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:

_____	__4/17/2013_____
Ian R. Fried	Date

Effects of socio-ecological variability on patterns of gastrointestinal virus prevalence and diversity in the lemur community of Ranomafana National Park, Madagascar

By

Ian R. Fried
Bachelor of Science
Environmental Studies

Thomas R. Gillespie, PhD
Committee Chair

Uriel Kitron, PhD
Committee Member

Christine Moe, PhD
Committee Member

2013

Effects of socio-ecological variability on patterns of gastrointestinal virus prevalence and diversity in the lemur community of Ranomafana National Park, Madagascar

By

Ian R. Fried

Dr. Thomas Gillespie
Advisor
Department of Environmental Studies

An abstract of
A thesis submitted to the Faculty of the
Department of Environmental Studies
of Emory University in partial fulfillment
of the requirements for the degree of
Bachelor of Science with Honors

Department of Environmental Studies

2013

ABSTRACT

Effects of socio-ecological variability on patterns of gastrointestinal virus prevalence and diversity in the lemur community of Ranomafana National Park, Madagascar
By Ian R. Fried

Diarrheal viruses are amongst one of the most common causes of morbidity in humans in developing countries; however, they have seldom been studied in wild non-human primates (NHPs). Due to a similar evolutionary history between humans and NHPs, as well as the increasing proximity of human populations to shrinking NHP territories, an understanding of NHP disease has the potential to inform decision-making for primate conservation and human health. In the first study to examine diarrheal viruses in wild lemur taxa, we screened individual lemurs for adenovirus, enterovirus, rotavirus, and norovirus (genogroups GI and GII), which are found in human populations in Madagascar. Eighty-four fecal samples were non-invasively collected from the seven dominant species of lemurs of Ranomafana National Park (RNP), Madagascar, during the 2011 and 2012 dry seasons (May-August). Of the seven taxa tested, all were infected with one or more diarrheal viruses. Norovirus GII, the most commonly exhibited virus, was found in 26.7% of individuals, followed by adenovirus in 25%. Because these viruses are transmitted via the fecal-oral route, we associated lemur dietary habits with infection rates and found that individuals with more terrestrial feeding patterns had lower viral loads than more arboreal feeding species. Given the nature of transmission of these viruses, changes in prevalence patterns will likely differ in the rainy season. Transmission of these viruses from humans to lemurs poses a serious risk for these already endangered species, while transmission from lemurs to humans may represent a health risk for tourists and local inhabitants. Future sequence-based approaches will help to resolve the zoonotic potential of the viruses recovered from lemurs.

Effects of socio-ecological variability on patterns of gastrointestinal virus prevalence and diversity in the lemur community of Ranomafana National Park, Madagascar

By

Ian R. Fried

Dr. Thomas Gillespie
Advisor
Department of Environmental Studies

A thesis submitted to the Faculty of the
Department of Environmental Studies
of Emory University in partial fulfillment
of the requirements for the degree of
Bachelor of Science with Honors

Department of Environmental Studies

2013

ACKNOWLEDGMENTS

This study was carried out at Emory University in the departments of Environmental Studies (College of Arts and Science) and Environmental Health (Rollins School of Public Health) and at Centre Valbio in Madagascar. I would like to thank the Department of Environmental Studies for all of the financial, intellectual, and emotional support that they have provided me with over the last four years. Dr. Gillespie, my advisor, has made this entire thesis, as well as my love of research, possible and without him I am not sure what I would have done with my college career. His patience, advice, and guidance are always appreciated and have helped me immensely since I have been at Emory. I especially want to thank Dr. Sarah Zohdy, my unofficial co-advisor. I cannot thank you enough for holding my hand from the beginning and finally throwing me out of the nest (figuratively) when you thought the time had come. Your direction and time have been invaluable. I would also like to thank the entire Centre Valbio Infectious Disease Program team that Dr. Gillespie coordinates for their help with all stages of this work. Dr. Wright, a collaborator, has been one of my inspirations for this project and I will consider my life complete when I have accomplished half of the things that she has. Centre Valbio (founded by Dr. Wright) provided me with the research base station and lab necessary while in Madagascar. I would like to thank Eileen Larney for helping with the logistics of this project as well as exchanging well over 100 emails prior to me even arriving in Madagascar. MICET provided me with more necessary logistics that helped me not to get lost (literally and figuratively) from the moment I arrived in Madagascar. Madagascar National Parks has made all of this possible and without them I would have nothing to study.

I could not have accomplished any part of this project without Zakamanana Francois, Solo Justin, or Dina Andrianoely—the three best research technicians that I could have asked for. Endless hours spent with each one of you have provided me with a different view of life and brought me into worlds that I never could have imagined existed. Thank you to the 2011 field team, Dr. Deanna Bublitz, Dr. Fidisoa Rasambainarivo, and Emily Hess. And thank you to the 2012 field team who I spent countless hours working with—Rachel Kearns, Chris Wegner, and Ze Zhang, and Sarah Zohdy (again).

This project was made possible by grants/stipends from the Herrnstein Foundation, International Research for Emory Students (Howard Hughes Grant No. 52005873), and Scholarly Inquiry for Research at Emory (Emory College of Arts and Sciences). For the facilitation of everything in regards to those grants I would like Pat Paladines, Leah Roesch, Jerry Byrd, and Cathy Quiñones. I would also like to thank Jo Williams-Newkirk for teaching me lab ethics, proper treatment of samples, and really just about everything else lab-related.

Thank you to my committee members Dr. Uriel Kitron and Dr. Christine Moe for taking the time out of your extremely busy schedules to help me defend my thesis; it will forever be appreciated. Finally I would like to thank my friends, roommates, family, and Muzzy for helping me make it through all of this, whether listening to me vent about lab work, thesis writing, or a broken computer charger two days prior to my thesis due date.

TABLE OF CONTENTS

Introduction……..1

Methods……..……13

Results……22

Discussion……..……27

Tables……..……32

Figures……..……...37

Literature Cited……..……41

LIST OF TABLES

Table 1. Gastrointestinal viruses of the lemurs of Ranomafana National Park, Madagascar
Table 2. Description of lemur species of Ranomafana National Park, Madagascar
Table 3. Lysis buffer reagents for RNA extractions of fecal samples
Table 4. Reagents and reagent quantities for mastermix of RNA polymerase chain reactions
Table 5. Forward and reverse primers used in polymerase chain reactions
Table 6. Reagents and reagent quantities for mastermix of DNA polymerase chain reactions
Table 7. Comparison of lemur species and individual virus infection rates
Table 8. Percentage of individuals infected with ≥1 virus (stratified by virus)
Table 9. Percentage of lemurs infected with ≥1 of five viruses
Table 10. Percentage of individuals with zero through five viral infections
Table 11. Comparison of lemur species and co-infection rates
Table 12. Percentage of individuals infected with ≥1 virus in 3 sites (stratified by site)
Table 13. Comparison of geophagy and non-geophagy practicing lemurs and virus infection rates
Table 14. Comparison of virus infection rate for large and small grouped lemurs

LIST OF FIGURES

Figure 1. Map of forests of Madagascar and location of Ranomafana National Park
Figure 2. Virus classification tree including adenovirus, enterovirus, norovirus, and rotavirus
Figure 3. Primate family tree including lemurs of Ranomafana National Park
Figure 4. Map of RNP including field sites, trails, and forest cover
Figure 5. Prevalence of viruses stratified by geophagy vs. non-geophagy practicing lemurs
Figure 6. Prevalence of viruses (grouped) for small vs. large-grouped lemurs
Figure 7. Prevalence of five viruses for combined lemur species
Figure 8. Prevalence of five viruses for *Eulemur rubriventer*
Figure 9. Prevalence of five viruses for *Eulemur rufifrons*
Figure 10. Prevalence of five viruses for *Hapalemur aureus*
Figure 11. Prevalence of five viruses for *Microcebus rufus*
Figure 12. Prevalence of five viruses for *Propithecus edwardsi*
Figure 13. Prevalence of five viruses for *Prolemur simus*
Figure 14. Prevalence of viruses (grouped) for bamboo vs. non-bamboo eating lemurs
Figure 15. Prevalence of five viruses for bamboo lemurs
Figure 16. Prevalence of five viruses for non-bamboo eating lemurs

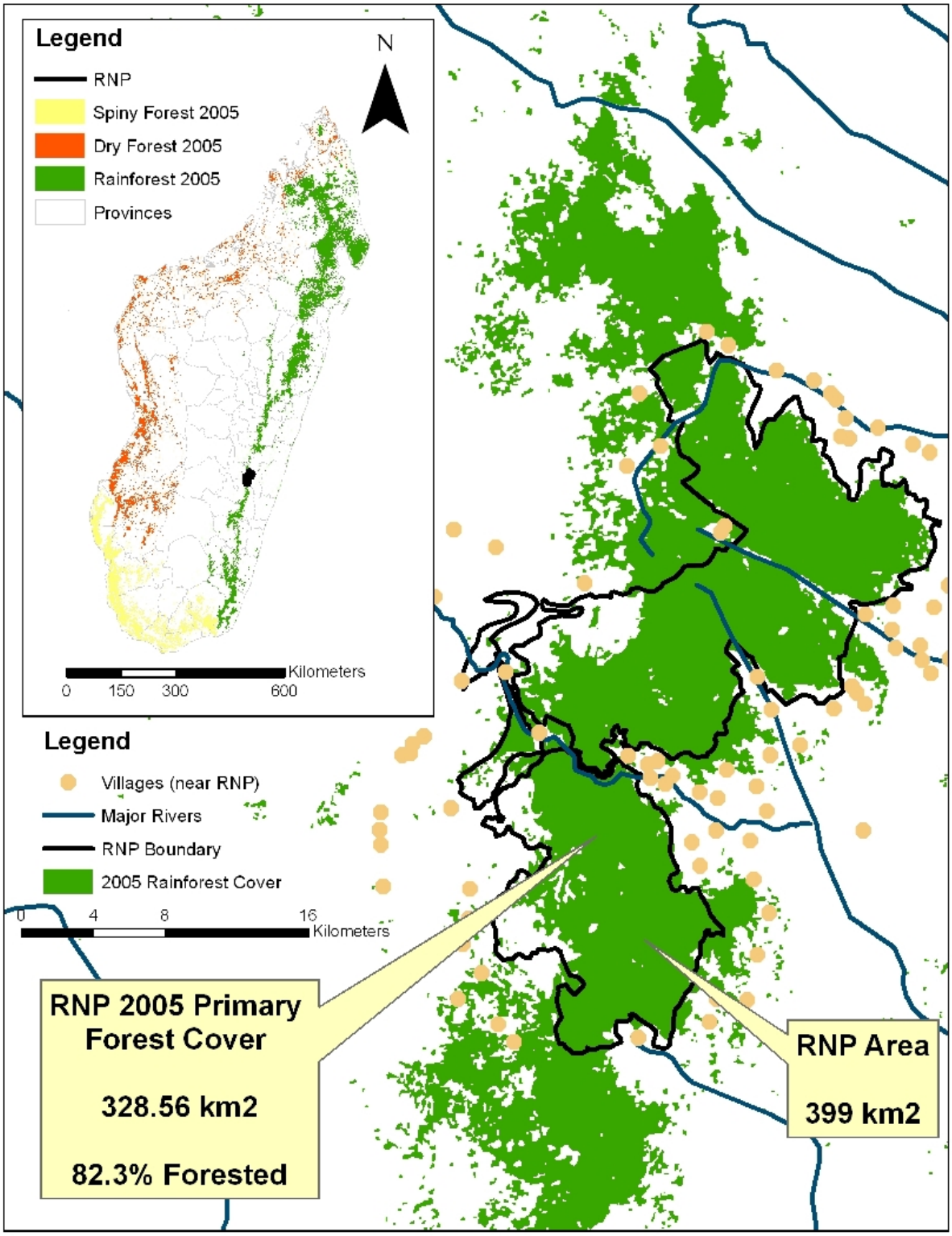
INTRODUCTION

Recent evidence of emerging infectious diseases with human and nonhuman primate (NHP) origins has raised awareness of the potential impact of zoonotic pathogen transmission on human and NHP health. As human population density continues to increase near edges of protected areas (Wittemyer et al. 2008), reduction and fragmentation of primate habitats is accelerating too (Meyer and Turner 1992). Growing human-primate contact is inevitable, which translates to more opportunity for pathogen transfer (Wolfe et al. 1998). Consequently, interest has grown in collecting baseline data on patterns of pathogen infections in wild primate populations in order to provide an index of population health and to begin to assess and manage disease risks.

Recently, simian gastrointestinal viruses 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 (Wevers et al. 2011). Although in most cases these simian viruses were shed by individuals demonstrating no clinical symptoms, they have been implicated in fatal respiratory outbreaks (Chi et al. 2007), as well as gastroenteritis (Wevers et al. 2011), in wild chimpanzees in Tai National Park in Cote d'Ivoire. To improve our understanding of this interplay, the current study examined the diversity and prevalence of gastrointestinal viruses in wild lemurs (a taxon that has previously not been investigated in this way). The current study also examined variability in these patterns among several lemur species that differ in key socio-ecological variables such as diet, ranging, and group size. Both of these are important in addressing potential conservation threats to endangered species and considering these viruses as potential zoonotic sources of human infection.

Madagascar, an island of just over 587,000 km^2, is considered one of the top three biodiversity hotspots in the world. With over 770 endemic vertebrate species, it holds 2.8% of the world's endemic vertebrates (Myers et al. 2000). Of these vertebrates, lemurs are a group of primates that consist of over 110 species. Since humans arrived in Madagascar ~2,000 years ago, 17 known species of lemurs have gone extinct (Mittermeier et al. 2010); thus it is vital that we support conservation efforts there to prevent losing further species. Over the last 60 years alone, Madagascar has been subject to more than a 40% loss in forest cover, which has been detrimental to many of these endemic species and has also continued to push humans closer to wildlife (Harper et al. 2007). With an expanding population, deforestation in eastern Madagascar is due to subsistence farming rather than to large scale logging operations. Plots of land are frequently cleared and then abandoned after soil nutrients have been leached out, and then sowed again before forests can grow back (Sussman et al. 1994). However, on account of Madagascar's extremely varied landscape, there are many niches that lemurs to take up, whether it is the montane rainforest of the East, the dry forest of the West, or the spiny forest of the South (Fig. 1).

Fig. 1. Map of forests of Madagascar and location of Ranomafana National Park



This map was created by and used with permission from Brian Gerber who has created free use GIS maps of RNP, which are available online at http://filebox.vt.edu/users/bgerber/maps.htm (accessed 1/13).

Ranomafana National Park (RNP), located in eastern Madagascar, is home to 13 species of lemurs. Many of these lemur species are categorized as "Endangered" or "Critically Endangered" on the 2012 IUCN Red List, and preserving their health is a important for their conservation. RNP was inaugurated in 1991, and today the park is 107,500 acres of montane rainforest with rainfall averaging around 73 inches per year (WorldClim). The Ranomafana area of Madagascar is subject to two distinct seasons: the drier, cooler season between May and September, and the wetter, cooler season between October and March (Overdorff et al. 1997).

Although several studies have examined gastrointestinal parasites in lemurs (Hogg et al. 2006, Junge et al. 2008, Irwin and Raharison 2009b, Clough et al. 2010), previous studies have not investigated enteric viruses in wild lemurs. Enterovirus, rotavirus, norovirus genogroups GI and GII are all zoonotically transmissible, RNA viruses found to cause gastroenteritis in humans (Häfliger et al. 2000, Rodriguez-Baez et al. 2002, Goller et al. 2004). The fifth virus examined in this study, adenovirus, is a DNA virus that also causes gastroenteritis, or inflammation of the gastrointestal system can result in both diarrhea and / or vomiting (Singh and Fleurat 2010). These five viruses are found in children and adults across Madagascar (Cassel-Beraud et al. 1993, Cunliffe et al. 1998, Rakoto-Andrianarivelo et al. 2005, Papaventsis et al. 2007, Randremanana et al. 2012).

Globally, diarrheal diseases are responsible for around 2.5 million child deaths annually (Kosek et al. 2003) and in Madagascar, diarrheal diseases remain a leading cause of death. Zoonotic transmission of diarrheal viruses has been largely overlooked despite the important role it may play. Historically, recorded viruses that have been transmitted from primates to humans include viruses such as HIV, Ebola (Gao et al. 1999, Rizkalla et al. 2007), Herpes, Influenza, and

Pox virus (Kalter and Heberling 1971). There has been no evidence to suggest that these transmission events have ceased or slowed, and so further transmission is likely. Close human contact with nonhuman primates (NHPs), as well as our phylogenetic similarity to primates, greatly facilitates zoonotic transfer of diseases (Gillespie et al. 2008).

Because of high rates of diarrheal disease in Madagascar in humans, we examined the five aforementioned viruses in lemurs in five sites with varying degrees of human disturbance and visitation. Of the five viruses, none has been studied in wild lemurs before. There has been no prior evidence of the transfer of these viruses from humans to lemurs; however, in a 2011 study, *Cryptosporidium hominis*, a species of *Cryptosporidium* that is restricted to reproducing in humans (Xu et al. 2004), was found in lemur feces in RNP (Bodager *et al*. 2011). This paper exemplifies zoonotic disease transfer from humans to lemurs in RNP.

Viruses

All viruses considered in this study are transmitted via the fecal-oral route. Infected humans will frequently spread the virus to food or household items through inadequate hand washing post-excretion. Animals will spread it by defecating in, on, or near food and water sources (Crawford 2011). Once the contaminated item is ingested, the viruses may quickly begin to reproduce in the body. These viruses are very prolific and, once released from the body again, are able to survive for long periods of time. These viruses are also transmissible via aerosols, meaning that an infected individual who vomits may spread the virus to nearby individuals and contaminate nearby objects (Couch et al. 1966, Goodgame 2006). Relative to other viruses, very few virus particles are sufficient to infect the next host (Crawford 2011). View Fig. 2 (page 8) for virus classification and Table 1 for summarized information about viruses.

Adenovirus

Adenoviruses are non-enveloped, double-stranded, icosahedral, DNA viruses of approximately 25–45 kbp (Acheson 2007) that infect a broad range of vertebrates (Davison et al. 2003). The specific species taxon is related to host origin, thus adenoviruses identified in lemurs can be classified under one of the human adenovirus species, HAdV, if humans were the primary host. However, a simian adenovirus species, SAdV has been identified in great apes and old world monkeys and is known to cause diarrhea (Kalter 1982, Wevers et al. 2011).

Enterovirus

Enteroviruses (family *Picornaviridae* family) are single-stranded, icosahedral, non-enveloped, RNA viruses that consists of ten different species (Acheson 2007, ICTV 2012). One of the species of *enterovirus* is poliovirus, the causative agent of Polio in humans; other species range in severity. Simian *Enterovirus* does exist and is known to cause gastroenteritis in primates (Kalter 1982).

Norovirus GI and GII

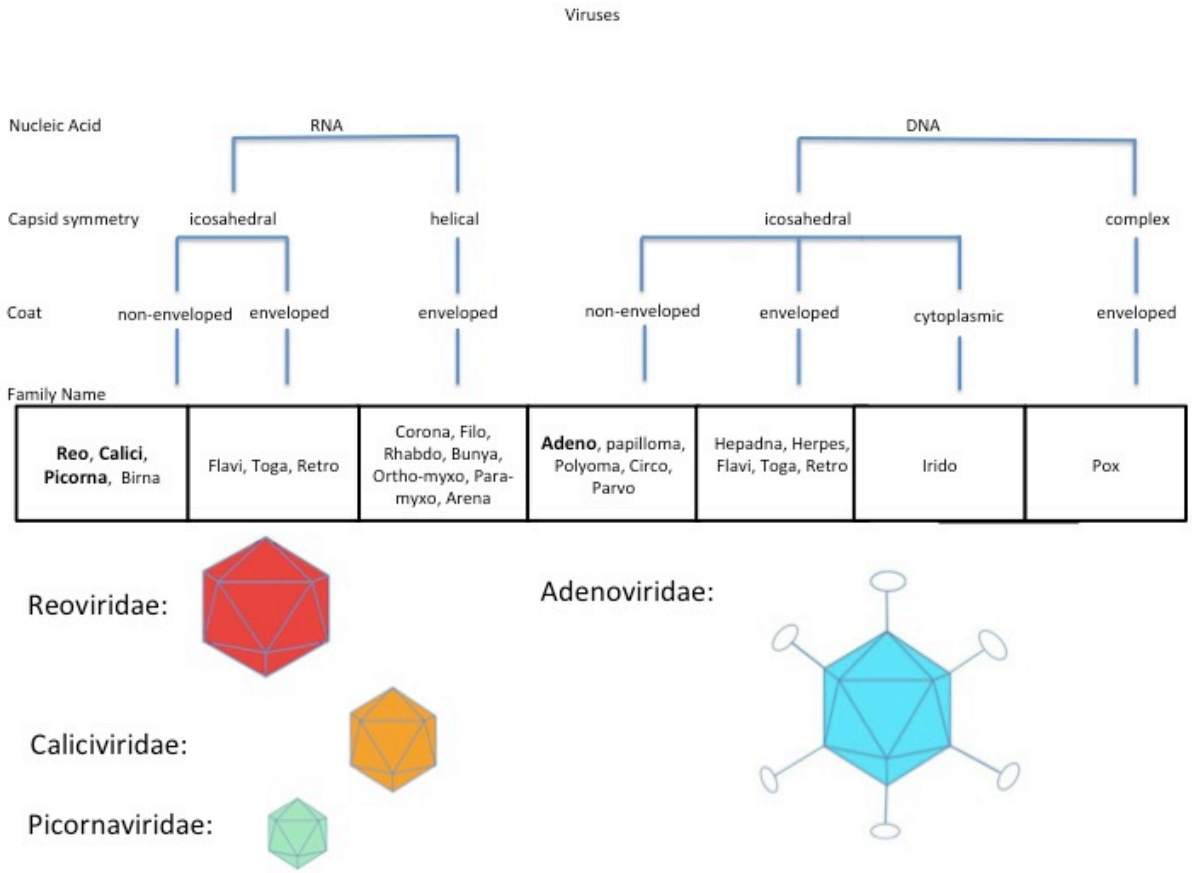
Noroviruses are in in the *Caliciviridae* family. Although there is only one species of norovirus, Norwalk, there are several genogroups that fall under this species (Acheson 2007, ICTV 2012). Norovirus GI and GII are positive-sense, single-stranded RNA viruses that are between 7 and 8 kb long (Acheson 2007, Papaventsis et al. 2007). Globally, norovirus is the second most common cause of viral gastroenteritis (after *Rotavirus*). In a 2007 Papaventsis *et al*.

study, they found norovirus GII to be the most common genogroup in Antananarivo, Madagascar, in children with acute gastroenteritis.

Rotavirus

Rotavirus is a virus in the *Reoviridae* family and is therefore an icosahedral, non-enveloped, double-stranded RNA virus. Rotavirus is the most common gastrointestinal virus present in children's daycare centers in the United States as well as the most common cause of viral gastroenteritis globally (Pickering et al. 1986, Crawford 2011). In humans, one milliliter of infected feces can contain up to one hundred billion virus particles; only ten are required to infect someone (Crawford 2011). The virus has five species: rotavirus A, rotavirus B, rotavirus C, rotavirus D, and rotavirus E (ICTV 2012). Simian strains do exist but have never been identified in lemurs.

Fig. 2. Virus classification tree including adenovirus, enterovirus, norovirus, and rotavirus



Lemurs

For the sake of simplicity, this section will be broken down into three sections, one for each family of lemurs sampled (Fig. 3, page 11). Lemurs are apart of the suborder Strepsirrhini, the infraorder Lemuriformes, and the superfamily Lemuroidea. All details, unless otherwise noted, will be in regards to the family. Further details on individual species can be seen in Table 2.

Cheirogaleidae

The *Cheirogaleidae* family consists of five genera—*Microcebus, Allocebus, Mirza, Cheirogaleus, and Phaner*—and 30 species of lemurs. All members of *Cheirogaleidae* are nocturnal and sleep in tree holes, burrows, or nests, some constructed by birds (Mittermeier et al. 2010, Thorén et al. 2010). This study only includes samples from *Microcebus rufus*, the Rufous Mouse Lemur. The *Microcebus* genus is home to the world's smallest primates, weighing in at as little as 30 grams. In order to conserve energy, *Microcebus* will enter states of torpor during winter months, with torpor times ranging from months to days. Their body temperatures will drop nearly to surrounding temperatures and they will become entirely inactive (Ortmann et al. 1997). One can find mouse lemurs in all forests in Madagascar, whether disturbed, primary, or secondary. Their diet consists of many types of insects, over 40 types of fruits, gum, and sap (Atsalis 1999). Predators include snakes, hawks, and mongooses (Mittermeier et al. 2010, Wright et al. 2012).

Lemuridae

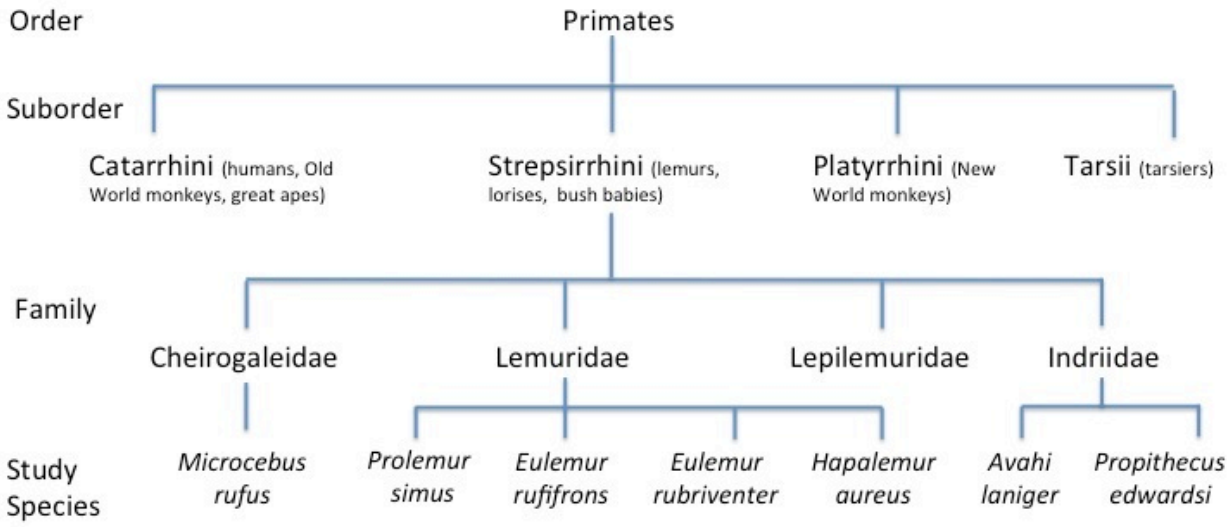
The *Lemuridae* family consists of five genera as well— *Eulemur, Hapalemur, Lemur, Prolemur,* and *Varecia.* We studied samples lemurs from the *Eulemur, Hapalemur, and Prolemur* genera, although all except *Lemur* may be found at RNP. *Eulemur*, or true lemurs, are typically cathemeral, moving primarily during the day, but are also active at night (Tattersall 1986). The majority of the *Eulemur* diet consists of fruits, leaves, and flowers, although they have been frequently seen practicing geophagy (Overdorff 1993). The *Eulemur* species studied are *E. rubriventer* and *E. ruffifrons*. The *Hapalemur* genus, or the bamboo lemurs, feed primarily on bamboo. Different species eat different parts of the plant, but *Hapalemur aureus*

(study species) feed primarily on the leaf base, vines, and shoots of bamboo (Glander et al. 1989), which contain high amounts of cyanide. *Prolemur* is a genus that consists of only one species, *Prolemur simus*, or the greater bamboo lemur. *P. simus* used to be in the *Hapalemur* family and since its removal is still considered to be a bamboo lemur (Mittermeier et al. 2010). *P. simus* are known to eat the culms, or stalks of giant bamboo, and this composes over 95% of their diet (Glander et al. 1989, Tan 1999).

Indriidae

The *Indriidae* family consists of three genera— *Avahi, Propithecus,* and *Indri*—of which the first two can be found at RNP. *Avahi*, or woolly lemurs, are nocturnal and generally sleep near the ground on leaves, and usually in groups. They are folivorous, although they have been observed eating fruits and flowers too. *Avahi laniger,* the study species, is found along the northern half of eastern Madagascar (Mittermeier et al. 2010). The second genus in the *Indriidae* family is *Propithecus*. *Propithecus,* or sifakas, are diurnal lemurs that can be found in both primary and secondary forests (Mittermeier et al. 2010). *P. edwardsi*, the study species, eat seeds, leaves, and fruit but have been observed eating soil and fungus too (Hemingway 1998).

Fig. 3. Primate family tree including lemurs of Ranomafana National Park



The current study examined the diversity and prevalence of gastrointestinal viruses in wild lemurs (a taxon that has previously not been investigated in this way). The current study also examined variability in these patterns among lemur species that differ in key socio-ecological variables such as diet, ranging, and group size.

Hypotheses

Prediction 1: Lemurs that practice geophagy will have higher viral infection rates because they are eating directly from the forest floor where other lemurs defecate.

Prediction 2: Lemurs that live in larger groups will have higher infection rates than lemurs that live in small groups because of how easily the five viruses transmit from member to member. In larger groups, more individuals are constantly in close contact.

Prediction 3: Site disturbance will have a direct relationship to virus prevalence. The more disturbed the site, the higher the infection rates we should expect to see because a more disturbed site offers a less diverse habitat for any one species.

METHODS

Field Site

Samples were collected from four areas at Ranomafana National Park (RNP), and one area located <1km from the park entrance. RNP is a 43,500 ha park and ranges from montane cloud rainforest to lowland rainforest (Overdorff 1996a). It is located in the southeast of Madagascar (between 21°02'–21°25'S and 47°18'–47°37'E) (Wright 1997) and spans from 600 m to 1513 m in altitude (fig. 1.). For the purpose of analysis, this study classifies the five collection areas (Fig. 4) into three distinct categories: least, intermediate, and most disturbed. Each category was based off of foot traffic of site as well as levels of deforestation.

Low Disturbance

The least disturbed site contained only one study area, Valohoaka (Valo). It was considered to be primary forest, and has never been logged or deforested (Balko and Underwood 2005). The approximately 9 km^2 site was located 6 km south of Talatakely and had an altitude of 1,100 m (Laakkonen et al. 2003). Because of its altitude, it is considered to be high montane rain forest (Overdorff 1996a). There is a trail system that runs through Valo.

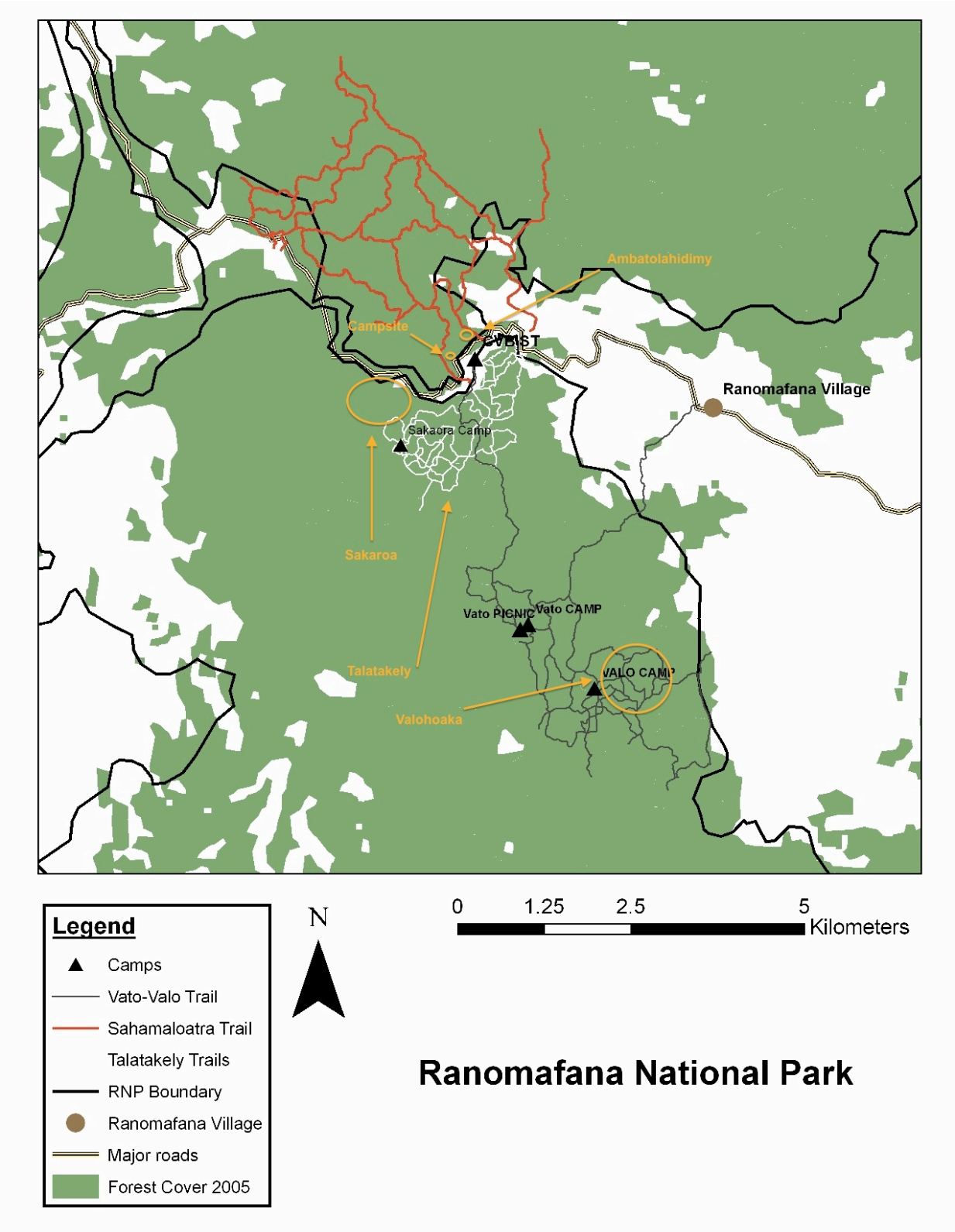
Intermediate Disturbance

There were two sites of intermediate disturbance, Talatakely and Sakaroa. The Talatakely (Tala) field site is in RNP, located between 21°15'20.0" S and 47°25' 15.3" E (Tan 1999), and sits at about 1000m (Wright 1995). During the 1930s and 1940s it used to be the site of a small market (Wright 1997), and then from 1986 to 1987 it was subject to deforestation for the purpose of logging. Today, the trees in Tala still have a smaller diameter at breast height

(DBH) than in Vatoharanana, and there is still relatively less tree species diversity (Tecot 2008). The majority of tourists going to RNP go to Tala, and it is considered to be the high tourist area, which was apparent by the well-maintained, non-overgrown, trail system. As it was most easily accessible to us from the field station, time and money permitting, it was the site that we collected the most samples from. The Sakaroa (SAK) field site was in RNP as well and was located directly to the west and shared a border with Talatakely. It is considered to be a combination of secondary forest and recovering disturbed forest (Irwin et al. 2009a).

High Disturbance

There were two sites that were considered to be most disturbed, Ambatolahidimy and Campsite. The two sites were approximately 0.5 km apart and had no physical barriers (such as roads or rivers) separating them. Ambatolahidimy (Amb) is a small unprotected forest approximately 1.5 km northeast from the park entrance (Norosoarinaivo et al. 2009). The forest bordered Ambatolahy village, and the forest fragment is frequented by villagers from Ambatolahy (Sarah Zohdy, pers. comm.). The Campsite (CS) field location is an outdoor area directly adjacent to the Centre Valbio Research Station. It was located across a river and a road (0.48 km) from Talatakely, Sakaroa, and Valo. Amb was mostly clear-cut in the 1990's and early 2000's to allow room for rice paddies but more recently has been utilized for researchers to camp out (King et al. 2012). The only samples collected at CS were from mouse lemurs.



This map was created by and used with permission from Bria(Gerber)n Gerber (2010) who has created free use GIS maps of RNP, which are available online at http://filebox.vt.edu/users/bgerber/maps.htm (accessed 1/13). This image was modified (in orange) to aid in visualization of field sites.

Sample Collection and Preservation

This field study was conducted over two field seasons from June to August of 2011 and 2012. All lemur fecal samples were collected non-invasively. Groups of lemurs were tracked daily until all or most members of groups had defecated. Upon defecation, feces was collected from forest floor with a plastic spoon and placed into a vial. Vials were then labeled with time, location, species, sex of lemur, and age of lemur. At the end of each day, fecal samples were transferred to Nunc 1.8 mL cryovials [Cat# V7634] (Sigma-Aldrich, St. Louis, MO) containing 0.8 mL of RNAlater® [Cat# 76104] (Qiagen, Hilden, Germany). Approximately 0.6-0.9 mL of feces was then inserted into the cryovial and pushed down so as to cover the entire sample in RNAlater®. Samples were stored at room temperature until nucleic acid extractions.

Mouse lemur samples were collected in Sherman Traps (Tallahassee, FL), using bananas and crushed peanuts as bait. When collected two hours later, traps usually contained sufficient amounts of feces. All traps were tied to trees so as to avoid also trapping rodents. Mouse lemurs were immediately released without handling if possible.

Lab Work

DNA Extractions

DNA extractions were performed using the FastDNA® SPIN Kit for Soil [Cat# 6560-200] (MP Biomedicals, LLC, Solon, OH). Protocol used was a modified version of protocol described in da Silva *et al*. 1999 and Bodager 2011. Fecal samples were removed from cryovials containing RNAlater with a Disposable Transfer Pipettes [Cat# 13-711-7M] (Fisher Scientific, Hampton, NH) and transferred into a Lysing Matrix E Tubes. Samples were then washed twice with 800 µL of ddH_2O to eliminate any RNAlater®. Proper washing consisted of rinsing with

ddDH_2O, re-suspending, centrifuging for six minutes at 14,000 RCF, and then removing the supernatant. Prior to re-suspension, 978 µL of sodium phosphate buffer and 122 µL of MT Buffer were added. Tubes were then placed in a FastPrep®-24 Instrument [Product No. 116004500] (MP Biomedicals, Carlsbad, CA) for 30 seconds at a speed of 5.5 m/s followed by one minute of centrifuging at 14,000 RCF. Supernatant was transferred to a new microcentrifuge tube and 250 µL of Protein Precipitation Solution (PPS) was added. Tubes were then shaken by hand ten times before centrifuging at 1,400 RCF for five minutes. Supernatant was added to falcon tubes along with 1mL of binding matrix suspension. Solution had to be inverted for two minutes to allow binding of DNA matrix before allowing to settle for five minutes. 650 µL of supernatant were removed then discarded, and a resuspended solution was added to a SPIN filter tube and centrifuged at 13,400 RCF for two minutes. This was repeated until all solution had been centrifuged, at which point Salt/Ethanol Wash Solution was run through the SPIN filter to eliminate excess PPS at 13,400 for four minutes. After spin, the SPIN filter was put in a clean catch tube, and the solution was air dried for five minutes to allow any excess liquid to evaporate. 100 µL of DNase free water was pipetted into top of SPIN filter and gently stirred with the tip to allow solid to liquefy. The solution was vortexed for 1 minute at 13,400 RCF and final product remained in catch tube. All DNA samples were then placed in -80°C freezer until subsequent polymerase chain reaction (PCR) analysis.

RNA Extractions

RNA extractions were performed using the QIAamp Viral RNA Mini Kit [Cat# 52906] (Qiagen, Hilden, Germany). The protocol used was a modified version of the protocol included with kit. To prepare fecal samples for extractions, fecal suspensions were prepared by adding

100 µL of sample feces to 400 µL of sterile water and vortexing for 15 seconds. 500 µL of Vertrell was then added to the mixture and samples were vortexed again before placed in a refrigerator for four hours. At this point, the samples were ready for RNA extraction. To begin extractions, 310 µL of AVE Buffer was mixed with 310 µg of carrier RNA to produce 620 µL of carrier RNA-AVE mix, which was used multiple times. The lysis buffer was then created using reagents specified in Table 3. Aforementioned fecal sample mix was spun in a centrifuge for 10 minutes at 13,200 rpm at 4 °C. Upon completion of spin, 140 µL of supernatant was removed from top of fecal mix and mixed in a new microcentrifuge tube with 560 µL of lysis buffer and incubated for 10 minutes at room temperature. 560 µL of ethanol was then added to samples, which were vortexed for 15 seconds and briefly spun down. All samples were then transferred to new Mini Spin Columns on manifold and washed with 750 µL of AW1 and 750µL of AW2 (concentrate included in kit, diluted with 100% ethanol). Mini Spin Columns were placed inside of collection tubes and spun at 13,200 rpm for 1 minute at 4 °C. Collection tubes and contents were discarded and Mini Spin Columns were placed inside of sterile microcentrifuge tubes. Remaining RNA was eluted with 50 µL of AVE Buffer and collected product was centrifuged for one minute at 8,000 rpm. Final product was stored in -80°C freezer until subsequent polymerase chain reaction (PCR) analysis.

RNA Polymerase Chain Reactions

All polymerase chain reactions (PCRs) took place inside of an ISO class 5 PCR workstation [Cat# 09-681-202] (Fisher Scientific, Hampton, NH). PCR performed on RNA viruses utilized OneStep RT-PCR kit [Cat# 210212] (Qiagen, Hilden, Germany), and followed a modified Qiagen protocol. To create the mastermix, RNase inhibitor was listed as optional and

was not used because it made results appear far less clear on gels. Q-solution was also not used for the same reason (see results section). Reaction components of mastermix can be seen on Table 4. 1 µL of extracted DNA or RNA was then combined with 23.5 µL of mastermix in PCR tubes [Cat# 14-230-214] (Fisher Scientific, Hampton, NH). Primers were obtained via Integrated DNA Technologies (Coralville, IA) (Table 5) and positive controls were obtained via the Center for Global Safe Water at Emory University. Immediately following this, samples were placed in a thermocycler with the following settings: 50 °C for 30 minutes; 95 °C for 15 minutes; *94 °C for 30 seconds; *50 °C for 30 seconds; 72°C for 1 minute; 4 °C hold. The asterisk represents a cycle that was repeated 30 times. Upon completion of the cycle, PCR samples were moved to a cold room at 5°C until they were run on an agarose gel in an electrophoresis chamber within 48 hours. All positive samples will be stored and used as positive controls in the future.

DNA Polymerase Chain Reactions

To create the mastermix for DNA PCR, a modified Qiagen protocol was followed. Reaction components of mastermix can be seen on Table 6. 1 µL of extracted DNA or RNA was then combined with 23.5 µL of mastermix in PCR tubes [Cat# 14-230-214] (Fisher Scientific, Hampton, NH). Primers were obtained via Integrated DNA Technologies (Coralville, IA) (Table 5) and positive controls were obtained via the Center for Global Safe Water at Emory University. Immediately following this, samples were placed in a thermocycler with the following settings: 95 °C for 5 minutes; *95 °C for 30 seconds; *55 °C for 30 seconds; *72 °C for 30 seconds; 72°C for 5 minutes; 4 °C hold. The asterisk represents a cycle that was repeated 35 times. Upon completion of the cycle, PCR samples were moved to a cold room at 5°C until they were run on

an agarose gel in an electrophoresis chamber within 48 hours. All positive samples will be stored and used as positive controls in the future.

Gel Electrophoresis

To view results of the PCR, we ran all samples on 2% agarose gels. Gels were made using 30ml of 1x Tris-Borate-EDTA buffer [Cat# T4415-10L] (Sigma-Aldrich, St. Louis, MO), 0.5 grams of agarose powder [Cat# 50004] (Lonza, Basel, Switzerland), and 1.25 µL of diluted 2.5mg/mL Ethidium Bromide solution [Cat# E1510] (Sigma Aldrich, St. Louis, MO). All gels were run with a 100 bp ladder [Cat# SM0243](Thermo Scientific, Waltham, MA) on both ends of both rows. A 5 µL aliquot of PCR sample was mixed with 1µL of 6x loading dye [Cat# R0611] (Thermo Scientific, Waltham, MA). All samples were run for 30 minutes at 80 volts. After running the gels, results were viewed on a computer using a Molecular Imager® Gel Doc™ XR System [Cat# 170-7950EDU] (Bio-Rad, Hercules, CA).

Statistical Analysis

There were a total of 84 lemur fecal samples in this study. Of the 84 samples, all were screened for adenovirus but only 77 were screened for enterovirus, norovirus GI and GII, and rotavirus due to a lack of sufficient amounts of feces for RNA extraction. Of the seven samples that did not have enough feces, two of them were positive for adenovirus. In any table or figure that counted infection rates by at least one virus, the five samples that were negative for adenovirus were excluded, while the other two were included. In any table or figure that compared prevalences of viruses, the seven samples were included for adenovirus and removed

for all other viruses. When comparing co-infection rates, none of the seven samples were included because any sample that was screened for only one virus would skew the results.

All statistical tests involving two groups were run with Mann-Whitney U tests in Microsoft Excel using XLSTAT Version 2013.1.01 (Addinsoft, New York, NY). Analysis of variance (ANOVA) tests were run for any statistical test involving more than two groups in JMP version 10.0.0 (JMP, Cary, NC). All graphs used were created in Prism 6.0b (Graphpad, San Diego, CA), with confidence intervals of 95%. On all graphs involving two groups, an asterisk was used to denote a statistical difference. On all graphs involving more than two groups, letters (i.e. "A," "B," "AB") were used to denote a statistical difference. Groups with the letters "AB" would not be statistically different than letters "A" or "B," but the latter two would be statistically different. For all tests, the significance level, α, was set to 0.05, and groups were not considered significantly different unless the p-value was <0.05.

RESULTS

Prior to running electrophoresis gels for RNA PCR, assays were run to maximize clarity of bands on gels. 0.5, 1, and 2 μL of positive control (10 mM) for norovirus GII and enterovirus were run, half of the samples with q-solution and half with standard 5x buffer. All three of the quantities of positive control showed up equally as clearly, but almost none of the samples that were run using Q-solution appeared on the gel. For this reason, we did not use Q-solution in the mastermix for PCR.

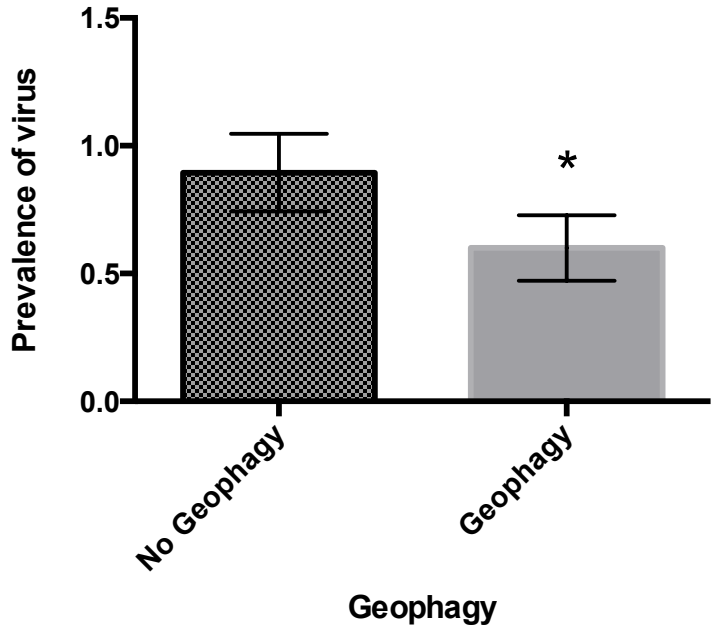
A total of 84 lemurs' fecal samples, representing seven species, were screened for five gastrointestinal viruses (adenovirus, enterovirus, norovirus GI and GII, and rotavirus). All lemur species were positive for at least two and at most four viruses (Table 7). However, the species that was positive for only two viruses, *Avahi laniger,* had one individual sampled in this study; all other species were positive for three or more. Rotavirus was the sole virus present in less than four species, and it appeared uniquely in *Eulemur rufifrons* with a prevalence of 38.1% (n=21). When lemur species were all grouped (Table 8), virus prevalence ranged from 10.7% (rotavirus) to 26.7% (norovirus GII). When viruses were all grouped (Table 9), lemur species that were infected with at least one virus ranged from 28.6% for *H. aureus* to 100% for *P. simus* (n=6). In total, 68.4% of collected individuals were infected with one or more virus.

In regards to co-infection, 20.5% of lemurs were infected with two viruses, 5.1% were infected with three viruses, and 1.3% were infected with four viruses; no individuals were found to be infected with all five viruses (Tables 10 and 11). Every species had at least one individual with a two-virus co-infection, and *A. laniger* (n=1) and H. aureus (n=7) only had individuals that displayed co-infection.

When grouping samples by disturbance levels (least, intermediate, and most), we are able to observe at which sites viruses are present (Table 12). Rotavirus was only present in the intermediate disturbance site and was completely absent from both the sites of most and least disturbance. Adenovirus was not present at the site of least disturbance but was prevalent in 23.1% and 46.2% of samples with intermediate and most disturbance, respectively. In the site of least disturbance (adenovirus n=6, all other viruses n=2), viruses were either present in all individuals (enterovirus, norovirus GI and GII) or in no individuals (adenovirus, rotavirus).

Lemurs that practice geophagy were significantly less likely to be infected with one or more of the five viruses than those that did not—Infection rates were 60% and 89.5%, respectively (Table 13, Fig. 5). Of the five viruses, only Norovirus GI displayed a significant difference in prevalence between groups; however, with the exception of rotavirus, lemurs that did not practice geophagy consistently had higher infection rates than those that did.

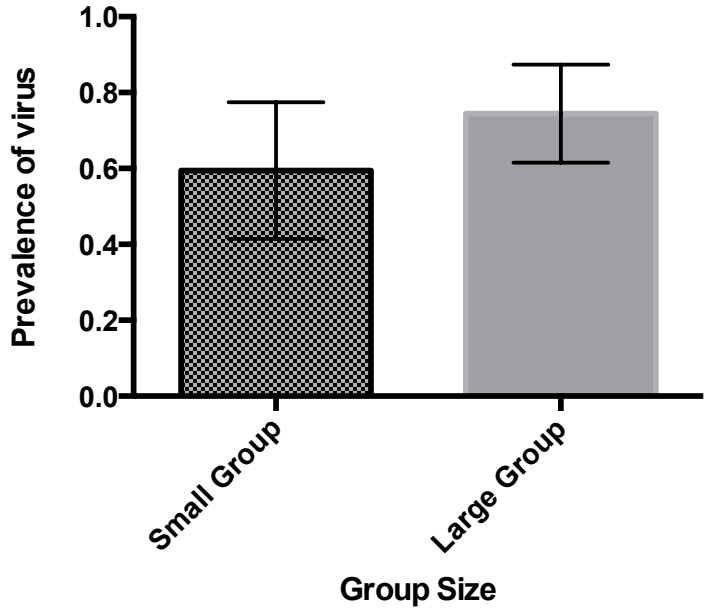
Fig. 5. Prevalence of viruses stratified by geophagy vs. non-geophagy practicing lemurs



Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs (separated by geophagy vs. non-geophagy practicing). The error bars represent a 95% confidence interval. Alpha = 0.05. The asterisk represents a significant difference between the two columns ($p<0.05$). No Geophagy n=19; geophagy n=60

Lemur group size (over versus under 5.5 individuals per group) showed no significant differences in virus prevalence when viruses were combined (Table 14, Fig. 6). When separated by virus, prevalences of *rotavirus* were significantly lower in small groups than in large groups (0% versus 17.4%); however, prevalences in small groups for norovirus *GI* and *GII* were significantly higher.

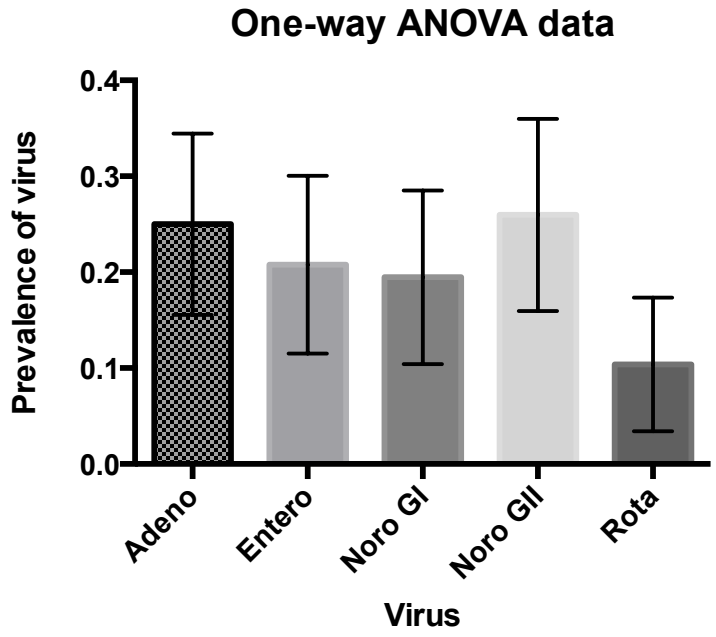
Fig. 6. Prevalence of viruses (grouped) for small vs. large-grouped lemurs



Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs (separated by small vs. large group sizes). The error bars represent a 95% confidence interval. Alpha = 0.05. There is no significant difference in values between the two columns ($p>0.05$). Small group n=32; large group n=47

When comparing all lemurs and all viruses, there was not a statistically significant difference in prevalence in any of the five viruses (Table 8, Fig. 7). However, four separate lemur species (*E. rubriventer, M. rufus, P. edwardsi,* and *P. simus)* did display statistically significant differences across the five viruses (Figs. 8-13). *Rotavirus* was not present in any species of lemurs except for *E. rufifrons,* where it was present in 38.1% of individuals.

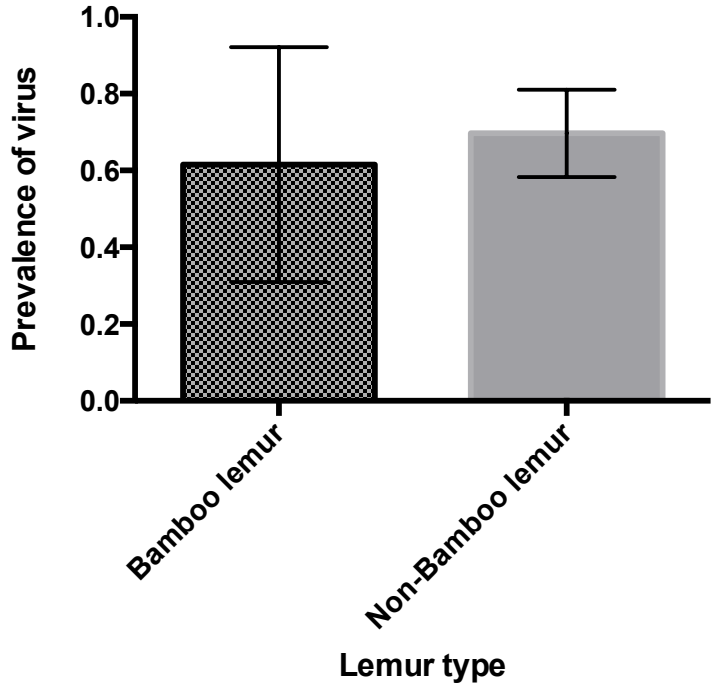
Fig. 7. Prevalence of five viruses for combined lemur species



Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs. The error bars represent a 95% confidence interval. Alpha = 0.05. There is no significant difference in values between any of the five columns ($p>0.05$). Adenovirus n=84; enterovirus, norovirus GI and GII, and rotavirus n=77.

Bamboo lemurs did not exhibit a difference in virus prevalence (Fig. 14). Virus prevalence for bamboo lemurs was 61.5% and virus prevalence was 69.7% for all other lemurs. When comparing different virus infection rates across bamboo and non-bamboo lemurs, there was no significant difference between any of the five viruses (Figs. 15 and 16). However, no bamboo lemurs were infected with either enterovirus or rotavirus.

Fig. 14. Prevalence of viruses (grouped) for bamboo vs. non-bamboo eating lemurs



Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs (separated by bamboo vs. non-bamboo-eating lemurs). The error bars represent a 95% confidence interval. Alpha = 0.05. There is no significant difference in values between the two columns ($p>0.05$). Bamboo lemur n=13; non-bamboo lemur n=66

Samples collected in 2011 and samples collected from 2012 showed no significant difference in the proportion of individuals with ≥1 virus detected. We detected one or more virus in 85% of 2011 individuals and 61% of 2012 samples. Between 2011 and 2012, adenovirus, enterovirus, and rotavirus did not show a statistically significant difference in prevalence rates, while 2011 rates were statistically different for norovirus GI and GII ($p<0.0001$ and $p=0.019$ respectively).

DISCUSSION

Gastrointestinal viruses have never before been studied in wild lemur populations. This study provides new findings and information on the presence and patterns of gastrointestinal viruses infecting wild lemurs in Ranomafana National Park. Although sample sizes were small for certain species, this study provides insight for characterizing lemur viruses. One of the lemurs studied, *Prolemur simus,* was ranked amongst the top 25 most endangered primates of 2008-2010 (IUCN), and with less than 200 remaining individuals, sample sizes much larger would be very difficult and time-consuming to obtain. Other than *M. rufus* and *A. laniger*, all lemurs species in this study are threatened (IUCN 2012).

We tested lemur individuals for five viruses (adenovirus, enterovirus, norovirus GI and GII, and rotavirus) and found that 68.35% of individuals were infected with one of these five viruses. In a 2007 Wang *et al*. study researching viral infection rates in captive monkeys, 68% of 92 specimens were positive for either adenovirus, enterovirus, picobirnavirus, or rotavirus, a number that is strikingly similar to ours, especially considering that captive primates have veterinary access. Our study found 25% of individuals to be positive for adenovirus, which is quite low compared to 59% in a 2011 Wevers *et al*. meta-analysis that tested 1,285 primate samples from 52 species (wild, captive, and sanctuary-kept). In our study norovirus GII was found in 26.7% of individuals, and was the most prevalent virus examined. Sample collection took place during the dry season, a time when water quality is usually better (Webber 2009). In a two-year, 2007 Papaventsis *et al*. study on children in children, prevalence of norovirus in people with gastroenteritis was only 6%. Considering that all lemurs were selected randomly in the dry season, and not on account of gastroenteritis, rates for all norovirus *GI* and *GII* were very high. For rotavirus, however, results were not far from expected, as rotavirus has higher

infection rates in the dry season in temperate climates, and is generally found to have a lower prevalence all year round (Cassel-Beraud et al. 1993, Cunliffe et al. 1998). In a 2009 Whittier study, 9% of free-ranging mountain gorillas were found to have rotavirus, again a number quite similar to our 10.7%. Overall variation in viral prevalences in our study, although not statistically significant, is most likely linked to choice of lemur species. *Rotavirus*, for instance, was only present in *E. rufifrons* and had the study not sampled that species, it would have had an overall infection rate of zero. Tables 8 and 9 describe which lemurs have which viruses and show individual virus prevalence; Figs. 7-13 show virus presence in different lemur species.

Lemurs that practiced geophagy had a significantly lower viral prevalence than lemurs that did not. Although geophagy has been observed to counteract endoparasite infection (Bicca-Marques and Calegaro-Marques 1994, Knezevich 1998), actual effectiveness against endoparasites is still unclear (Krishnamani and Mahaney 2000). Because geophagy involves eating soil, accidental consumption of parasites such as helminths has been suggested (Pebsworth et al. 2012), which leaves open the possibility of virion consumption. It was unexpected to see that lemur species that practiced geophagy had lower infection rates throughout the study. One explanation could be that there were only two species of lemurs that did not practice geophagy, *A. laniger* and *M. rufus*. There was only one fecal sample from *A. laniger*, and *M. rufus* may have exhibited high rates of viruses because of frequent proximity to humans and nighttime visits to Ambatolahy village (Sarah Zohdy, pers. comm.). Coincidentally, the two non-geophagous lemurs are also both nocturnal, and although there should be no relation between nocturnal sleeping patterns and virus prevalence, the statistics indicate that there is a significant difference.

Of the three catagories of sites (least, middle, and most disturbed) we hypothesized that the sites that had been most anthropogenically disturbed would have the most infected individuals, the site that had the middle amount of disturbance would have an intermediate amount of infected individuals, and the site with the least amount of disturbance would have the lowest amount of infected individuals. Because only two samples from the least disturbed site had sufficient amounts of feces for RNA extractions (a necessary component in order to test for enterovirus, norovirus G1, norovirus GII, and rotavirus) we were not able to compare that site to the other two. The site of intermediate disturbance had a lower viral prevalence than the site of most disturbance although there was no statistically significant difference.

Smaller groups displayed higher virus prevalence, but the difference was not statistically different (view Fig. 6). Individual viruses, however, did show significant differences between small and large groups. For norovirus GI and GII, larger groups exhibited significantly less viral infection. One explanation for these results could be that because larger groups deplete food resources so quickly, they are unlikely to stay in one area for a long time. This constant movement reduces the risk of fecal contamination of their food and living areas thus reducing viral infection (Freeland 1976). The opposite results were seen for rotavirus: smaller groups exhibited significantly more viral infection. These results could be attributed to higher amounts of lemur-lemur contact in larger groups, which could aid in transmission of viruses (Freeland 1976). Alternatively, these results could be attributed to the manner in which these viruses are spread. Although there is no significant trend in the five viruses in relation to group size, it is possible (even though these are all gastrointestinal viruses transmitted via the fecal-oral route) that ease of transmission or particles necessary is variable depending upon the individual virus.

This variation could facilitate transmission of certain viruses in small groups and other viruses in large groups.

When comparing viral infection in bamboo lemurs and non-bamboo lemurs, bamboo lemurs did not exhibit a difference in infection rates. There were two species of bamboo lemurs in this study, *H.aureus* and *P. simus*. All *H. aureus* individuals tested negative for all five viruses, and all *P. simus* individuals tested positive for at least one virus. Besides the single *Avahi laniger* sample, no other species had every sample with at least one virus. In 2008, there were an estimated 60 wild *P. simus* individuals remaining in Madagascar (Wright et al. 2008). Because their diet consist primarily of only one food type (bamboo), they are at especially at risk for extinction due to habitat destruction (Jernvall and Wright 1998, Wright and Jernvall 1999). Additionally, a lemur with low variation in diet that remains in one home range is likely to revisit locations where fecal contamination has built up over time (Freeland 1976); this does not distinguish the two bamboo lemurs. What does distinguish the two species, however, is that *P. simus* are the only lemurs in RNP that have been observed regularly drinking from streams (Wright et al. 2008), a possible source of transmission. Because of the difference in viral infection between the two species of bamboo lemurs, comparing viral infection rates in bamboo lemurs and non-bamboo lemurs in this study is not a useful comparison given how few (~20) habituated *P. simus* individuals live in RNP.

Many of the gels in this study were subject to non-specific ~75kb contamination. It was unclear where this contamination came from, but it was suspected to be from the norovirus GII and adenovirus primers. However, all actual positive results for these viruses could be viewed and distinguished from the contamination bands. All positive samples were directly in line with the positive control.

The results of this paper have the potential to influence future lemur conservation strategies in Madagascar. Location of infected lemurs, as well as life history traits (geophagy, group size), will help to better understand the health of some of the world's most endangered primate species. Knowing the prevalence and patterns of the five viruses may provide information that reveals zoonotic transmission routes to humans. This information, in turn, could help influence public health research. Future studies need to examine patterns of gastrointestinal virus infection in overlapping human and domestic animal populations to better understand zoonotic potential for these pathogens. In addition, use of sequencing technologies will clarify gastrointestinal virus transmission dynamics within the Greater Ranomafana Ecosystem.

TABLES

Table 1. Gastrointestinal viruses of the lemurs of Ranomafana National Park, Madagascar

Genus	Genome size (kbp)	Capsid symmetry	Coat	Family	Nucleic Acid	Base pair length on gel
Adenovirus	25–45	Icosahedral	Naked	*Adenoviridae*	Double stranded DNA	482 bp (Yan et al. 2004)
Enterovirus	7-9	Icosahedral	Naked	*Picornaviridae*	Single stranded RNA	440 bp (Zoll et al. 1992)
Norovirus GI	7-8	Icosahedral	Naked	*Caliciviridae*	Single stranded RNA	330 bp (Yan et al. 2003)
Norovirus GII	7-8	Icosahedral	Naked	*Caliciviridae*	Single stranded RNA	387 bp (Yan et al. 2003)
Rotavirus	10-12	Icosahedral	Naked	*Reoviridae*	Double stranded RNA	569 bp (Khamrin et al. 2011)

Table 2. Description of lemur species of Ranomafana National Park, Madagascar

	Conservation status (IUCN 2012)	Geophagy (Y/N)	Diet	Foraging Group size	Activity pattern	Weight (kg)
Avahi laniger	Least Concern	N	Primarily leaves, fruits, flowers (Ganzhorn et al. 1985)	≤5 (Mittermeier et al. 2010)	Nocturnal (Harcourt 1991)	1.0-1.3 (Glander et al. 1992)
Eulemur rubriventer	Vulnerable	Y (Overdorf f 1993)	Fruit, flowers, nectar, leaves, Chinese guava (Mittermeier et al. 2010)	Approximately 10 (Mittermeier et al. 2010)	Cathemeral (Overdorff and Rasmussen 1995)	1.6-2.4 (Glander et al. 1992)
Eulemur rufifrons	Near Threatened	Y (Overdorf f 1993)	Chinese guava (Garbutt 2007), flowers, leaves, harongana fruit (Overdorff 1996a)	6-18 (avg of 8) (Mittermeier et al. 2010)	Cathemeral (Overdorff and Rasmussen 1995)	2.2-2.3 (Glander et al. 1992)
Hapalemur aureus	Endangered	Y (Tan 1999)	Giant bamboo (Meier and Rumpler 1987)	3-4 (Tan 1999)	Diurnal (Mittermeier et al. 2010)	1.3-1.7 (Mittermeier et al. 2010)
Microcebus rufus	Least Concern	N	Fruits, insects, gums (Atsalis 1998a)	1 (Atsalis 2000)	Nocturnal (Atsalis 1998a)	0.0395-0.0479 (Mittermeier et al. 2010)
Propithecus edwardsi	Endangered	Y (Hemingw ay 1998)	Leaves, fruits, seeds, flowers (Mittermeier et al. 2010)	4.8 (Wright 1998)	Diurnal (Mittermeier et al. 2010)	5.0-6.5 (Glander et al. 1992)
Prolemur simus	Critically Endangered	Y (Tan 1999)	Giant bamboo (primarily), traveler's palm flowers, jackfruit, fig, *Dyspsis spp.* fruits (Meier and Rumpler 1987)	Up to 28 (Tan 1999, Tan 2000)	Cathemeral (Tan 1999)	2.2-2.5 (Meier et al. 1987)

Table 3. Lysis buffer reagents for RNA extractions of fecal samples

Reagent	AVL Buffer (µL)	Carrier RNA-AVE mix (µL)	Total (µL)
Quantity per fecal sample	560	5.6	565.6

Table 4. Reagents and reagent quantities for mastermix of RNA polymerase chain reactions

Reagent	**Quantity per reaction**
5x Buffer	5.0 µL
dNTP (10mM)	1.0 µL
Primer F1	0.5 µL
Primer R1	0.5 µL
One Step RT PCR Enzyme Mix	1.0 µL
RNase Free water	15.5 µL
Template RNA	1 µL
TOTAL	24.5 µL

Table 5. Forward and reverse primers used in polymerase chain reactions

Virus	**Forward Primer**	**Sequence**	**Reverse Primer**	**Sequence**	**Reference**
Adenovirus	AdJFWD2	5'-GCC GCA GTG GTC TTA CAT C-3'	AdJRVS2	5'-CAG CAC GCC GCG GAT GTC AAA GT-3'	(Jothikumar et al. 2005)
Enterovirus	Enterovirus F1	5'-TCC TCC GGC TGA ATG CG-3'	Enterovirus R1	5'-ATT GTC ACC ATA AGC AGC CA-3'	(Donaldson et al. 2002)
Norovirus GI	Norovirus G1-SKF	5'-CTG CCC GAA TTY GTA AAT GA-3'	Norovirus G1-SKR	5'-CCA ACC CAR TTR TACA-3'	(Kageyama et al. 2003)
Norovirus GII	Norovirus COG2F	5'-CAR GAR BCN ATG TTY AGR TGG ATG AG GA-3'	Norovirus G2-SKR	5'-CCR CCN GCA TRH CCR TTR TACAT -3'	(Kageyama et al. 2003)
Rotavirus	Rota-A-F	5'-GGC TTT AAA AGA GAG AAT TTC CGT CTG-3'	Rota-A-R	5'-GGT CACATCATA CAA TTCTAA TCT AAG-3'	(Logan et al. 2006)

Table 6. Reagents and reagent quantities for mastermix of DNA polymerase chain reactions

Reagent	**Quantity per reaction**
5x green gotaq buffer	5 ul
dNTP (10mM)	0.5 ul
Forward Primer	0.25ul
Revers Primer	0.25 ul
Polymerase	0.25 ul
bovine serum albumin (10mg/ml) from powder	0.25 ul
DNase free water	13.5 ul
Template DNA	5ul
TOTAL	**25 ul**

Table 7. Comparison of lemur species and individual virus infection rates

	Total individuals sampled (n)	*Adenovirus %*	*Enterovirus %*	*Norovirus GI %*	*Norovirus GII %*	*Rotavirus %*
Avahi laniger	1	0	0	100	100	0
Eulemur rubriventer	21	23.8	40	15	10	0
Eulemur rufifrons	21	33.3	4.8	0	9.5	38.1
Hapalemur aureus	7	0	28.6	14.3	28.6	0
Microcebus rufus	12	50	30	50	40	0
Propithecus edwardsi	12	0	16.7	25	41.7	0
Prolemur simus	6	33.3	0	33.3	66.7	0

Table 8. Percentage of individuals infected with ≥1 virus (stratified by virus)

Virus	Adenovirus	Enterovirus	Norovirus GI	Norovirus GII	Rotavirus	Total
Percentage of individuals infected	25%	20.78%	19.48%	26.67%	10.67%	68.35%

Table 9. Percent lemurs infected with ≥1 of five viruses

	Avahi laniger	*Eulemur rubriventer*	*Eulemur rufifrons*	*Hapalemur aureus*	*Microcebus rufus*	*Propithecus edwardsi*	*Prolemur simus*
Sample size	*n=1*	*n=21*	*n=21*	*n=7*	*n=12*	*n=16*	*n=6*
Percent infected	*100%*	*71.4%*	*66.7%*	28.6%	83.3%	37.5%	100%

Table 10. Percentage of individuals with zero through five viral infections

Number of viruses infected with	0	1	2	3	4	5
% of individuals	33.77%	38.96%	20.78%	5.19%	1.30%	0%

Table 11. Comparison of lemur species and co-infection rates

Number of viruses per species(%)	*Eulemur rubriventer* % (n=21)	*Eulemur rufifrons* % (n=21)	*Hapalemur aureus* % (n=7)	*Microcebus rufus* % (n=13)	*Propithecus edwardsi* % (n=16)	*Prolemur simus* % (n=6)
0	25	38.1	71.4	20	50	0
1	15	38.1	0	30	25	66.7
2	60	23.85	14.3	30	8.3	33.3
3	0	0	14.3	10	16.7	0
4	0	0	0	10	0	0
5	0	0	0	0	0	0
Total	100	100	100	100	100	100

Table 12. Percentage of individuals infected with ≥1 virus in 3 sites (stratified by site)

Disturbance levels	Val (least) %	Sak/Tal (intermediate) %	CS/Amb (most) %
Adenovirus	0	23.1	46.2
Enterovirus	100	17.2	27.3
Norovirus GI	100	10.9	54.5
Norovirus GII	100	20.3	45.5
Rotavirus	0	12.5	0
% individuals infected with ≥1 virus	100	62.5	83.3

Table 13. Comparison of geophagy and non-geophagy practicing lemurs and virus infection rates

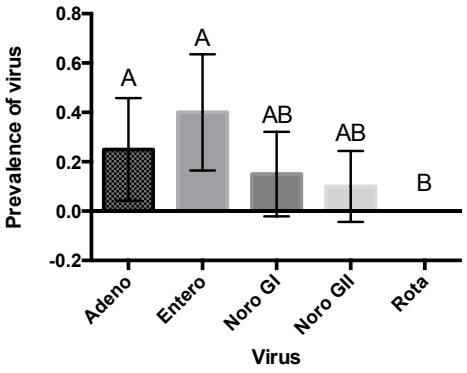
	Adenovirus %	Enterovirus %	Norovirus GI %	Norovirus GII %	Rotavirus %	% individuals infected with 1+ virus
No Geophagy	44.45	27.27	44.45*	44.45	0	89.47*
Geophagy	22.22	20.31	14.06	23.44	12.50	60.00
p-value	0.103	0.613	0.015	0.132	0.246	0.018

Table 14. Comparison of virus infection rates for large and small grouped

	Adenovirus %	Enterovirus %	Norovirus GI %	Norovirus GII %	Rotavirus %	% individuals infected with 1+ virus
Small Group	20	24.147	31.03*	41.38*	%*	89.47
Large group	29.17	19.57	10.87	17.39	17.39	60.00
p-value	0.349	0.646	0.031	0.023	0.019	0.161

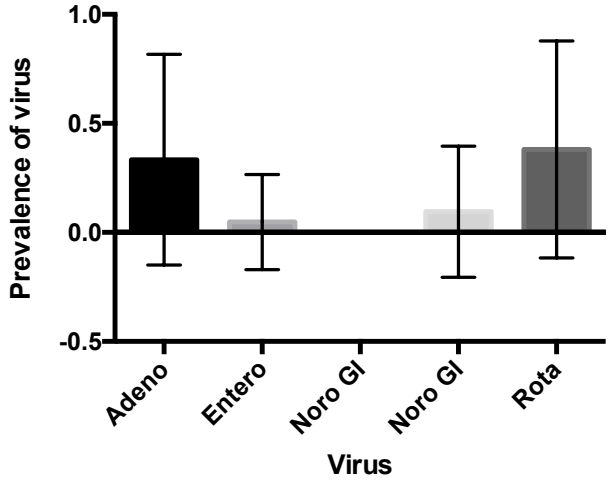
FIGURES

Fig. 8. Prevalence of five viruses for *Eulemur rubriventer*



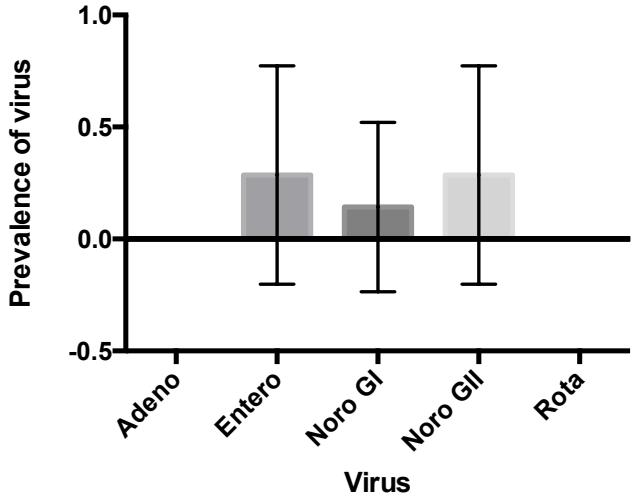
Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs the error bars representing a 95% confidence interval. Alpha = 0.05. Means with different letters are significantly different (p>0.05). *E. rubriventer*, n=21.

Fig. 9. Prevalence of five viruses for *Eulemur rufifrons*



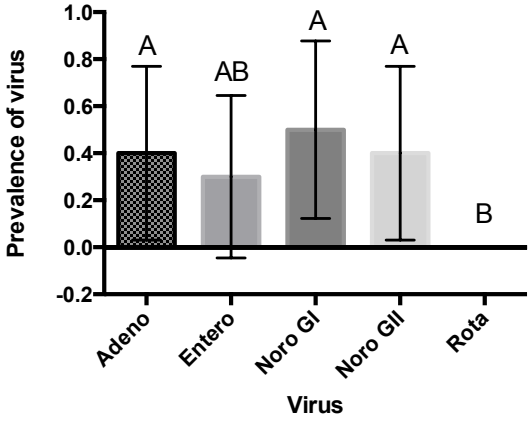
Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs the error bars representing a 95% confidence interval. Alpha = 0.05. Means with different letters are significantly different (p>0.05). *E. rufifrons*, n=21

Fig. 10. Prevalence of five viruses for *Hapalemur aureus*



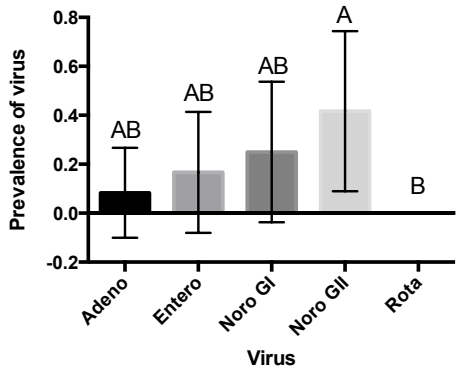
Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs the error bars representing a 95% confidence interval. Alpha = 0.05. Means with different letters are significantly different (p>0.05). *H. aureus*, n=7.

Fig. 11. Prevalence of five viruses for *Microcebus rufus*



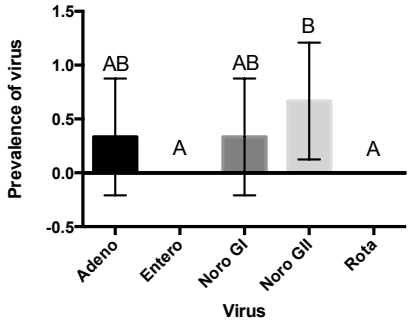
Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs the error bars representing a 95% confidence interval. Alpha = 0.05. Means with different letters are significantly different (p<0.05). *M. rufus*, n=12.

Fig. 12. Prevalence of five viruses for *Propithecus edwardsi*



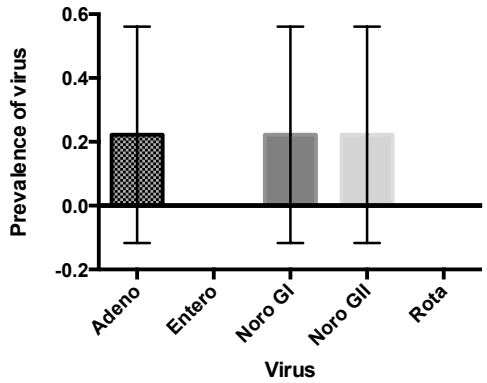
Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs the error bars representing a 95% confidence interval. Alpha = 0.05. Means with different letters are significantly different ($p<0.05$). *P. edwardsi*, n=16.

Fig. 13. Prevalence of five viruses for *Prolemur simus*



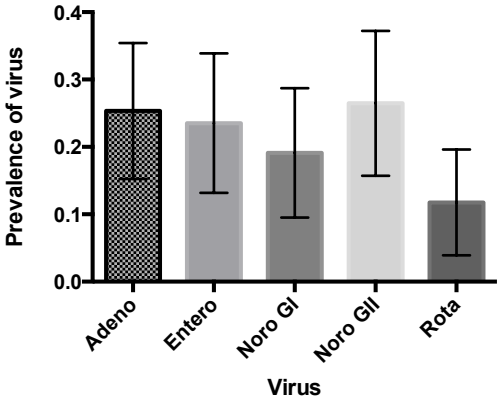
Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs the error bars representing a 95% confidence interval. Alpha = 0.05. Means with different letters are significantly different ($p<0.05$). *P. simus*, n=6.

Fig. 15. Prevalence of five viruses for bamboo lemurs



Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs. The error bars represent a 95% confidence interval. Alpha = 0.05. There is no significant difference in values between any of the five columns ($p>0.05$). Bamboo lemurs, n=13.

Fig. 16. Prevalence of five viruses for non-bamboo eating lemurs



Bars in the middle of the graphs are the means for all prevalences amongst all species of lemurs. The error bars represent a 95% confidence interval. Alpha = 0.05. There is no significant difference in values between any of the five columns ($p>0.05$). Non-bamboo lemurs, n=66.

LITERATURE CITED

Acheson, N. H. 2007. Fundamentals of molecular virology. John Wiley & Sons, Inc.

Atsalis, S. 1998a. Feeding ecology and aspects of life history in Microcebus rufus (family Cheirogaleidae, order Primates). Unpublished Ph. D. dissertation, City University of New York, New York.

Atsalis, S. 1999. Diet of the Brown Mouse Lemur (Microcebus rufus) in Ranomafana National Park, Madagascar. International Journal of Primatology **20**:193-229.

Atsalis, S. 2000. Spatial distribution and population composition of the brown mouse lemur (Microcebus rufus) in Ranomafana National Park, Madagascar, and its implications for social organization. American Journal of Primatology **51**:61-78.

Balko, E. A. and Underwood. 2005. Effects of forest structure and composition on food availability for Varecia variegata at Ranomafana National Park, Madagascar. American Journal of Primatology **66**:45-70.

Bicca-Marques, J. and C. Calegaro-Marques. 1994. A case of geophagy in the black howling monkey Alouatta caraya. Neotropical Primates **2**:7-8.

Bodager, J. R. 2012. Eco-epidemiology of diarrheal disease with an emphasis on Cryptosporidium in and around Ranomafana National Park, Madagascar. Methods **5**:6.

Cassel-Beraud, A., P. Michel, and A. Garbarg-Chenon. 1993. Epidemiological study of infantile rotavirus diarrhoea in Tananarive (Madagascar). Journal of diarrhoeal diseases research **11**:82-87.

Chi, F., M. Leider, F. Leendertz, C. Bergmann, C. Boesch, S. Schenk, G. Pauli, H. Ellerbrok, and R. Hakenbeck. 2007. New Streptococcus pneumoniae clones in deceased wild chimpanzees. Journal of bacteriology **189**:6085-6088.

Clough, D., M. Heistermann, and P. M. Kappeler. 2010. Host intrinsic determinants and potential consequences of parasite infection in free−ranging red−fronted lemurs (Eulemur fulvus rufus). American journal of physical anthropology **142**:441-452.

Couch, R. B., T. R. Cate, R. G. Douglas Jr, P. J. Gerone, and V. Knight. 1966. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. Bacteriological reviews **30**:517.

Crawford, D. H. 2011. Viruses: A Very Short Introduction. OUP Oxford.

Cunliffe, N., P. Kilgore, J. Bresee, A. Steele, N. Luo, C. Hart, and R. Glass. 1998. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. Bulletin of the World Health Organization **76**:525.

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

Davison, A. J., M. Benkő, and B. Harrach. 2003. Genetic content and evolution of adenoviruses. Journal of general virology **84**:2895-2908.

Donaldson, K., D. Griffin, and J. Paul. 2002. Detection, quantitation and identification of enteroviruses from surface waters and sponge tissue from the Florida Keys using real-time RT–PCR. Water research **36**:2505-2514.

Freeland, W. 1976. Pathogens and the evolution of primate sociality. Biotropica **8**:12-24.

Ganzhorn, J. U., J. Abraham, and M. Razanahoera-Rakotomalala. 1985. Some aspects of the natural history and food selection of Avahi laniger. Primates **26**:452-463.

Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, and G. M. Shaw. 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature **397**:436-440.

Garbutt, N. 2007. Mammals of Madagascar: a complete guide. Yale University Press.

Gerber, B. D. (2010) Madagascar GIS: spatial information to aid research and conservation. Accessed 18 Jan 2013.

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

Glander, K. E., P. C. Wright, P. S. Daniels, and A. M. Merenlender. 1992. Morphometrics and testicle size of rain forest lemur species from southeastern Madagascar. Journal of Human Evolution **22**:1-17.

Glander, K. E., P. C. Wright, D. S. Seigler, V. Randrianasolo, and B. Randrianasolo. 1989. Consumption of cyanogenic bamboo by a newly discovered species of bamboo lemur. American Journal of Primatology **19**:119-124.

Goller, J., A. Dimitriadis, A. Tan, H. Kelly, and J. Marshall. 2004. Long-term features of norovirus gastroenteritis in the elderly. The Journal of hospital infection **58**:286-291.

Goodgame, R. 2006. Norovirus gastroenteritis. Current Gastroenterology Reports **8**:401-408.

Häfliger, D., P. Hübner, and J. Lüthy. 2000. Outbreak of viral gastroenteritis due to sewage-contaminated drinking water. International journal of food microbiology **54**:123-126.

Harcourt, C. 1991. Diet and behaviour of a nocturnal lemur, Avahi laniger, in the wild. Journal of Zoology **223**:667-674.

Harper, G. J., M. K. Steininger, C. J. Tucker, D. Juhn, and F. Hawkins. 2007. Fifty years of deforestation and forest fragmentation in Madagascar. Environmental Conservation **34**:325-333.

Hemingway, C. A. 1998. Selectivity and Variability in the Diet of Milne-Edwards' Sifakas (Propithecus diadema edwardsi): Implications for Folivory and Seed-Eating. International Journal of Primatology **19**:355-377.

Hijmans, R., S. Cameron, J. Parra, P. Jones, and A. Jarvis. WORLDCLIM - a set of global climate layers (climate grids).

Hogg, K., S. Wade, and P. Wright. 2006. Parasites of nine lemur species at Ranomafana National Park, Madagascar.

ICTV. 2012. International Committee on Taxonomy of Viruses.

Irwin, M., J. Raharison, and P. Wright. 2009a. Spatial and temporal variability in predation on rainforest primates: do forest fragmentation and predation act synergistically? Animal Conservation **12**:220-230.

Irwin, M. T. and J.-L. Raharison. 2009b. A review of the endoparasites of the lemurs of Madagascar. Malagasy Nat **2**:66-93.

IUCN. 2012. The IUCN Red List of Threatened Species. Version 2012.2. http://www.iucnredlist.org. Downloaded on 12 January 2013.

Jernvall, J. and P. C. Wright. 1998. Diversity Components of Impending Primate Extinctions. Proceedings of the National Academy of Sciences of the United States of America **95**:11279-11283.

Jothikumar, N., J. Lowther, K. Henshilwood, D. Lees, V. Hill, and J. Vinjé. 2005. Rapid and sensitive detection of noroviruses using TaqMan-based one-step RT-PCR assays and application to clinical and naturally contaminated shellfish samples. Appl. Environ. Microbiol **71**:1870-1875.

Junge, R. E., C. J. Dutton, F. Knightly, C. V. Williams, F. T. Rasambainarivo, and E. E. Louis. 2008. Comparison of biomedical evaluation for white-fronted brown lemurs (Eulemur fulvus albifrons) from four sites in Madagascar. Journal of Zoo and Wildlife Medicine **39**:567-575.

Kageyama, T., S. Kojima, M. Shinohara, K. Uchida, S. Fukushi, F. B. Hoshino, N. Takeda, and K. Katayama. 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. Journal of clinical microbiology **41**:1548-1557.

Kalter, S. 1982. Enteric viruses of nonhuman primates. Veterinary Pathology Online **19**:33-43.

Kalter, S. and R. Heberling. 1971. Comparative virology of primates. Bacteriological reviews **35**:310.

Khamrin, P., M. Okame, A. Thongprachum, N. Nantachit, S. Nishimura, S. Okitsu, N. Maneekarn, and H. Ushijima. 2011. A single-tube multiplex PCR for rapid detection in feces of 10 viruses causing diarrhea. Journal of virological methods **173**:390-393.

King, S. J., D. M. Boyer, S. Tecot, S. G. Strait, S. Zohdy, M. B. Blanco, P. C. Wright, and J. Jernvall. 2012. Lemur habitat and dental senescence in Ranomafana National Park, Madagascar. American journal of physical anthropology **148**:228-237.

Knezevich, M. 1998. Geophagy as a therapeutic mediator of endoparasitism in a free−ranging group of rhesus macaques (Macaca mulatta). American Journal of Primatology **44**:71-82.

Kosek, M., C. Bern, and R. L. Guerrant. 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. BULLETIN-WORLD HEALTH ORGANIZATION **81**:197-204.

Krishnamani, R. and W. C. Mahaney. 2000. Geophagy among primates: adaptive significance and ecological consequences. Animal Behaviour **59**:899-915.

Laakkonen, J., J. T. Lehtonen, H. Ramiarinjanahary, and P. C. Wright. 2003. Trypanosome parasites in the invading Rattus rattus and endemic rodents in Madagascar. ACIAR MONOGRAPH SERIES **96**:37-39.

Logan, C., J. J. O'Leary, and N. O'Sullivan. 2006. Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. Journal of clinical microbiology **44**:3189-3195.

Meier, B., R. Albignac, A. Peyriéras, Y. Rumpler, and P. Wright. 1987. A New Species of Hapalemur (Primates) from South East Madagascar. Folia Primatologica **48**:211-215.

Meier, B. and Y. Rumpler. 1987. Preliminary survey of Hapalemur simus and of a new species of Hapalemur in eastern Betsileo, Madagascar. Primate Conserv **8**:40-43.

Meyer, W. B. and B. L. I. Turner. 1992. Human Population Growth and Global Land-Use/Cover Change. Annual Review of Ecology and Systematics **23**:39-61.

Mittermeier, R. A., E. E. Loiuis, M. Richardson, C. Schwitzer, O. Langrand, A. B. Rylands, F. Hawkins, S. Rajaobelina, J. Ratsimbazafy, R. Rasoloarison, C. Roos, P. Kappeler, and J. Mackinnon. 2010. Lemurs of Madagascar. 3rd edition. Conservation International.

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

Norosoarinaivo, J. A. e., C. Tan, L. Rabetafika, and D. Rakotondravony. 2009. Impact du tourisme sur Prolemur

simus à Talatakely, dans le Parc National de Ranomafana. Lemur News **14**:43.

Ortmann, S., G. Heldmaier, J. Schmid, and J. U. Ganzhorn. 1997. Spontaneous Daily Torpor in Malagasy Mouse Lemurs. Naturwissenschaften **84**:28-32.

Overdorff, D. J. 1993. Similarities, differences, and seasonal patterns in the diets ofEulemur rubriventer andEulemur fulvus rufus in the Ranomafana National Park, Madagascar. International Journal of Primatology **14**:721-753.

Overdorff, D. J. 1996a. Ecological correlates to social structure in two lemur species in Madagascar. American journal of physical anthropology **100**:487-506.

Overdorff, D. J. and M. A. Rasmussen. 1995. Determinants of nighttime activity in "diurnal" lemurid primates. Pages 61-74 Creatures of the Dark. Springer.

Overdorff, D. J., S. G. Strait, and A. Telo. 1997. Seasonal variation in activity and diet in a small−bodied folivorous primate, Hapalemur griseus, in southeastern Madagascar. American Journal of Primatology **43**:211-223.

Papaventsis, D. C., W. Dove, N. A. Cunliffe, O. Nakagomi, P. Combe, P. Grosjean, and C. A. Hart. 2007. Norovirus infection in children with acute gastroenteritis, Madagascar, 2004–2005. Emerging infectious diseases **13**:908.

Pebsworth, P. A., C. E. Archer, C. C. Appleton, and M. A. Huffman. 2012. Parasite Transmission Risk From Geophagic and Foraging Behavior in Chacma Baboons. American Journal of Primatology **74**:940-947.

Pickering, L. K., A. V. Bartlett, and W. E. Woodward. 1986. Acute infectious diarrhea among children in day care: epidemiology and control. Review of Infectious Diseases **8**:539-547.

Rakoto-Andrianarivelo, M., D. Rousset, R. Razafindratsimandresy, S. Chevaliez, S. Guillot, J. Balanant, and F. Delpeyroux. 2005. High frequency of human enterovirus species C circulation in Madagascar. Journal of clinical microbiology **43**:242-249.

Randremanana, R., F. Randrianirina, M. Gousseff, N. Dubois, R. Razafindratsimandresy, E. R. Hariniana, B. Garin, A. Randriamanantena, H. C. Rakotonirina, and L. Ramparany. 2012.

Case-Control Study of the Etiology of Infant Diarrheal Disease in 14 Districts in Madagascar. PLOS ONE **7**:e44533.

Rizkalla, C., F. Blanco-Silva, and S. Gruver. 2007. Modeling the impact of Ebola and bushmeat hunting on Western Lowland Gorillas. EcoHealth **4**:151-155.

Rodriguez-Baez, N., R. O'Brien, S.-Q. Qiu, and D. M. Bass. 2002. Astrovirus, adenovirus, and rotavirus in hospitalized children: prevalence and association with gastroenteritis. Journal of pediatric gastroenterology and nutrition **35**:64-68.

Singh, A. and M. Fleurat. 2010. Pediatric Emergency Medicine Practice Acute Gastroenteritis — An Update. EB Medecine **7**.

Sussman, R. W., G. M. Green, and L. K. Sussman. 1994. Satellite imagery, human ecology, anthropology, and deforestation in Madagascar. Human Ecology **22**:333-354.

Tan, C. L. 1999. Group Composition, Home Range Size, and Diet of Three Sympatric Bamboo Lemur Species (Genus Hapalemur) in Ranomafana National Park, Madagascar. International Journal of Primatology **20**:547-566.

Tan, C. L. 2000. Behavior and ecology of three sympatric bamboo lemur species (genus Hapalemur) in Ranomafana National Park, Madagascar. State University of New York at Stony Brook.

Tattersall, I. 1986. Notes on the Distribution and Taxonomic Status of Some Subspecies of< i> Propithecus</i> in Madagascar. Folia Primatologica **46**:51-63.

Tecot, S. 2008. Seasonality and predictability: the hormonal and behavioral responses of Eulemur rubriventer in Southeastern Madagascar [dissertation]. Austin, TX: University of Texas-Austin.

Thorén, S., F. Quietzsch, and U. Radespiel. 2010. Leaf nest use and construction in the golden-brown mouse lemur (Microcebus ravelobensis) in the Ankarafantsika National Park. American Journal of Primatology **72**:48-55.

Wang, Y., X. Tu, C. Humphrey, H. McClure, X. Jiang, C. Qin, R. I. Glass, and B. Jiang. 2007. Detection of viral agents in fecal specimens of monkeys with diarrhea. Journal of medical primatology **36**:101-107.

Webber, R. 2009. Communicable disease epidemiology and control: a global perspective. Cabi.

Wevers, D., S. Metzger, F. Babweteera, M. Bieberbach, C. Boesch, K. Cameron, E. Couacy-Hymann, M. Cranfield, M. Gray, and L. A. Harris. 2011. Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. Journal of virology **85**:10774-10784.

Whittier, C. A. 2009. Diagnostics and Epidemiology of Infectious Agents in Mountain Gorillas.

Wittemyer, G., P. Elsen, W. T. Bean, A. C. O. Burton, and J. S. Brashares. 2008. Accelerated human population growth at protected area edges. Science **321**:123-126.

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

Wright, P. C. 1995. Demography and life history of free-rangingPropithecus diadema edwardsi in ranomafana national park, madagascar. International Journal of Primatology **16**:835-854.

Wright, P. C. 1997. The future of biodiversity in Madagascar: A view from Ranomafana National Park. Natural change and human impact in Madagascar:381-405.

Wright, P. C. 1998. Impact of Predation Risk on the Behaviour of Propithecus diadema edwardsi in the Rain Forest of Madagascar. Behaviour **135**:483-512.

Wright, P. C., E. M. Erhart, S. Tecot, A. L. Baden, S. J. Arrigo-Nelson, J. Herrera, T. L. Morelli, M. B. Blanco, A. Deppe, S. Atsalis, S. Johnson, F. Ratelolahy, C. Tan, and S. Zohdy. 2012. Long-Term Lemur Research at Centre Valbio, Ranomafana National Park, Madagascar. Pages 67-100 *in* P. M. Kappeler and D. P. Watts, editors. Long-Term Field Studies of Primates. Springer Berlin Heidelberg.

Wright, P. C. and J. Jernvall. 1999. The future of primate communities: A reflection of the present? Primate communities:295.

Wright, P. C., S. E. Johnson, M. T. Irwin, R. Jacobs, P. Schlichting, S. Lehman, E. E. Louis Jr, S. J. Arrigo-Nelson, J.-L. Raharison, and R. R. Rafalirarison. 2008. The crisis of the critically endangered greater bamboo lemur (Prolemur simus). Primate Conservation **23**:5-17.

Xu, P., G. Widmer, Y. Wang, L. S. Ozaki, J. M. Alves, M. G. Serrano, D. Puiu, P. Manque, D. Akiyoshi, and A. J. Mackey. 2004. The genome of Cryptosporidium hominis. Nature **431**:1107-1112.

Yan, H., T. A. Nguyen, T. G. Phan, S. Okitsu, Y. Li, and H. Ushijima. 2004. Development of RT-multiplex PCR assay for detection of adenovirus and group A and C rotaviruses in diarrheal fecal specimens from children in China. Kansenshogaku zasshi. The Journal of the Japanese Association for Infectious Diseases **78**:699.

Yan, H., F. Yagyu, S. Okitsu, O. Nishio, and H. Ushijima. 2003. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. Journal of virological methods **114**:37-44.

Zoll, G., W. Melchers, H. Kopecka, G. Jambroes, H. Van der Poel, and J. Galama. 1992. General primer-mediated polymerase chain reaction for detection of enteroviruses: application for diagnostic routine and persistent infections. Journal of clinical microbiology **30**:160-165.