkin, W. I. (2011). Human metapneumovirus infection in wild mountain gorillas, Rwanda. *Emerging Infectious Diseases, 17*(4), 711–713. DOI: 10.3201/eid1704.100883

Pantó, L., Podgorski, I. I., Jánoska, M., Márkó, O., & Harrach, B. (2015). Taxonomy proposal for old world monkey adenoviruses: Characterisation of several non-human, non-ape primate adenovirus lineages. *Archives of Virology, 160,* 3165–3177. DOI: 10.1007/s00705-015-2575-z

Parrish, C. R., Holmes, E. C., Morens, D. M., Park, E. C., Burke, D. S., Calisher, C. H., … Daszak, P. (2008). Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiology and Molecular Biology Reviews*, *72*(3), 457–470. DOI: 10.1128/MMBR.00004-08

Parsons, M. B., Travis, D., Lonsdorf, E. V., Lipende, I., Roellig, D. M. A., Kamenya, S., … Gillespie, T. R. (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, 9*(2), 1-13. DOI: 10.1371/journal.pntd.0003529

Patz, J. A., Daszak, P., Tabor, G. M., Aguirre, A. A., Pearl, M., Epstein, J., … Bradley, D. J., & Working Group on Land Use Change and Disease Emergence. (2004). Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. *Environmental health perspectives*, *112*(10), 1092–1098. DOI: 10.1289/ehp.6877

Plumptre, A. J., Cox, D., & Mugume, S. (2003). The status of chimpanzees in Uganda. Albertine Rift Technical Report Series. Wildlife Conservation Society, Kampala, Uganda.

Plumptre, A. J ., Rose, R., Nangendo, G., Williamson, E. A., Didier, K., Hart, J., … Bennett, E. (2010). *Eastern Chimpanzee (Pan troglodytes schweinfurthii): Status Survey and Conservation Action Plan 2010-2020*. Gland, Switzerland. IUCN. pp. 52.

Pusey, A. E., Pintea, L., Wilson, M. L., Kamenya, S., & Goodall, J. (2007). The contribution of long-term research at Gombe National Park to chimpanzee conservation. *Conservation Biology*, *21*(3), 623-634. DOI: 10.1111/j.1523-1739.2007.00704.x

Pusey, A. E., Wilson, M. L., & Collins, D. A. (2008). Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. *American Journal of Primatology*, *70*(8), 38–744. DOI: 10.1002/ajp.20567

Rachwal, P. A., Rose, H. L., Cox, V., Lukaszewski, R. A, Murch, A. L., & Weller, S. A. (2012). The potential of TaqMan Array Cards for detection of multiple biological agents by real-time PCR. *PLoS One, 7*(4), e35971. DOI: 10.1371/journal.pone.0035971

Reynolds, V. (2005). *The Chimpanzees of the Budongo Forest*. Oxford: Oxford University Press.

Robbins, M. M., & Robbins, A. M. (2018). Variation in the social organization of gorillas: Life history and socioecological perspectives. *Evolutionary Anthropology*, *27*(5), 218– 233. DOI: 10.1002/evan.21721

Roy, S., Vandenberghe, L. H., Kryazhimskiy, S., Grant, R., Calcedo, R., Yuan, X., …Wilson, J. M. (2009). Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. *PLoS pathogens*, *5*(7), e1000503. DOI: 10.1371/journal.ppat.1000503

Rudicell, R. S., Jones, J. H., Wroblewski, E. E., Learn, G. H., Li, Y., Robertson, J. D., … Wilson, M. L. (2010). Impact of simian immunodeficiency virus infection on chimpanzee population dynamics. *PLoS Pathogens*, *6*(9), e1001116. DOI: 10.1371/ journal.ppat.1001116

Rwego, I. B., Isabirye-Basuta, G., Gillespie, T. R., & Goldberg, T. L. (2008). Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. *Conservation Biology*, *22*(6), 1600-1607. DOI: 10.1111/j.1523-1739.2008.01018.x

Sarkar, R., Tate, J. E., Ajjampur, S. S. R., Kattula, D., John, J., Ward, H. D., & Kang, G. (2014). Burden of diarrhea, hospitalization and mortality due to cryptosporidial infections in Indian children. *PLoS Neglected Tropical Diseases*, *8*(7), e3042. DOI: 10.1371/journal.pntd.0003042

Sapolsky, R. M., & Else, J. G. (1987). Bovine tuberculosis in a wild baboon population: Epidemiological aspects. *Journal of Medical Primatology*, *16*(4), 229–235. DOI: 10.1111/(ISSN)1600-0684

Sawyer, S. C. (2012). Subpopulation range estimation for conservation planning: A case study of the critically endangered Cross River gorilla. *Biodiversity and Conservation*, *21*(6), 1589–1606. DOI: 10.1007/s10531-012-0266-6

Seimon, T. A., Olson, S. H., Lee, K. J., Rosen, G., Ondzie, A., Cameron K., … Lipkin, W. I. (2015). Adenovirus and herpesvirus diversity in free-ranging great apes in the Sangha region of the Republic of Congo. *PLoS ONE*, *10*(3): e0118543. DOI: 10.1371/journal. pone.0118543

Shrivastava, A. K., Kumar, S., Mohakud, N. K., Suar, M., & Sahu, P. S. (2017). Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. *Gut Pathogens, 9*(16), 1-12. DOI: 10.1186/s13099-017-0166-0

Silva, M. M. O., Tauro, L. B., Kikuti, M., Anjos, R. O., Santos, V. C., Gonçalves, T. S. F., ... Ribeiro, G. S. (2019). Concomitant transmission of Dengue, Chikungunya, and Zika viruses in Brazil: Clinical and epidemiological findings from surveillance for acute febrile illness. *Clinical Infectious Diseases*, *69*(12), 2238. DOI: 10.1093/cid/ciz588

Streicker, D. G., Turmelle, A. S., Vonhof, M. J., Kuzmin, I. V., McCracken, G. F., & Rupprecht, C. E. (2010). Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. *Science*, *329*(5992), 676–679. DOI: 10.1126/science.1188836

Sugiyama, Y. (2004). Demographic parameters and life history of chimpanzees at Bossou, Guinea. *American Journal of Physical Anthropology*, *124*(2), 154–165. DOI: 10.1002/ajpa.10345

Sullivan, K. M., Mannucci, A., Kimpton, C. P., & Gill, P. (1993). A rapid and quantitative DNA sex test: Fluorescence-based PCR analysis of XY homologous gene amelogenin. *Biotechniques*, *15*(4), 636-638, 640-641. PMID: 8251166

Sunderland-Groves, J. L., Ekinde, A., & Mboh, H. (2009). Nesting Behavior of *Gorilla gorilla diehli* at Kagwene Mountain, Cameroon: Implications for assessing group size and density. *International Journal of Primatology, 30*(2), 253–266. DOI: 10.1007/s10764-009-9340-7

Tan, B., Wu, L., Yang, X., Li, B., Zhang, W. Lei, Y., … Shi, Z. (2016). Isolation and characterization of adenoviruses infecting endangered golden snub-nosed monkeys (*Rhinopithecus roxellana*). *Virology Journal, 13*(190), 1-5. DOI 10.1186/s12985-016-0648-6

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. (2001). Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, *356*(1411), 983–989. DOI: 10.1098/rstb.2001.0888

Thalmann, O., Wegmann, D., Spitzner, M., Arandjelovic, M., Guschanski, K., Leuenberger, C., … Vigilant, L. (2011). Historical sampling reveals dramatic demographic changes in western gorilla populations. *BMC Evolutionary Biology*, *11*, 85. DOI: 10.1186/1471-2148-11-85

Travis, D. A., Lonsdorf, E. V., & Gillespie, T. R. (2018). The grand challenge of great ape health and conservation in the Anthropocene. *American Journal of Primatology, 80*(1), 1-5. DOI: 10.1002/ajp.22717

van den Beld, M. J., & Reubsaet, F. A. G. (2012). Differentiation between *Shigella*, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. *European Journal of Clinical Microbiology & Infectious Diseases*, *31*(6), 899–904. DOI: 10.1007/s10096-011-1395-7

van den Beld, M., & Reubsaet, F. A. G. (2012). Differentiation between *Shigella*, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. *European Journal of Clinical Microbiology & Infectious Diseases,* 31(6), 899–904. DOI: 10.1007/s10096-011-1395-7

van Zijll Langhout, M., Reed, P., & Fox, M. (2010). Validation of multiple diagnostic techniques to detect *Cryptosporidium* sp. and *Giardia* sp. in free-ranging western lowland gorillas (*Gorilla gorilla gorilla*) and observations on the prevalence of these protozoan infections in two populations in Gabon. *Journal of Zoo and Wildlife Medicine, 41*(2), 210–217. DOI: 10.1638/2009-0051R1.1

Vernet, G., Saha, S. K., Satzke, C., Burgess, D. H., Alderson, M., Maisonneuve, J. F., … Klugman, K. P. (2011). Laboratory-based diagnosis of pneumococcal pneumonia: State of the art and unmet needs. *Clinical Microbiology and Infection*, *17*(Supplement 3), 1–13. DOI: 10.1111/j.1469-0691.2011.03496.x

Wachtman, L. M., & Mansfield, K. G. (2008). Opportunistic infections in immunologically compromised nonhuman primates. *Institute for Laboratory Animal Research Journal*, *49*(2), 191–208. DOI: 10.1093/ilar.49.2.191

Wasimuddin, S. D. B., Tschapka, M., Page, R., Rasche, A., Corman, V. M., Drosten, C., & Sommer, S. (2018). Astrovirus infections induce age-dependent dysbiosis in gut microbiomes of bats. *The ISME Journal, 12,* 2883–2893. DOI: 10.1038/s41396-018-0239-1

Wasimuddin, S. D. B., Corman, V. M., Ganzhorn, J. U., Rakotondranary, J., Ratovonamana, Y. R., Drosten, C., & Sommer, S. (2019). Adenovirus infection is associated with altered gut microbial communities in a non-human primate. *Scientific reports*, *9*(1), 13410. DOI: 10.1038/s41598-019-49829-z

Weiss, R. A., & McMichael, A. J. (2004). Social and environmental risk factors in the emergence of infectious diseases. *Nature Medicine, 10*(12*),* S70–S76. DOI: 10.1038/nm1150

Weller, S. A., Cox, V., Essex-Lopresti, A., Hartley, M. G., Parsons, T. M., Rachwal, P. A., … Lukaszewski, R. A. (2012). Evaluation of two multiplex real-time PCR screening capabilities for the detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis* in blood samples generated from murine infection models. *Journal of Medical Microbiology*, *61*(11), 1546–1555. DOI: 10.1099/jmm.0.049007-0

Wevers, D., Metzger, S., Babweteera, F., Bieberbach, M., Boesch, C., Cameron, K., … Ehlers, B. (2011). Novel adenoviruses in wild primates: A high level of genetic diversity and evidence of zoonotic transmissions. *Journal of virology*, *85*(20), 10774–10784. DOI: 10.1128/JVI.00810-11

Williams, J. M., Lonsdorf, E. V., Wilson, M. L., Schumacher-Stankey J., Goodall, J., & Pusey, A. E. (2008). Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. *American Journal of Primatology, 70*(8), 766–777. DOI: 10.1002/ajp.20573

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A., & Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: The missing link? *Emerging Infectious Diseases*, *4*(2), 149-158. DOI: 10.3201/eid0402.980202

Wolfe, N. D., Dunavan, C. P., & Diamond, J. (2007). Origins of major human infectious diseases. *Nature*, *447*, 279–283. DOI: 10.1038/nature05775

Wrangham, R. W., Chapman, C. A., Clark-Arcadi, A. P., & Isabirye-Basuta, G. (1986). Social ecology of Kanyawara chimpanzees: Implications for understanding the costs of great ape groups. In: W.C. McGrew, L.F. Marchant, & T. Nishida (Eds.), *Great Ape Societies* (pp.45-57). Cambridge, UK: Cambridge University Press.

Wrangham, R. W., Chapman, C. A., & Chapman L. J. (1994). Seed dispersal by forest chimpanzees in Uganda. *Journal of Tropical Ecology, 10*(3), 355-368. DOI: 10.1017/S0266467400008026

Wroblewski, E. E., Murray, C. M., Keele, B. F., Schumacher-Stankey, J. C., Hahn, B. H., & Pusey, A. E. (2009). Male dominance rank and reproductive success in chimpanzees, *Pan troglodytes schweinfurthii*. *Animal Behavior*, *77*(4), 873–85. DOI: 10.1016/j.anbehav.2008.12.014

Wroblewski, E. E., Norman, P. J., Guethlein, L. A., Rudicell, R. S., Ramirez, M. A., Li, Y., … Parham, P. (2015). Signature patterns of MHC diversity in three Gombe communities of wild chimpanzees reflect fitness in reproduction and immune defense against SIVcpz. *PLoS Biology*, *13*(5), e1002144. DOI:10.1371/journal. pbio.1002144

Xiao, L. H., Fayer, R., & Upton, S. J. (2004). *Cryptosporidium* taxonomy: Recent advances and implications for public health. *Clinical Microbiology Reviews*, *17*(1), 72-97. DOI: 10.1128/cmr.17.1.72-97.2004

Xiao, L. H., & Fayer, R. (2008). Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *International Journal of Parasitology*, *38*(11), 239–55. DOI: 10.1016/j.ijpara.2008.03.006

Zhang, S., Zhou, Y., Xu, W., Tian, L., Chen, J., Chen, S., … Zhou, X. (2016). Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China. *Infectious Diseases of Poverty*, *5*(64), 1–13. DOI: 10.1186/s40249-016-0157-2

Zhao, N., Li, M., Luo, J., Wang, S., Liu, S., Wang, S., … He, H. (2017). Impacts of canine distemper virus infection on the giant panda population from the perspective of gut microbiota. *Scientific Reports, 7*(39954), 1-10. DOI: 10.1038/srep39954

Zohdy, S., Grossman, M. K., Fried, I. R., Rasambainarivo, F. T., Wright, P. C., & Gillespie, T. R. (2015). Diversity and prevalence of diarrhea-associated viruses in the lemur community and associated human population of Ranomafana National Park, Madagascar. *International Journal of Primatology*, *36*(1), 143–153. DOI: 10.1007/s10764-015-9817-5

Tables

Table 1. Enteric pathogen targets (n = 39) validated for use on TaqMan array cards. Distinct gene targets for the same pathogen were grouped together for simplicity (adapted from Diaz *et al*. 2019).

Bacteria (n = 16)		
Aeromonas spp.	*Enterococcus faecium*	*Salmonella enterica/bongori*
Campylobacter coli	Enteroaggregative *Escherichia coli (aaiC, aatA)*	Shiga toxin (*stx1, stx2)*
Campylobacter jejuni	Enteroinvasive *E. coli/Shigella* (*ipaH)*	*Vibrio cholerae* (non-toxigenic, toxigenic)
Clostridioides difficile, nontoxigenic	Enteropathogenic *E. coli (bfpA, eae)*	*Yersinia* spp.
C. difficile toxin A *tcdA,* toxin B *tcdB*	Enterotoxigenic *E. coli* (LT, ST)	
Enterococcus faecalis	*Mycobacterium tuberculosis*	
Viruses (n = 12)		
Adenovirus	Norovirus genogroup GI	Rotavirus C
Adenovirus serotype 40/41	Norovirus genogroup GII	Rotavirus nontypeable
Astrovirus	Rotavirus A	Sapovirus I/II/IV
Enterovirus	Rotavirus B	Sapovirus V
Parasites (n = 5)		
Ascaris lumbricoides	*Entamoeba histolytica*	*Trichuris trichiura*
Cryptosporidium parvum	*Giardia* spp.	

Table 2. Contingency table comparing the observed and expected (in parenthesis) values of coinfection for SIV and C. parvum in eastern chimpanzees (*Pan troglodytes schweinfurthii*) in the Kasakela and Mitumba communities of Gombe National Park, Tanzania.

	C. parvum		**Total**
	Not Infected	Infected	
SIV			
Not infected	38 (39.1)	7 (5.9)	45
Infected	8 (6.9)	0 (1.1)	8
Total	46	7	53

Table 3. Enteric pathogen prevalence of Cross River gorillas (*Gorilla gorilla diehli)* in Kagwene Gorilla Sanctuary and Mone River Forest Reserve, Cameroon (n=18).

Assay	**Organisms Detected**	**Pathogen Group**	**No. (%) Positive Individuals**
Cryptosporidium parvum	*Cryptosporidium parvum*	Parasite	12 (66.67%)
Adenovirus	All Adenovirus serotypes except 40 and 41	Virus	7 (38.89%)
All diarrheagenic *E. coli*		Bacteria	5 (27.78%)
Enterotoxigenic *E. coli*	*E. coli* carrying the virulence gene for a heat-labile (LT) OR heat-stable (ST) enterotoxin		4 (22.22%)
Enteropathogenic *E. coli*	*E. coli* carrying the gene (*eae*) encoding the outer membrane protein intimin and causing pathogenesis through attachment and effacement of human epithelial cells		1 (5.56%)
Enterococcus faecalis	*Enterococcus faecalis*	Bacteria	5 (27.78%)
Escherichia coli/Shigella (*ipaH* gene)	*Escherichia coli* and *Shigella* species carrying the invasion plasmid antigen H gene	Bacteria	2 (11.11%)
Salmonella spp.	*Salmonella bongori* and all subspecies of *Salmonella enterica*	Bacteria	2 (11.11%)
Enterovirus	all Enterovirus serotypes with the *Enterovirus* genus	Virus	1 (5.56%)

Table 4. Comparison of enteric pathogen prevalence of two Cross River gorilla (*Gorilla gorilla diehli)* populations in Cameroon (Kagwene Gorilla Sanctuary, n = 10; Mone River Forest Reserve, n = 8).

Assay	**Pathogen Group**	**No. (%) Positive Individuals**	
		Kagwene	**Mone**
Cryptosporidium parvum	Parasite	6 (60.00%)	6 (75.00%)
Adenovirus	Virus	5 (50.00%)	2 (25.00%)
All diarrheagenic *E. coli*	Bacteria	1 (10.00%)	4 (50.00%)
Enterotoxigenic *E. coli* (LT, ST)		0 (0.00%)	4 (50.00%)
Enteropathogenic *E. coli (eae)*		1 (10.00%)	0 (0.00%)
Enterococcus faecalis	Bacteria	1 (10.00%)	4 (50.00%)
Escherichia coli/Shigella (*ipaH* gene)	Bacteria	0 (0.00%)	2 (25.00%)
Salmonella spp.	Bacteria	1 (10.00%)	1 (12.50%)
Enterovirus	Virus	1 (10.00%)	0 (0.00%)

Table 5. Number of enteric pathogens detected in coinfected Cross River gorillas (*Gorilla gorilla diehli)* in Kagwene Gorilla Sanctuary and Mone River Forest Reserve, Cameroon (n = 10).

Pathogens Detected	**No. (%) Positive Individuals**
2 pathogens	6 (60.00%)
3 pathogens	2 (20.00%)
4 pathogens	2 (20.00%)

Table 6. Comparison of the number of enteric pathogens detected in coinfected Cross River gorillas (*Gorilla gorilla diehli)* in two Cameroon populations (Kagwene Gorilla Sanctuary, n = 4; Mone River Forest Reserve, n = 6).

Pathogens Detected	**No. (%) Positive Individuals**	
	Kagwene	**Mone**
2 pathogens	3 (75.00)	3 (50.00%)
3 pathogens	1 (25.00%)	1 (16.67%)
4 pathogens	0 (0.00%)	2 (33.33%)

Table 7. Enteric pathogens in coinfected Cross River gorillas (*Gorilla gorilla diehli*) in Kagwene Gorilla Sanctuary and Mone River Forest Reserve, Cameroon (n = 10). Red cells indicate bacterial pathogens, blue cells indicate viral pathogens, and yellow cells indicate parasitic pathogens.

	***E. coli/Shigella* (*ipaH* gene)**	***Enteropathogenic E. coli* (*eae* gene)**	**Enterotoxigenic *E. coli* (LT, ST)**	***Salmonella* spp.**	***E. faecalis***	**Enterovirus**	**Adenovirus**	***C. parvum***
G020	X				X			
G019					X			X
G004							X	X
G017		X					X	
G008						X		X
G015	X		X				X	
G001				X			X	X
G016			X					X
G013			X		X		X	X
G014			X	X	X			X

Table 8. Enteric pathogen prevalence of eastern chimpanzees (*Pan troglodytes schweinfurthii*) in the Kasakela, Mitumba, and Kalande communities of Gombe National Park, Tanzania (n = 56).

Assay	**Organisms Detected**	**Pathogen Group**	**No. (%) Positive Individuals**
Adenovirus	All Adenovirus serotypes except 40 and 41	Virus	29 (51.79%)
All diarrheagenic *E. coli*		Bacteria	9 (16.06%)
Enterotoxigenic *E. coli*	*E. coli* carrying the virulence gene for a heat-labile (LT) OR heat-stable (ST) enterotoxin		5 (8.93%)
Enteroaggregative *E. coli*	*Escherichia coli* carrying a virulence gene (*aaiC*) associated with causing pathogenesis through aggregation in the intestinal mucosa		4 (7.14%)
Enteropathogenic *E. coli*	*Escherichia coli* carrying the gene (*bfpA*) encoding the bundle-forming pilus and causing pathogenesis through attachment and effacement of human epithelial cells		1 (1.79%)
Cryptosporidium parvum	*Cryptosporidium parvum*	Parasite	7 (12.50%)
Enterovirus	All Enterovirus serotypes with the *Enterovirus* genus	Virus	5 (8.93%)
Giardia spp.	All *Giardia* species infecting humans	Parasite	5 (8.93%)
Trichuris trichiura	*Trichuris trichiura* (*Trichocephalus trichiuris)*	Parasite	3 (5.36%)
Escherichia coli/Shigella (*ipaH* gene)	*Escherichia coli* and *Shigella* species carrying the invasion plasmid antigen H gene	Bacteria	2 (3.57%)
Aeromonas spp.	*Aeromonas hydrophila, caviae, veronii, jandaei, salmonicida, schubertii, popofii*	Bacteria	1 (1.79%)
Enterococcus faecalis	*Enterococcus faecalis*	Bacteria	1 (1.79%)
Norovirus GII	Norovirus belonging to genogroup 2	Virus	1 (1.79%)
Rotavirus A	Rotavirus A species from the Rotavirus genus	Virus	1 (1.79%)

Table 9. Comparison of enteric pathogen prevalence in two eastern chimpanzee (*Pan troglodytes schweinfurthii*) communities in Gombe National Park, Tanzania (Kasakela, n = 38; Mitumba, n = 15).

Assay	**Pathogen Group**	**No. (%) Positive Individuals**	
		Kasakela	**Mitumba**
Adenovirus	Virus	21 (55.26%)	7 (46.67%)
All diarrheagenic *E. coli*	Bacteria	5 (13.16%)	4 (26.67%)
Enterotoxigenic *E. coli* (LT, ST)		4 (10.53%)	1 (6.67%)
Enteroaggregative *E. coli (aaiC)*		2 (5.36%)	2 (13.33%)
Enteropathogenic *E. coli (bfpA)*		0 (0.00%)	1 (6.67%)
Cryptosporidium parvum	Parasite	6 (15.79%)	1 (6.67%)
Enterovirus	Virus	5 (13.16%)	0 (0.00%)
Giardia spp.	Parasite	3 (7.89%)	1 (6.67%)
Trichuris trichiura	Parasite	2 (5.36%)	1 (6.67%)
Escherichia coli/Shigella (*ipaH* gene)	Bacteria	1 (2.63%)	1 (6.67%)
Aeromonas spp.	Bacteria	0 (0.00%)	1 (6.67%)
Enterococcus faecalis	Bacteria	0 (0.00%)	1 (6.67%)
Norovirus GII	Virus	1 (2.63%)	0 (0.00%)
Rotavirus A	Virus	1 (2.63%)	0 (0.00%)

Table 10. Number of enteric pathogens detected in coinfected eastern chimpanzees (*Pan troglodytes schweinfurthii*) in the Kasakela and Mitumba communities of Gombe National Park, Tanzania (n=19).

Pathogens Detected	**No. (%) Positive Individuals**
2 pathogens	13 (68.42%)
3 pathogens	5 (26.32%)
4 pathogens	1 (5.26%)

Table 11. Comparison of the number of enteric pathogens detected in coinfected eastern chimpanzees *(Pan troglodytes schweinfurthii*) in the Kasakela and Mitumba communities of Gombe National Park, Tanzania (Kasakela, n = 15; Mitumba, n = 4).

Pathogens Detected	**No. (%) Positive Individuals**	
	Kasakela	**Mitumba**
2 pathogens	12 (80.00%)	1 (25.00%)
3 pathogens	2 (13.33%)	3 (75.00%)
4 pathogens	1 (6.67%)	0 (0.00%)

Table 12. Enteric pathogens in coinfected eastern chimpanzees *(Pan troglodytes schweinfurthii*) in the Kasakela and Mitumba communities of Gombe National Park, Tanzania (n = 19). Red cells indicate bacterial pathogens, yellow cells indicate parasitic pathogens, and blue cells indicate viral pathogens.

	***E. coli/Shigella* (*ipaH* gene)**	***Aeromonas* spp.**	***Enteropathogenic E. coli* (*bfpA* gene)**	***Enteroaggregative E. coli* (*aaiC* gene)**	***Enterotoxigenic E. coli* (LT, ST)**	***C. parvum***	***Giardia* spp.**	***T. trichuria***	**Adenovirus**	**Enterovirus**
GM4680						X			X	
GM5178							X		X	
GM5123						X			X	
GM5181						X			X	
GM5006						X			X	
GM5161									X	X
GM5166									X	X
GM4973									X	X
GM5148								X	X	
GM5145				X					X	
GM5044				X					X	
GM5171					X				X	
GM5139							X			X
GM5107	X					X			X	
GM4968			X			X			X	
GM5177		X						X	X	
GM5136				X	X				X	
GM5162	X			X			X			
GM4674					X		X	X	X	