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Kristen E. Cross

April 15, 2013

"Factors Affecting Enteric Adenovirus Infection Dynamics in Wild Chimpanzees (*Pan troglodytes*) in Gombe National Park, Tanzania: Implications for Global Health and Biodiversity Conservation"

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

"Factors Affecting Enteric Adenovirus Infection Dynamics in Wild Chimpanzees (*Pan troglodytes*) in Gombe National Park, Tanzania: Implications for Global Health and Biodiversity Conservation"

By Kristen Cross

Adenoviruses have been isolated from human tissues since the early 1960s and are one of the most well studied viruses known today. However, our understanding of these viruses in other species remains largely unknown. Recently, adenoviruses were detected at high prevalence in wild non-human primate populations, raising concerns of the potential of zoonotic transmission to affect human health and wildlife conservation. Although in most cases these simian adenoviruses were shed from the gastro-intestinal tracts of individuals demonstrating no clinical symptoms, they have been implicated in fatal respiratory outbreaks, as well as gastroenteritis, in wild chimpanzees within Tai National Park in Cote d'Ivoire. To improve our understanding of this interplay, the current study examined factors that may affect the patterns of adenovirus infection in two chimpanzee communities residing in Gombe National Park, Tanzania. From March 2010 to March 2011, 251 samples were collected from 62 individually recognized chimpanzees from the Kasakela and Mitumba communities. Aliquots of these samples were preserved and later screened using appropriate preservatives and protocols for adenoviruses, SIV, and gastrointestinal viruses. In addition to patterns of co-infection with gastrointestinal parasites and SIV, chimpanzee community was also considered as a factor that may affect adenovirus infection dynamics since the chimpanzee communities examined differed in their patterns of overlap with people and domestic animals. None of the factors examined demonstrated significant associations with adenovirus shedding. Future studies should

use sequencing technologies to better understand adenovirus transmission dynamics within the Greater Gombe Ecosystem.

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Abstract

Adenoviruses have been isolated from human tissues since the early 1960s and therefore are one of the most well studied viruses known today. However, our understanding of these viruses in other species remains largely unknown. Recently, adenoviruses were detected at high prevalence in wild non-human primate populations, raising concerns of the potential of zoonotic transmission to affect human health and wildlife conservation. Although in most cases these simian adenoviruses were shed from the gastro-intestinal tracts of individuals demonstrating no clinical symptoms, they have been implicated in fatal respiratory outbreaks, as well as gastroenteritis, in wild chimpanzees within Tai National Park in Cote d'Ivoire. To improve our understanding of this interplay, the current study examined factors that may affect the patterns of adenovirus infection in two chimpanzee communities residing in Gombe National Park, Tanzania. From March 2010 to March 2011, 251 samples were collected from 62 individually recognized chimpanzees from the Kasakela and Mitumba communities. Aliquots of these samples were preserved and later screened using appropriate preservatives and protocols for adenoviruses, SIV, and gastrointestinal viruses. In addition to patterns of co-infection with gastrointestinal parasites and SIV, chimpanzee community was also considered as a factor that may affect adenovirus infection dynamics since the chimpanzee communities examined differed in their patterns of overlap with people and domestic animals. None of the factors examined demonstrated significant associations with adenovirus shedding. Future studies should use sequencing technologies to better understand adenovirus transmission dynamics within the Greater Gombe Ecosystem.

Introduction

Zoonoses and Emerging Infectious Diseases

Until recently, ape conservation and human health have been treated as separate fields, with one involving the work of field ecologists and the other being the focus of public health scientists and microbiologists. With 335 instances of emerging infectious diseases (EIDs) in the past 60 years and 60.3% of those attributed to zoonoses, the two fields are in crucial need of collaboration and co-operation (Jones et al. 2008). Of those EIDs originating in wildlife (71.8%), 25.4% are viral pathogens crossing over into new species. With the close phylogenic relationship between humans and non-human primates, great apes serve as an important sentinel species through which we can monitor not only the status of a highly endangered population, but also gain insight into which diseases are likely to cross the species gap into human populations (Leendertz et al. 2006, Calvignac-Spencer et al. 2012). A multitude of sampling methods have been developed in response to the growing need, including the methods used in this paper which involve non-invasive sampling and consistent and continuous monitoring of habituated populations (Gillespie and Chapman 2006, Leendertz et al. 2006, Gillespie et al. 2008). While the benefits of habituation have been called into question due to the frequency of fatal outbreaks in populations exposed to ecotourism and research, it is important to continue observations with strictly imposed regulations for the health of all parties involved (Wallis and Rick Lee 1999, Taylor et al. 2001, Rouquet et al. 2005, Lonsdorf et al. 2006, Pusey et al. 2007, Pusey et al. 2008). Systemic screening for pathogens residing in great ape populations is an important aspect for conservation and human health. This allows us to have a better understanding of

transmission dynamics of the pathogens and factors that put human and ape populations at risk, including co-infection with seemingly non-pathogenic species. While some strains of Simian Immunodeficiency virus (SIV) and adenovirus show no overt clinical symptoms, their effect on immune-compromised individuals is not well understood. This study aimed to improve our understanding of factors that may affect patterns of adenoviruses infection, including SIV_{cpz} (a strain specific to chimpanzees) and gastrointestinal parasite co-infection and human/domestic animal overlap, in chimpanzee communities residing in Gombe National Park, Tanzania.

Background of Gombe National Park and the Chimpanzees

Originally established as a game reserve by the British government, Gombe National Park is now a narrow strip of land approximately 14km long and 2-3.5km wide on the eastern most edge of Tanzania. Access to Lake Tanganyika is shared by the animals in the park in addition to the human settlements in the surrounding borderland (Pusey et al. 2008). The park is divided into three distinct chimpanzee communities: Kasakela, Mitumba and Kalande. Kasakela is the most naturally protected region of the park, and received most of its attention from the initial behavioral studies completed there in the 1960s by Jane Goodall and her team. The chimpanzees that reside there are completely surrounded by the borders of the park and their habitat does not touch the surrounding villages. The size of the population has fluctuated from 32 to 68 individuals over the past 40 years of study, but according to the most recent census in 2008, the population is now at 62 individuals. Mitumba, to the contrary, borders the northern most region of the park and touches a nearby village. The chimpanzees residing in this area are known to venture out into the

village, raiding crops and increasing their contact with humans and their domestic animals. The chimpanzees of the Mitumba community were habituated to humans later (in the mid-1980s as opposed to the early 1960s) and include a smaller number of individuals (approximately 25) (Pusey et al. 2008). The Kalande community of about 40 individuals has never been habituated, but has been consistently monitored since 1999.

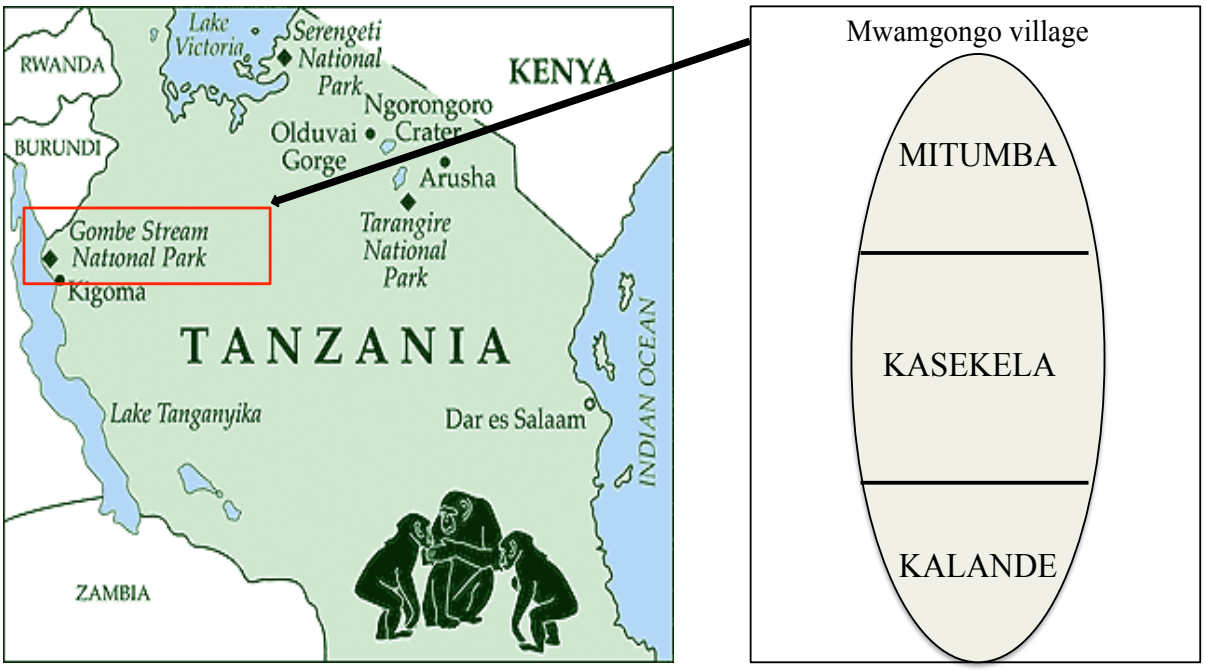


Figure 1. Map of Gombe National Park, Tanzania. Figure on the left shows the relative location of the park within Tanzania and the figure on the right depicts the relative locations of the three chimpanzee communities.

The habituation of many of the chimpanzees has led to closer and more frequent human-ape contact and may, as an unintended consequence, have resulted in the transfer of disease-causing agents between humans and primates in and near the 35km^2 park. While disease is merely one of the complex risk factors affecting the health and vitality of these endangered individuals, it has drawn significant attention in the past few years as virulent pathogens have been confirmed crossing the inter-species gap, in some cases causing

fatalities on both sides (Brack 1987, Daszak et al. 2000, Ferber 2000, Leendertz et al. 2006, Wittmann et al. 2007, Köndgen et al. 2008, Williams et al. 2008, Calvignac-Spencer et al. 2012). Although chimpanzees (*Pan troglodytes*) are the most wide-spread and commonly occurring of the great apes, they require a significant time to reach sexual maturity and reproduce at a staggered rate, making them especially vulnerable to habitat loss and disease spread (Pusey et al. 2007, Oates et al. 2010). The number of individual chimpanzees living in the wild is estimated to have decreased from approximately 1 million in 1900 (Teleki 1989) to an estimated 172,200–299,700 in 2000 (Butynski 2003). Much of this decline can be attributed to habitat loss and poaching, which prior to the establishment of the region as a national park were exceedingly common (Pusey et al. 2007). Morbidity and mortality from infectious diseases have not decreased as a result of the increased protection of the Gombe chimps. Ebola and anthrax epidemics have swept through chimpanzee populations, leaving a multitude of mortalities in their wake, and although these have not been traced directly to human origin, we should heed the warnings given to us by the histories of other habituated parks. The vulnerability of these populations requires our unyielding attention to safe healthcare practices when there is any form of contact with chimpanzees (Woodford et al. 2002, Wittmann et al. 2007).

Of the three Gombe chimpanzee communities, disease has been most thoroughly studied in Kasakela, where it is the major cause of death among the animals, accounting for 58% of the 86 deaths of known causes in the region over the last 40 years (Nishida et al. 2003, Williams et al. 2008). Many of these were the result of epidemics, such as polio-like disease in 1966 (six mortalities), mange in 1997 (three deaths), and respiratory disease outbreaks in 1968 (four mortalities), 1987 (nine mortalities), and 2000 (two mortalities).

Those individuals affected by respiratory illness showed symptoms similar to those described in primates in Taï National Park, Côte d'Ivoire (Köndgen et al. 2008). Despite these concerning trends, the Kasakela community has a growth rate of zero, as opposed to the negative rates measured by Hill et al. 2001 in other populations of chimpanzees in Mahale and Taï National park. These results have been attributed to the relatively sheltered nature of the Kasekela community, in addition to the care given by the researchers in the park upon discovery of a major epidemic. As logging and poaching decreased within the park boundaries, food availability increased, supporting population growth. In contrast, much less information is currently available for the Mitumba community. In 1996, six years after the onset of habituation via banana provisioning in the park, a respiratory outbreak killed 32% of the community. The provisioning of bananas by hand continued much later (until 2000) in the Mitumba community, compared to Kasekela, where it was replaced by sanitized machines in 1968 (Wallis and Rick Lee 1999). In addition, *Balantidium coli* and *Entamoeba histolytica* were found at higher prevalence in the Mitumba community as opposed to Kasekela, indicating increased human contact and potentially lower chimpanzee health (Gillespie et al. 2010). The Mitumba community also faces the threat of the encroaching larger Kasakela community, since smaller communities- particularly those that have lost adult male chimpanzees- are sometimes too weak to defend themselves against attack from larger communities that wish to claim their resources (Wilson and Wrangham 2003).

The historically divided communities within the park and the discrepancies in their past and current contact with humans create an interesting study site for differences in pathogen prevalence and transmission. In order to fully understand the dynamics and risk

factors associated with emerging zoonotic diseases, it is beneficial to study populations varying in human contact rates.

Background about Adenovirus

Adenoviruses (family *Adenoviridae*) consist of five subgenera (*Mastadenovirus, Atadenovirus, Aviadenovirus, Siadenovirus, and Ichtadenovirus*). These viruses are encased in an icosahedral non-enveloped shell containing double-stranded DNA. Although these viruses typically have very specific host ranges, they have been shown to jump between closely related hosts. Evidence of such host switching has primarily been seen between humans and great apes, with prevalence as high as 59% PCR-positive for serotypes belonging to the *Mastadenovirus* genus being found in populations of wild chimpanzees. These patterns suggest high zoonotic transmission potential for this virus in habitats shared between humans and wildlife (Roy et al. 2009, Wevers et al. 2010, Wevers et al. 2011, Roy et al. 2012). These recent findings have focused primarily on the phylogenetic characterization of these viral strains in order to better understand their transmission history. With non-human primates as an established reservoir for S-Adv and serotypes of H-Adv strains –B, -C, -E, -F and –G (Wevers et al. 2011), it is important to monitor potential spillback events and risk factors that may lead to infection or the development of overt clinical symptoms, as have been shown previously in human clinical studies (Kojaoghlanian et al. 2003).

Adenoviruses are thought to originate primarily in humans and therefore wildlife infection with adenoviruses may be a good indicator of the amount of transmission occurring between humans and wildlife in a given region. Though previous studies of

phylogeny have shown that several types of adenoviruses may infect chimpanzees, for this study we focus specifically on those serotypes that are know to be implicated in gastroenteritis, namely adenoviruses 40 and 41. The reasoning behind this focus was the vulnerable status of the population and the crucial need for non-invasive sampling via fecal specimen collection. Though it is possible to isolate other adenovirus serotypes that are implicated primarily respiratory ailments from stool, it is not as common. The Gombe Stream Research Centre is the perfect study site for understanding the influence of human interaction on adenovirus transmission, as the Gombe chimpanzee communities fall along a well-understood and documented gradient of human exposure. Viruses are thought to be the pathogen that transmits most easily between different species hosts, as its poor replication machinery leads to its ability to rapidly mutate (Köndgen et al. 2008).

Background about SIV Infection in Gombe

Although many strains of simian immunodeficiency viruses naturally infect wild NHP and many infected individuals are asymptomatic, there is growing concern in Gombe National Park as some of the infected individuals have shown marked lower birth rates and a 10 to 16 fold increased death risk as compared to those not infected with the virus (Keele et al. 2006). Once assays were developed that could detect viral pathogens from urine and fecal material, the chimpanzees of Gombe were screened on a consistent basis in order to better understand mortality and morbidity associated with infection (Keele et al. 2009). Sampling in 2009 showed a prevalence of almost 18% infected with SIV in the Kasakela and Mitumba populations. The observational data that was collected from the confirmed SIV_{cpz} positive individuals showed that infected chimpanzees do have an increased risk of death,

presumably by immune system suppression and resulting co-infection (Keele et al. 2006, Keele et al. 2009).

Gastro-intestinal parasites and immune response

Commensal parasites are an important component of the intestinal tracts of chimpanzees. The presence of particular ciliate protozoan species that have evolved with their hosts have been used as indicators of individual and environmental health, as they decrease inversely with the amount of stress the individual is experiencing. Their presence is affected by anthropogenic disturbance, with lower richness numbers in parks with higher rates of habitat disturbance (Howells et al. 2011). More pathogenic parasites can also be found shed from the gastro-intestinal tract of individuals living in less-pristine regions, including *Oesophagostomum* sp., *Necator* sp., *Strongylodies fulleborni*, *Cryptospordium* sp., *Giardia* sp. and *Balantidium coli*, though for the purposes of this study only the former three were examined. High prevalence of these parasites in an individual can cause malnutrition, dysentery, serious weight loss and death in their primate hosts (Harper et al. 2005). *Oesophagostomum* sp. are also an important parasite in human health, as infections with this parasite are frequently associated with abdominal pain, diarrhea and secondary bacterial infections (Brack 1987). In non-human primates, high infection rates can lead to nodular oesphagostomosis without severe clinical signs, but chimpanzees in Gombe have been observed with oesophagotomosis-related morbidity and mortality (Huffman et al. 1997). These parasites are also implicated in suppressed immune system function and reduced ability for reproduction in non-human primates (Gillespie and Chapman 2006, Howells et al. 2011). On the other hand, presence of commensal ciliates,

such as *Troglodytella abrassarti* and *Troglocorys cava* can act as useful indicators of ecosystem and dietary health (Modrý et al. 2009, Tokiwa et al. 2010). Changes in the presence and distribution of these commensal parasites can indicate stress in the environment and can also suppress the immune system, making the individual more susceptible to co-infection with deadly pathogens.

Introduction of hypothesis and the experiment

Based on past findings of adenovirus and SIV infections in wild chimpanzees, our research seeks to better understand the interactions of these viruses by the decreased immune function caused by shifts in gastro-intestinal parasite load. We would expect the more pristine Kasakela community to have lower rates of H-Adv strains, as well as lower prevalence of stress induced nematode loads. The Mitumba community, on the other hand, with their high rates of contact with humans and domestic animals in the nearby villages, are surmised to carry high a higher burden of infectious agents. In general, we were looking to see how the communities differ along this interaction gradient, as well as how much co-infection with SIV and nematode larvae affect the shedding of adenovirus in fecal material. This study seeks to test factors that may affect adenovirus shedding in wild-living, habituated chimpanzee populations.

Hypotheses:

1. Adenovirus shedding is higher in the Mitumba community than in the Kasekela community due to increased human contact along the border of the park.

2. Individuals with SIV are more likely to shed adenovirus particles.

3. Individuals with higher nematode species richness are more likely to have adenovirus shedding, and in contrast, those with higher ciliate species richness will have less shedding.

Methods

Sample Collection

Freshly voided fecal samples were collected from 62 individuals in 44 distinct locations within the Gombe Stream National Park. The sample set is a collection from 4 total sampling periods, beginning in March of 2010 and ending in March 2011. For this study, a subset of the samples were used, namely only those chimpanzees of the Kasakela and Mitumba groups and those samples from the first three sampling seasons. Individual shedding across multiple seasons was tested where possible in order to account for seasonal sampling bias. Samples were collected directly from the field and divided into one of three preservative methods. For gastro-intestinal parasite identification, the fecal material was transferred to ParaPak® containers (Meridian Bioscience,Cleveland, OH) labeled by individual and prefilled with 15 ml of 10% formalin solution. For samples to be tested for SIV infection, samples were preserved in RNA later and for adenovirus testing the samples were preserved in potassium dichromate. Upon arrival, nucleic acids were extracted from the fecal material preserved in potassium dichromate using the protocol outlined by the FastDNA® SPIN Kit for Soil (MP Biomedicals). The extracted material was stored in the -80 freezer until used for molecular analysis.

Determination of Adenovirus infection status

We used forward primers -*5'GCC GCA GTG GTC TTA CAT GCA CAT C 3'*- and reverse primers -*5'CAG CAC GCC GCG GAT GTC AAA GT 3'*- in a one-step PCR in order to amplify fragments of the hexon gene of HADV-A 40 and 41 (Allard 1990). The specificity of these primers has been shown as broad enough to detect all six subgenera of human adenovirus. Each reaction consisted of the following proportions of master mixture: 5µL 5x Green GoTaq® Reaction Buffer (Promega), 0.5µL of 10mM dNTPs (Promega), 0.25µL of each the forward and reverse primers at a concentration of 10mM, 0.25µL of Bovine Albumin Serum (diluted lyophilized powder from Beta South Technologies), 0.25µL of GoTaq® polymerase and the rest molecular water to make a total reaction mixture of 20µL. 5µL of template DNA was added to each reaction tube in duplicate in order to improve accuracy and help combat the unknown concentration of DNA in each sample. Reactions were run at an initial denaturation step at 95°C for 5mins, then 35 cycles of the following three steps: 30 seconds of denaturation at 95°C, 30 seconds of annealing at 55°C, with a 30 second elongation at 72°C. A final elongation step at 72°C for 5 minutes completed the cycle. Two positive and negative controls were run with each PCR set by adding 5µL of heat released HAdv to each positive master mix reaction and 5µL of sterile water to the negatives.

PCR samples were either run immediately after the completion of the thermocycler when possible, and otherwise were stored at 4°C for a maximum of 1 day until gel electrophoresis. Samples were run on a 2% SeaKem LE Agarose gel injected with 1.25µL of 2.5µg/mL of EtBr (Lonza, Inc.). 5µL of PCR product was added to each well at then run at 80V for 45 minutes. Gels were kept to one row per gel to prevent overlap of the green GoTaq® dye with the next row of product. Gels were visualized under UV light using GelDoc™ XR Software. The product was visualized as a 308bp fragment.

Determination of gastro-intestinal parasite infection status

A two-part method consisting of a floatation and sedimentation specialized for fecal material recovered from herbivores was used to maximize the number of parasites that can be detected via bright field microscopy (Gillespie and Chapman 2006). The flotation method isolates the less dense helminth eggs and larvae from fibrous material in the diet.. Formalin was first be washed from the sample by adding distilled water to standard test tube filled ½ to ⅓ of the way with fecal material (depending on the density and availability of the material). The sample was then spun at 1800rpm for 10 minutes until the fecal pellet had completed settled at the bottom of the tube and the resulting supernatant mixture of formalin and water can be discarded. An ionic mixture of NaNO3 (6.68mol) in 1L of distilled was added to the pellet. The mixture was re-suspended and then centrifuged once more at 1800rpms for 10 minutes with a glass cover slip placed on top of the test tube to catch helminth eggs (Fischer Scientific). The cover slip was then removed and placed on a glass slide with a drop of diluted Lugol's Iodine (half concentrated iodine and half distilled water). The samples were then screened under a compound microscope and parasites differentiated to the genus and species level where possible based on cyst size, shape and the morphology and location of the larval contents. Each parasite of a given genus/species was counted and measured on the 40x objective to the 0.1µm size (length/width). Any unknown species were photographed and sent to parasitology specialists for identification (Gillespie and Chapman 2006).

The fecal sedimentation protocol allows for the isolation of heavier parasites (i.e., Trematodes) and commensals (i.e., entodiomorph ciliates) from the fecal mass. The pellet

and NaNO3 suspension was then washed with and dilute soapy solution of soap and distilled water (Fischer Scientific) and filtered through a thin strip of cheese cloth using reusable plastic cups into a 50mL centrifuge tube. The pellet was then left to reform and once the solution had completely settled (about an hour), the supernatant was removed and discarded using a disposable micropipette. The remaining pellet was washed a final time with the dilute soapy mixture and filtered through the same cheesecloth back into the same 50mL tube. Once the pellet had reformed, three drops of the settled solution (taken from the most dense part of the mixture) were added to a slide with a drop of Lugol's iodine and covered with coverslips. Parasites were then identified and counted in the same methodology as before. This second component to the procedure helps to visual larger parasites, mainly ciliate and also reinforces the prevalence of species seen in the floatation. Final counts of parasites from the flotation and sedimentation analysis were then entered into a database matched with the individual and the date, time and location of the sample collection (Gillespie and Chapman 2006).

Determination of SIV Status

Fecal samples from individuals across Gombe national park were collected from March 2010- January 2011. The samples were associated with the individual by careful observation, but in cases where the individual could not be affiliated with the sample by direct observation, molecular analysis was done to ensure host DNA identification. Samples were run through western blot analysis and screened for SIVcpz antibodies. A subset of the antibody positive samples was then screened additionally via reverse transcriptase polymerase chain reaction (RT-PCR) for confirmation. The data compilation from this study

was used to associate values from individuals screened additionally for gastrointestinal parasites and adenovirus. Samples that had been collected around the same time (within one month) were used to determine the SIV status of that individual. A subset of individuals were tested multiple times for SIV and had the same status up to 7 months later, indicating that the assumption holds true.

Data Analysis/ Statistical Methods

Multiple datasheets were compiled for various tests and for all analyses except for comparisons of seasonal shedding of parasites and adenovirus, individuals were not duplicated. Sixty-two distinct individuals were tested in total from the park; forty-five from Kasakela and seventeen from Mitumba. Twenty-four individuals were tested multiple times with samples from different season collections (sampling period 1,2,3,or 4, shown in Table 1) in order to act as an indicator of viral shedding and how seasonality may affect it. Chi-square values were calculated to determine the trends in shedding between seasons.

Table 1. Dates for sample collection by sampling period

Sampling Period	Dates	No. of Samples Tested
1	March 2-May 8, 2010	56
2	July 10-July 23, 2010	0
3	November 4- November 26, 2010	6
4	January 15-February 5, 2011	1

Individuals were compared across between communities (Kasekela and Mitumba) in relation to adenovirus, SIV, and parasite prevalence using the chi-square test of independence. Co-infection rates were then examined by comparing the likelihood of adenovirus infection based on the SIV infection status of the individual using the Pearson correlation coefficient. As there were only 7 positive SIV individuals, a monte-carlo

randomization was performed before the chi-square. For analysis of co-infection with SIV and adenovirus, no individuals who had not been tested for both were included. This accounts for the difference in sample sizes for the communities between the different tests.

A chi-square test for parasite prevalence was done for each species of parasite between the two parks. Species richness was determined using the Shannon Weaver index and then compared between the two parks using a Mann-Whitney U test. These richness values were also tested for correlation with individuals infected with one or both of the viruses.

Parasite richness was broken down into ciliate richness and nematode species richness and then compared across communities, as well as between sexes and across sampling periods. The Pearson correlation coefficient was used to determine the relationship between SIV infected individuals and adenovirus-infected individuals. SPSS and Excel were both used in the statistical analyses.

Samples that were duplicates of the same individual were examined to see if individuals were more likely to be positive in one sampling period over another via a chi-square test. No significant difference was detected, indicating that for this study, one season was not more likely to have more shedding than the others. This is why we chose to include samples from various seasons and only exclude duplicates of individuals. The overwhelming majority of the samples were from sampling period 1, so we chose this sample to represent the duplicated individuals. As the number of parasite eggs shed from the intestinal tract is not a good measure of infection rates, we used species richness

instead of parasite egg counts to compare overall health in the two communities (Gillespie and Chapman 2006).

Results

Sixty-two chimpanzee individuals from Gombe National Park were tested for adenovirus presence in their stool. Forty-five of these were from the Kasekela community and seventeen were from Mitumba. These sample sizes represent a large portion of the community, though are not exhaustive. We tested for difference in viral load between communities and for co-infection rates between SIV and adenovirus.

Viral Infection

There was no significant difference in prevalence of adenovirus between the two Gombe communities, though the rate of positive individuals was higher by about 10% in the Mitumba community (Figures 2). Overall adenovirus prevalence rates for each tested individual were 58.1% positive and 41.9%. The presence of SIV was not correlated with a difference in infection by adenovirus (Figure 6, Pearson=-.007).

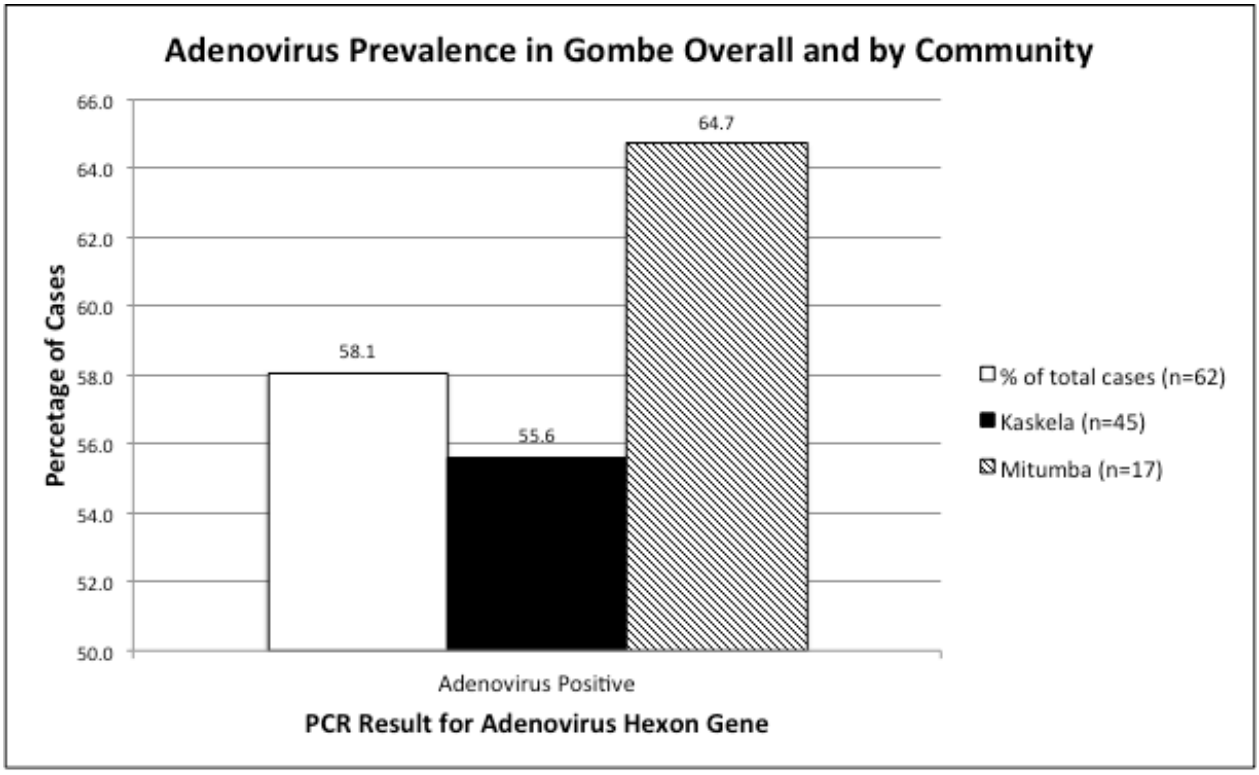


Figure 2. Bar chart showing the prevalence of adenovirus positive individuals total (open bar), those in the Kasekela community (closed bar) and those in the Mitumba community (striped bar). Number of tested individuals is shown to the right of the graphic.

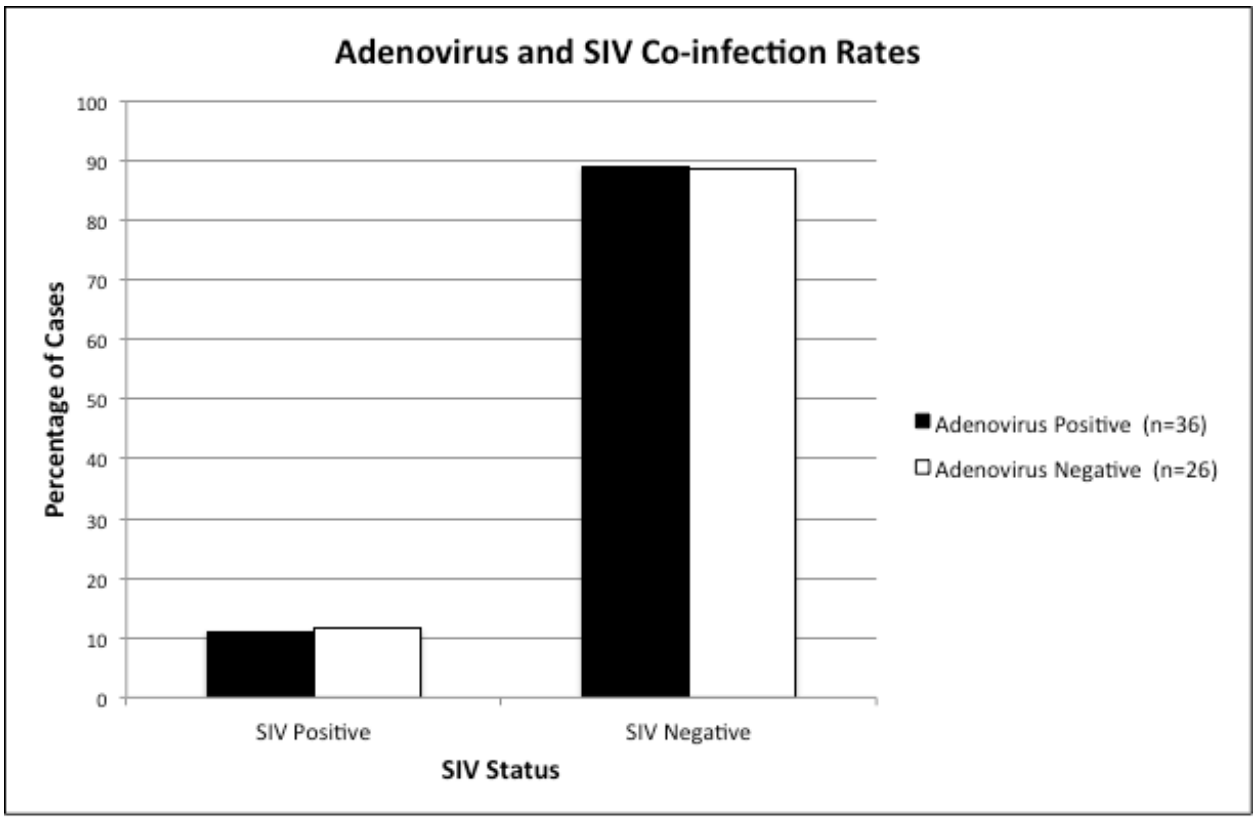


Figure 3. Results of chi-square test of independence for relationship between SIV infection and adenovirus particle shedding.

Gastro-Intestinal Parasites

The prevalence of each species of gastro-intestinal parasite did not differ across communities for most of the parasites, excluding a higher prevalence of *Physaloptera spp.* in Kasekela versus Mitumba (Table 2, p=.03). The mean number of parasites per individual gave too high of a variance, as the majority of the parasite load was harbored in a small percent of the population. We chose to compare medians instead and through this comparison, found that the communities showed similar trends in number of nematode and ciliate species.

Table 2. Comparison of prevalence of all parasite species and viruses between the two Gombe communities using chi-square test (*p<.05)

Parasite/ Virus Species	% Prevalence in Kasekela n=37	% Prevalence in Mitumba n=15	% Prevalence for total sample size n=52	Significance
Oesophagostumum sp.	86.5	93.3	88.5	
Necator sp.	24.3	26.7	25.0	
Probstmayria	2.7	0.0	1.9	
Stronglyloides foulbourni	21.6	26.7	23.1	
Physaloptera sp.	32.4	6.7	25.0	*
Trichuris sp.	2.7	0.0	1.9	
Trichostrongylus sp.	8.1	26.7	13.5	
Entamoeba coli	5.4	6.7	5.8	
Troglodytella abrassarti	73.0	80.0	75.0	
Troglocorys cava	5.4	0.0	3.8	
Adenovirus	55.6	64.7	58.1	
SIV	11.8	11.8	11.3	

Testing of the Pearson correlation coefficient showed no significant correlation between adenovirus infection and ciliate or helminths species richness (Figures 4 and 5).

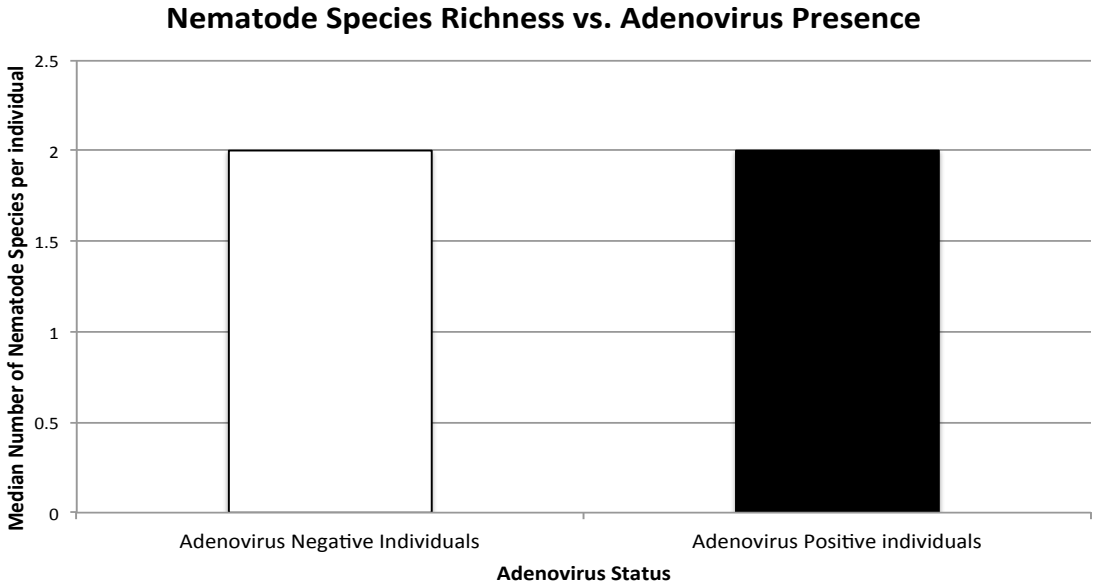


Figure 4. Bar chart shows the median number of nematode species per individual as compared between individuals determined to be adenovirus positive (closed bar) and negative (open bar).

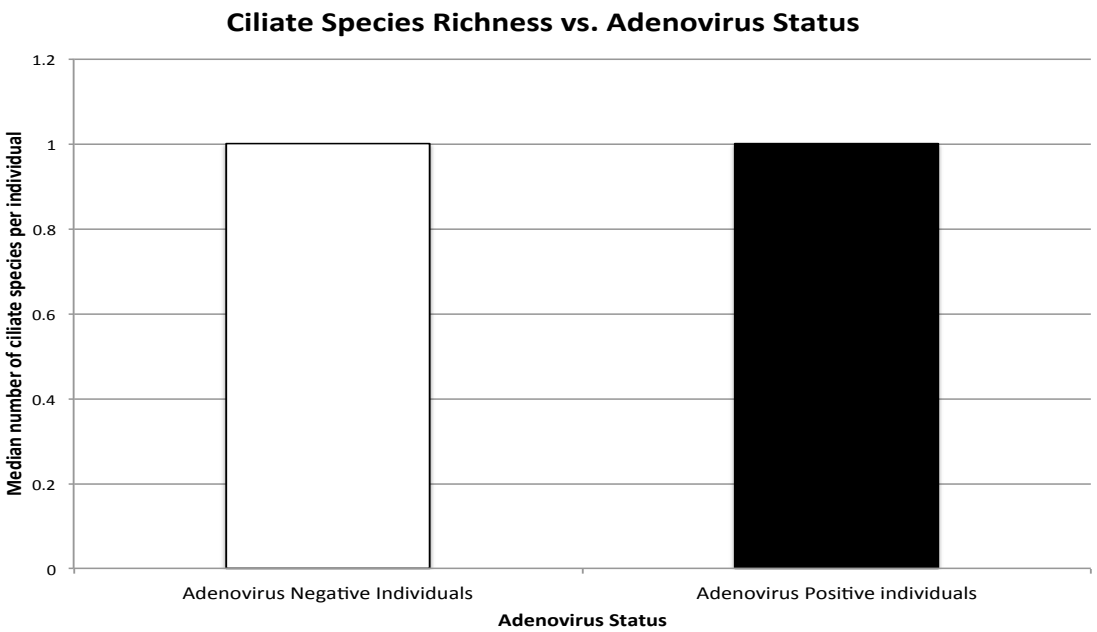


Figure 5. Bar chart shows the median number of ciliate species per individual as compared between individuals determined to be adenovirus positive (closed bar) and negative (open bar).

Other factors affecting adenovirus shedding

Sampling period was not correlated with ciliate richness or helminth richness (r^2=.005, .030). The same seasonal pattern could not be tested for SIV infected individuals because once they contracted the virus they were positive for the rest of the testing period.

Sex was also not correlated with ciliate richness or helminth richness (Figure 7, r^2=.027 and .002, respectively). There was also no significant difference between adenovirus or SIV infection between sexes (Chi-sqaure, P-value= 0.62 and 0.71 respectively). Age class revealed a trend of higher rates of infection in the sub-adult population (10 years of age or younger) as compared with the two other age classes.

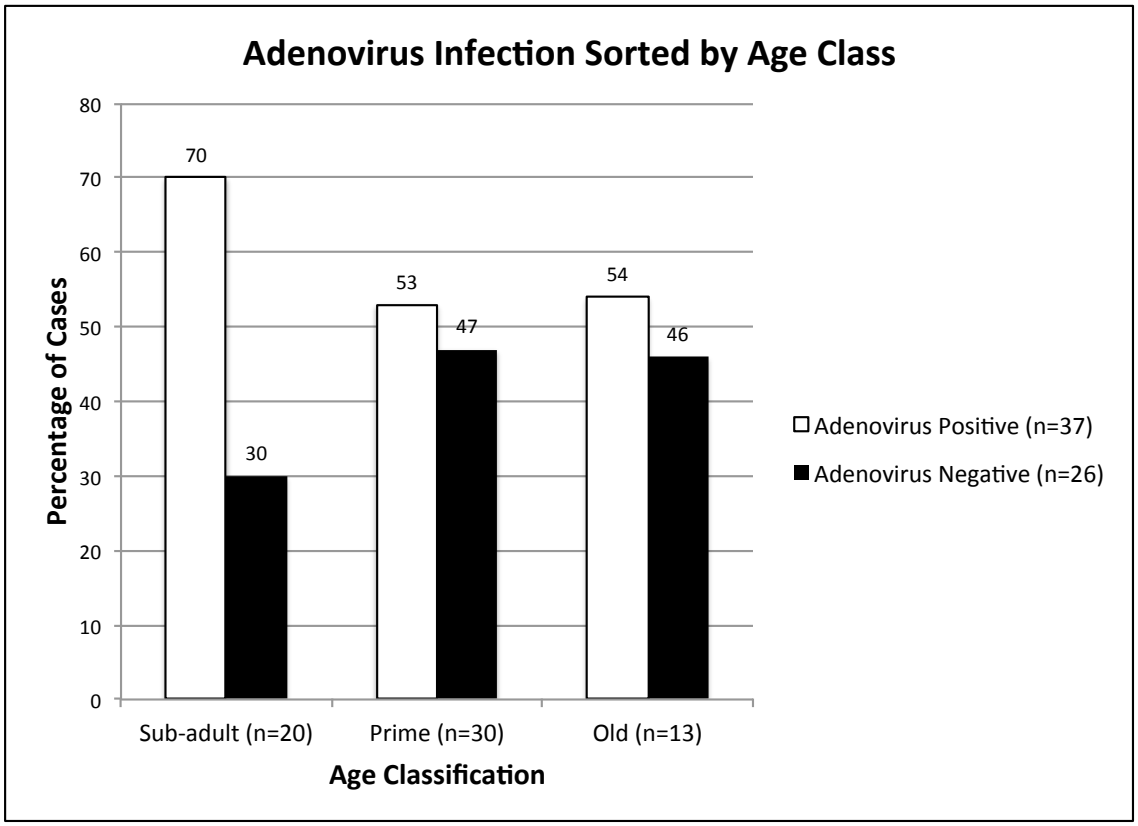


Figure 6. Adenovirus shedding differences in chimpanzees across age classes in Gombe National Park, Tanzania. Sub-adult= 10 years and younger; prime= 11-29; old= 30 years or older

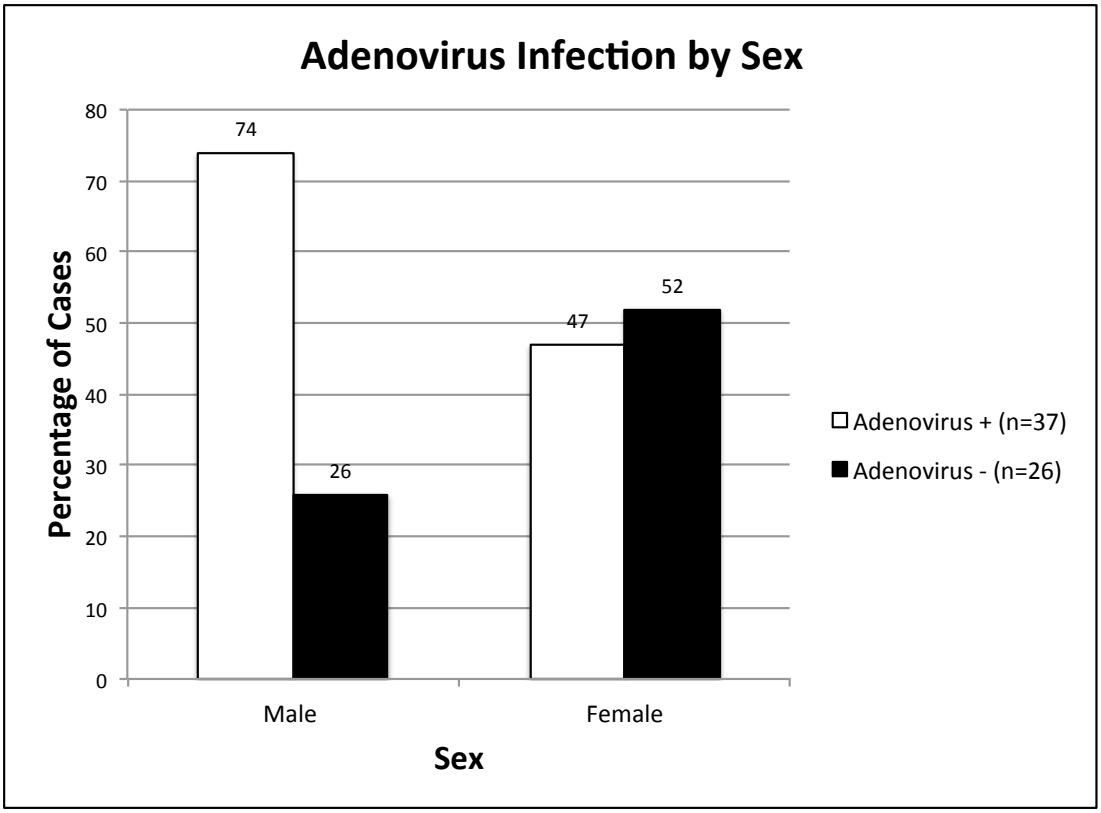


Figure 7. Adenovirus shedding differences between males and female chimpanzees in Gombe National Park, with adenovirus positive individuals represented by open bars and adenovirus negative individuals represented by darkened bars.

Discussion

Adenovirus shedding in the chimpanzee population of Gombe National Park are consistent with recent findings of high rates of adenovirus infection in non-human primates (58.1% positive, n= 62, Figure 6, Roy et al. 2009, Tong et al. 2010, Wevers et al. 2010a, Wevers et al. 2010b, Wevers et al. 2011, Roy 2012)). No significant differences in viral infection were found between Gombe chimpanzee communities. Overall, SIV infection rates were much lower (only 7 positive, n=62) and also showed no difference between communities. This result was surprising, as the individuals of the Mitumba community

frequent human villages and thus we expected that they would show a higher prevalence of infection by zoonotic pathogens. While the communities are distinct, perhaps they are not as separated in terms of the parasite community. Depending on the main route of adenovirus infection (we cannot be clear of the particular serotypes due to lack of genetic sequencing), the environment may have enough overlap to introduce the pathogens widely across the park (Pirtle and Beran 1991). There may also be multiple sources that allow adenovirus to infect individuals in both communities. In addition, we were also limited by determining simply presence or absence of the virus in the stool of an individual. Viral load could not be determined from this protocol, just as parasitic load could also not be determined. The burden of infection per individual would allow us to more completely understand the transmission dynamics of this population.

The lack of variability observed in patterns of parasitism between the two chimpanzee communities may be the result of the short-term nature of this sampling effort, as a previous long-term study of parasitism in these communities did find significant variability in parasitism (Gillespie et al. 2010). Many of the parasites recovered from chimpanzees in this study are transmitted primarily through the fecal-oral route and thus rely on density-dependent factors. As the Kasakela community is significantly larger than the Mitumba community, the Mitumba community experiences encroachment from Kasakela community patrols, as well as human populations moving into the forest from the north end of the park. It is important to note that for most parasite species on average, the prevalence was higher in Mitumba than in Kasakela and the mean number of parasites per individual was also higher. Although these results are not statistically significant, they may indicate an increased prevalence from previous surveys.

The limited variation in seasonality of these samples may also explain this difference, as many of the samples for which we had adenovirus data did not also have gastro-intestinal parasite data from the same sampling period (only 44 individuals out of 62). The prevalence of gastrointestinal parasites has previously been shown to vary significantly across seasons, with the lowest prevalence occurring between April and October, which encompasses the time span of the majority of the samples we tested for adenovirus (Gillespie et al. 2010). Data for the gastro-intestinal parasites was collected separately from the adenovirus data, so not all of the individuals that were tested for adenovirus also had information about gastro-intestinal parasites and vice versa.

The interaction between adenoviruses and SIV are still not well known. There have been fatal cases of co-infection in humans and an increased rate of adenovirus in correlation with the increased rate of HIV prevalence in particular regions (Kojaoghlanian et al. 2003, Kooriyama et al. 2012). Infection of the gastro-intestinal tract is the most common type of adenovirus infections (30% of serotypes) (Kojaoghlanian et al. 2003). Adenoviruses may only confer a disadvantage to the host when they are active, and we cannot be sure from the PCR result of positive shedding for particles that the virus is currently active. One of the main factors of pathogenesis from adenoviruses is that they stay in the body for extended periods of time and this can represent a constant threat to the host immune system should it be invaded by another pathogen (Kojaoghlanian et al. 2003). Prolonged shedding of the virus in HIV patients has been shown due to the longer period of time it takes for the immune system to clear the primary infection. This can also be transmitted sexually, so perhaps the mating structure of the population is leading to the multitude of infection (Kojaoghlanian et al. 2003).

Although not statistically significant, the Mitumba community did show a 10% higher shedding rate of adenovirus than the Kasakela community. This is especially concerning as the prevalence of several species of helminths was higher in this community as well. These higher burdens of helminthic organisms have previously been implicated in increased stress burden and lower immune response, therefore increasing the infection rates and pathogenicity of viruses such as SIV in chimpanzees (Ashford et al. 2000, Borkow and Bentwich 2006).

Increased Physaloptera spp. Prevalence in Kasakela

The higher prevalence of *Physaloptera* spp. observed in Kasakela compared to Mitumba does not seem to be driven by adenovirus shedding or SIV infection in the individuals. *Physaloptera* was found in 6 individuals who were negative for adenovirus and 6 individuals who were positive for adenovirus. The pathogenicity of this parasite has yet to be elucidated, and it is considered asymptomatic in the other mammal species in which it is found. The higher prevalence of this parasite may be driven simply by the larger population size in Kasakela, which allows access to more hosts and more opportunities for transmission.

The lack of variability in viral shedding between the two communities within Gombe, suggest that anthropogenic disturbance may not be the major driver of adenovirus infection in these chimpanzees. As there was no historical data on adenovirus infection from Gombe National Park, we cannot determine at this point whether adenovirus infection is a recent phenomenon in Gombe chimpanzees or is a chronic infection that began early in the park's history. The high prevalence of shedding from the individuals may

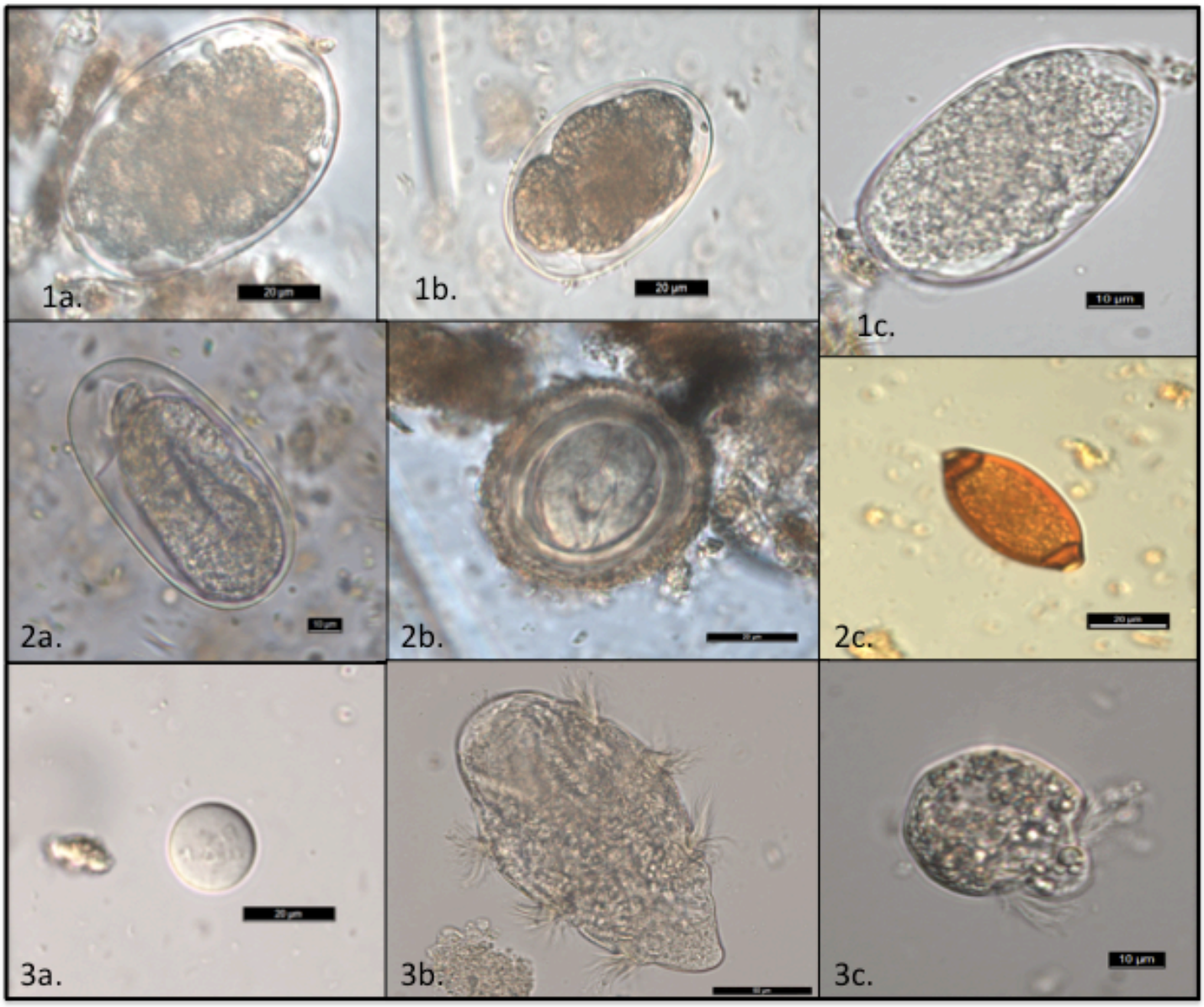
be asymptomatic, but is still concerning with the decreased ciliate richness of the individuals in this park versus more pristine parks, such as the chimpanzees of Goualougo Triangle. These chimpanzees can harbor up to 8 species of commensal gut bacteria, whereas captive chimps and the chimpanzees of Gombe have been shown to have only 1 to 2 (Gillespie et al. 2010, Howells et al. 2011).

The results of this study provide a baseline of adenovirus infection for the chimpanzees of Gombe National Park. Continual screening should take place to look for shifts in the infection rates or increases in pathogenic parasites and/or SIV infection. Though our study does not indicate any negative health impacts of adenovirus shedding, we provide an important comparison for studies of adenovirus infection in other parks and the factors that may influence its introduction and transmission. In light of recent studies confirming a high rate of shedding of adenovirus in non-human primates, it is crucial that we understand the long-term effects this chronic infection may have on the health of vulnerable primate population, as well as the threat it presents to human populations (Roy et al. 2009, Wevers et al. 2010b, Wevers et al. 2011, Roy 2012). Understanding the dynamics of disease transmission in this population is additionally important in order to be better prepared when known infectious agents cross into the population. Once the primary carriers of the parasite are identified, more effective methods of intervention can be implemented.

Future Directions

To continue this study, we would like to sequence some of the PCR samples to verify adenovirus shedding as well as confirm the species. Determination of phylogeny would help us determine zoonotic transmission and give insight into the origin of adenovirus at Gombe. GIS data would also allow us to look at transmission patterns in the park. Though none of our selected co-factors appeared to have a significant effect on adenovirus shedding, we would like to continue investigating the effect of viral load on primate health, as well as the rates of spill back into the human population.

Appendix I



1a. Oesophagostumum sp., 1b. Necator sp., 1c. Trichostrongylus sp.

2a. Stronglyloides foulbourni, 2b. Physaloptera sp., 2c. Trichuris sp.

3a. Entamoeba coli, 3b. Troglodytella abrassarti, 3c. Troglocorys cava

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