A PILOT STUDY OF GASTRO-INTESTINAL PARASITES IN TWO U.S. CAPTIVE FREE-RANGING LEMUR POPULATIONS

By

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ABSTRACT

PHILIP B. CORBETT II. A PILOT STUDY OF GASTRO-INTESTINAL PARASITES IN TWO U.S. CAPTIVE FREE-RANGING LEMUR POPULATIONS.
(Under the direction of DR. LYDIA LIGHT)

A comparative gastrointestinal parasite study was conducted looking at different lemur species and their parasite prevalence and diversity at two Association of Zoo & Aquarium accredited facilities, the Duke Lemur Center and Lemur Conservation Foundation. The study compared parasite prevalence and diversity among three different host lemur species, *Lemur catta*, *Eulemur mongoz*, and *Varecia rubra*. A total of 54 fecal samples were collected noninvasively from the lemurs. These samples were prepared for microscopic analysis using a standard fecal flotation method (RUSVM 2020). A total of six different parasite taxa were identified during microscopic analysis. Although no statistical significance (defined as $p > 0.05$) was found, there are indications that location and diet possibly play a role in differences of parasite prevalence and diversity among the different lemur species that could not be detected with the small sample size. A total of eight parasite-positive samples were found: 5 in *L. catta*, 2 in *E. mongoz*, and 1 in *V. rubra*. Few published studies have conducted comparative parasitology between lemur species; this is especially the case for captive lemurs. Parasitology studies are important due to the relationship between primate parasitology, primate health, and conservation. As such, this study fills a problematic gap in the literature and will hopefully evoke parasitology centered conversations between the Duke Lemur Center and Lemur Conservation Foundation.

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LIST OF ABBREVIATIONS

AZA	Association of Zoos and Aquariums
CSD	Cat scratch disease
DLC	Duke Lemur Center
G	Gravitational constant
GI	Gastrointestinal
IACUC	Institutional Animal Care and Use Committee
LCF	Lemur Conservation Foundation
μm	Micrometer
NHP	Nonhuman primate
PCR	Polymerase chain reaction
SCI	St. Catherine's Island
spg	Specific gravity

SECTION 1: INTRODUCTION

The primary goals of this study are to identify which gastrointestinal parasites affect different captive free-ranging lemur species living in multiacre enclosures within the United States and to identify any differences in gastrointestinal parasite diversity between the species. The lemurs in this study were located at the Lemur Conservation Foundation and Duke Lemur Center. Fecal samples were collected from members of each lemur species at the faciltities, which is important because there may be differences in parasite diversity between locations. Predictors for parasite diversity will provide supplemental information to help explain why there might be differences in diversity or prevalence between different lemur species.

Approximately 94% of lemurs are considered threatened species (Schwitzer et al., 2014); therefore, studies focused on their health provide essential information for lemur conservation. Recent studies on gastrointestinal microbiomes have shown scientists the importance of gut communities in relation to the health of a host organism (Clayton et al., 2018; Stumpf et al., 2016; Hollister et al., 2014; Yildirim et al., 2010). Parasites, which often make their way into organisms' gastrointestinal systems, undoubtedly can have an impact on the overall health and behavior of their host (Nguyen et al., 2015; Johnson-Delaney, 2009; Nunn & Altizer, 2006). While many studies have looked at parasite diversity in various primate species, few published studies have compared parasite diversity or prevalence between captive free-ranging lemur species, particularly in the United States. This study may, therefore, be beneficial to understanding the relationship between parasites and captive free-ranging lemur populations.

SECTION 2: BACKGROUND INFORMATION

2.1 Lemur Diversity

Strepsirrhines are a group of nonhuman primates (**NHP**s) characterized by their wet rhinarium. Among the strepsirrhines are lemurs, a group of NHPs which are believed to have colonized the island of Madagascar 60 to 50 million years ago (Irwin & Raharison, 2009; Tattersall, 2006; Poux et al., 2005). It is suggested that Madagascar broke off from the mainland well before the origin of primates; therefore, scientists believe lemurs made their way to the island by rafting on large mats of vegetation and hibernating to survive the long journey (Kappeler, 2000). Some primatologists suggest the number of wild lemur species to be around 100 (Mittermeier et al., 2008; "Research Overview," n.d.), however, the number of lemur species represented in captivity is notably lower. The number of lemur species in existence are constantly changing due to the discovery of new species, extinction of other species, and revisions to taxonomic classifications. Despite their small geographic range, lemurs represent more than 20% of NHP species (Schwitzer et al., 2014). The harsh climate and various unique habitats scattered across Madagascar have allowed for many unique species to evolve over time, filling a variety of niches (Tattersall, 2006; Wright, 1999). For example, the nocturnal aye-aye (*Daubentonia madagascariensis*) evolved to be a large, primarily insectivorous lemur with unique dental and finger morphology. These traits enable this species to fill the percussive foraging niche that woodpeckers fill in other areas of the world (Sterling & McCreless, 2006). Significant size variations also exist between lemur species. The pygmy mouse lemur (*Microcebus myoxinus*) is the smallest known primate, with average adults weighing only 32 grams (Schmid et al., 2000). Sifakas (*Propithecus* spp.) and indris (*Indri indri*), both members of the Indriidae family, are the largest lemurs remaining in Madagascar. The indri is primarily

folivorous (Powzyk & Mowry, 2006; Irwin, 2006), a trait shared with many other large NHPs. Sifakas primarily eat leaves and supplement their diets with fruits and nuts; however, their digestive tracts are long and complex, making them morphologically folivorous (Irwin, 2006; Campbell et al., 2000). These traits allow these species to survive during the fruitless dry seasons.

Lemur catta

Lemur catta are some of the most well-known lemur species and are heavily represented in United States' zoos and other facilities. They serve as a flagship species for Madagascar (Villers et al., 2008) and have even made their way into famous Hollywood films. They are known for their black and white ringed tails, white faces, and grey bodies. These largely terrestrial lemurs live in female dominated groups with males that tend to leave their natal troop upon adulthood (Sauther, 1998; Sussman, 1991; Sauther, 1989; Taylor and Sussman, 1985). Studies show that adult ring-tailed lemurs tend to weigh anywhere from 2 to 2.8 kilograms (Campbell et al., 2000; Keith-Lucas et al., 1999), making them medium-sized compared to other lemur species. Despite their limited range in southern Madagascar, they live in a variety of habitats. As such, the diet of ring-tailed lemurs tends to be quite varied. They are classified as frugivorous/folivorous and have also been reported as occasionally omnivorous (Gould, 2006; Simmen et al., 2006; Sauther, 1998). The diets of *L. catta* are based on various factors such as season, lactation, and geography. *Lemur catta* are noted to eat more fruit during the wet season or during lactation, while in the dry season they notably eat more leaves, grasses, and herbs. However, it was found that *L. catta* at the Beza Mahafaly Reserve in Madagascar eat leaves and herbs 62% of the time during the wet season (Simmen et al., 2006). The varied diet of ring-tailed lemurs has led primatologists to refer to them as generalists (Rushmore et al., 2012). The

gastrointestinal (**GI**) tract of *L. catta* is described as being moderately complex relative to other lemur species and is reflective of their diet and body size (Campbell et al., 2000).

Eulemur mongoz

The mongoose lemur (*Eulemur mongoz*), much like *L. catta*, lives in female dominated groups, however, group size is comparably smaller, usually 2-6 individuals (Curtis, 2004). Like many other lemur species, mongoose lemurs have seasonal diets. They are primarily frugivorous and supplement their diets with a variety of leaves and flowers (Curtis, 2004; Tattersall & Sussman, 1975). At Anjamena (northwestern Madagascar), it was found that *E. mongoz* eats fruits and seeds 65% of the time during the dry season, with leaves making up 17% of their diet (Curtis, 2004). They are slightly smaller than the medium-sized lemurs, such as *Eulemur fulvus* and *L. catta*, and are named after their mongoose-like appearance. Although monomorphic, sexes can be identified by different colored beards. Females have white colored beards while males' beards are orange. Although typically considered a diurnal species, one study found mongoose lemurs to be more active at night (Tattersall & Sussman, 1975).

Varecia spp.

Critically endangered ruffed lemurs (*Varecia* spp.), including red ruffed lemurs (*Varecia rubra*) and black-and-white ruffed lemurs (*Varecia variegata*), are proven seed dispersers and play a key role in maintaining the health of their ecosystems in Madagascar (Razafindratsima et al., 2012). There are ongoing debates about whether *V. rubra* and *V. variegata* are the same species or separate species. One study found that the color variations of ruffed lemur species are not geographically unique, thus they should be considered the same species (Vasey & Tattersall, 2002). However, a more recent study concluded that there is enough genetic diversity between color variations to suggest they are different species (Louis et al., 2005). Regardless, the ecology

and biology of the two species is quite similar and as they have often been discussed collectively in the literature, their ecology is reviewed here in a similar manner (Rushmore et al., 2012). Ruffed lemurs live in large multimale multifemale groups with females serving as the dominate sex (Overdorff et al., 2005; Vasey, 2004). These species are highly frugivorous (Rushmore et al., 2012; Razafindratsima et al., 2012; Dutton et al., 2008; Overdorff et al., 2005; Vasey, 2004) and are larger than previously mentioned medium-sized lemurs. A study at Ranomafana National Park found the diet of *V. variegata* to contain 82-86% fruit (Overdorff et al., 2005). This would make *Varecia* spp. the most frugivorous of the captive free-ranging lemurs in the United States. The average adult *Varecia* spp. weighs in at approximately 3 kilograms (Dutton et al., 2008; Campbell et al., 2000). Their digestive system is characteristic of frugivorous species and is simple compared to folivorous species (Campbell et al., 2000).

2.2 U.S. Lemur Conservation & Research Colonies

The Lemur Conservation Foundation (**LCF**) and Duke Lemur Center (**DLC**) are two Association of Zoos & Aquariums (**AZA**) accredited facilities. While both facilities are based in the United States, they are fully engaged with conservation efforts in Madagascar. These U.S. based facilities present an opportunity to conduct research with lemurs without having to travel to Madagascar. Although these lemurs are in captive situations, individuals with access to captive free-range enclosures have been shown to express similar behaviors to those found in the wild (Keith-Lucas et al., 1999). These multiacre fenced-off habitats containing U.S. native flora and fauna can be found at DLC and LCF. As such, these facilities provide an environment that can be useful in understanding both captive and wild lemur species. Although both facilities

notably house a variety of species only the species mentioned before (*L. catta*, *E. mongoz*, and V. *rubra*) are found in free-ranging enclosures at both facilities.

Duke Lemur Center

Founded in 1966 at Duke University, DLC is a lemur conservation and non-invasive research center in Durham, North Carolina. The facility is 85 acres and, as of 2021, houses 14 species of lemur ("History and Mission," n.d.). In addition to the center's diverse collection of living lemurs, the center's Division of Fossil Primates has an expansive collection of fossil NHPs and other non-primate fossils ("Division of Fossil Primates," n.d.). As of 2021, DLC has 11 multiacre free-range enclosures. Lemurs in these enclosures, although provisioned by the center, can eat and interact with North Carolina's flora, fauna, and soil.

Lemur Conservation Foundation

The Lemur Conservation Foundation was founded by Penelope Bodry-Sanders in 1996. Although the facility started off at 30 acres in 1997, the foundation currently sits on 130 acres in Myakka City, Florida. As of 2021, LCF houses 5 species of lemurs, only 3 of which are granted access to 3 different multiacre free-ranging enclosures. Various types of fruits and vegetation are planted in each enclosure to supplement the native Florida flora and fauna consumed by the lemurs while foraging ("Florida," n.d.). Similar to DLC, LCF also provisions their lemurs with a mixture of chow, fruits, and vegetables.

St. Catherine's Island

Of the locations that lemurs inhabit in the United States, St. Catherine's Island (**SCI**) in Georgia is perhaps the most unique. St. Catherine's Island is a barrier island (over 22,000 acres) off the coast of Georgia and is home to a variety of different U.S. native wildlife. Although various nonnative species have been introduced to the island, lemurs are the most notable. In

1985, the Wildlife Conservation Society introduced six ring-tailed lemurs (*Lemur catta*) from the Bronx Zoo to SCI (Parga & Lessnau, 2005). Other lemur species were also introduced to the island such as the blue-eyed black lemur (*Eulemur flavifrons*) and the black-and-white ruffed lemur (*Varecia variegata*) (Yabsley et al., 2007; Yabsley et al., 2004). Today the population of ring-tailed lemurs in SCI has grown substantially with the lemurs forming several separate troops (Parga, 2010; Yabsley et al., 2007; Parga & Lessnau, 2005).

Similar to both LCF and DLC, lemurs at SCI are provisioned with Mazuri (manufacturer) primate chow, fruits, and vegetables and also have the ability to forage on and interact with the island's native ecosystem (Hall et al., 2007; Parga & Lessnau, 2005). What makes the lemurs at SCI different from lemurs at the two other facilities is the ability for the lemurs to freely enter and exit provided shelters (Parga & Lessnau, 2005). Lemurs at SCI are also only restricted by geographical boundaries, while the lemurs at LCF and DLC are limited by fences and can be locked outside of their free-ranging enclosure. This unique management style puts lemurs at SCI somewhere between a captive and 'wild' free-ranging population. Studies on lemur behavior (Parga, 2010; Parga & Lessnau, 2008; Parga & Lessnau, 2005) and lemur parasitology (Hall et al., 2007; Yabsley et al., 2007; Junge & Sauther, 2006; Yabsley et al., 2004) at SCI have shown us that U.S. free-ranging lemurs have parasite diversity and exhibit behaviors also found in wild populations. It is important to note that the majority of published parasite studies from SCI focus on non-GI endoparasites. Due to the uniqueness of the situation at SCI and financial/logistical constraints, including the SCI lemur population was beyond the scope of this project. A future lemur parasitology study may benefit from including the SCI lemur population.

2.3 Zoonoses & Anthroponoses

Approximately 60% of past, present, and emerging human infectious diseases are considered zoonotic, which is when a pathogen originates in animals and spreads to humans (Quammen, 2013; Pourrut et al., 2011; Muriuki et al., 1998). Primatologists have taken the initiative to look at these issues because NHPs and people commonly interact via pet trade, tourism, captivity, and consumption of bushmeat, which makes them ideal 'study subjects' for zoonotic disease and parasite transfer (Pourrut et al., 2011; Gillespie, 2006; Michaud et al., 2003; Wallis & Lee, 1999). Increased interactions between humans and animals increases parasite and disease transmission risk. One study in Africa showed that there are significant numbers of gastrointestinal parasites in both bushmeat and pet trade NHP individuals (Pourrut et al., 2011). During the skinning and butchering process of infected primate bushmeat, hunters could contract a disease through cuts on their hands (Michaud et al., 2003). Zoonotic retrovirus can also infect people who have made direct contact with fresh NHP bushmeat (Chomel et al., 2007). The close evolutionary and genetic relationship between humans and NHPs allows for relatively easy pathogen transfer across species (Nunn & Altizer, 2006; Michaud et al., 2003; Woodford et al., 2002; Wallis & Lee, 1999).

Humans are also capable of transmitting pathogens to NHPs. These events are called anthroponoses. Perhaps the most devastating examples of anthroponoses are due to ecotourism and can be seen in chimpanzees (*Pan troglodytes*). In the 1980-90's there were several cases where chimpanzees were infected with respiratory infections; this was thought to be caused by tourists who were ill with the common cold when they participated in ecotourism. Approximately 36 individual chimpanzees died as a result during this time period (Woodford et al., 2002). Since apes are closely related to humans, they are susceptible to many of the same

diseases and parasites (Woodford et al., 2002) However, the effects that diseases such as the common cold can have on NHPs are often significantly more catastrophic than in humans (Woodford et al., 2002). As such, studying pathogens that can transfer between humans and NHPs, such as lemurs, is important to both conservation and public health (Rushmore et al., 2017; Cleaveland et al., 2001).

2.4 Introduction to Parasitology

Nunn and Altizer (2006, pg. 3) define parasites as "any organism that lives on or draws nutrients from another living organism." Pathogenic parasites can alter their hosts' behaviors, cause disease, and produce problematic clinical signs. Although the term 'parasite' tends to have a negative connotation, not all parasites are bad. Several species of parasites are nonpathogenic, meaning they do not cause harm to their host. *Entamoeba coli* is a great example of a nonpathogenic parasite that is commonly found in a variety of animal species, including primates (Issa, 2014). Some parasites can be beneficial to their host by regulating the population of other parasites through the competition; this competition can sometimes be seen between trematode species (Poulin, 1999).

Anthropogenic factors such as habitat degradation from agriculture and other human activities have been proven to alter NHP parasite ecology and parasite diversity (Junge & Sauther, 2006; Nunn & Altizer, 2006; Michaud et al., 2003). There may be several reasons for this with one being that forest fragmentation due to agriculture and deforestation brings NHPs closer to each other and humans. This has notably affected NHP parasite ecology in wild populations (Mbora & McPeek, 2009; Gillespie & Chapman, 2008). Close proximity of individuals has independently been proven to increase parasite transmission risk (Rushmore et

al., 2017; Nunn & Altizer, 2006). In captivity, individuals of the same species, different species, and humans are often in close proximity with each other. Although it was previously believed that parasites were highly host-specific, it is now known that 60-68% of known NHP parasites affect multiple primate species, including humans (Pedersen et al., 2005; Cleaveland et al., 2001). Therefore, if one species of captive NHP is infected with a parasite, it is possible for another primate species to contract that parasite, including humans. In Peru, helminth parasites have been reportedly shared between captive NHPs and humans (Michaud et al., 2003). As such, humans are also capable of transmitting parasites to and between captive NHP species if not careful. Free-range enclosures also add a new dynamic to NHP parasite ecology. The environment provides a non-sterile location for environmental stages of parasites to thrive, for paratenic hosts to exist, etc. This is in comparison to enclosed more sterilized enclosures.

This project does not focus on bacteria, fungi, or other "nontraditional" parasites. On the most basic level, parasites can be split into two categories: ectoparasites and endoparasites. Ectoparasites are organisms that live outside their host's body, such as lice, ticks, or mites. Endoparasites are organisms that live within the host's body, such as helminths and protozoa (Nunn & Altizer, 2006). Although parasites are broadly suggested to include bacteria, viruses, and fungi (Nunn & Altizer, 2006; Pedersen et al., 2005), the term is more typically used to refer to protozoa and helminths, with this project focusing on GI protozoa and helminths.

2.5 NHP Parasites

Ectoparasites

Ectoparasites can be defined as any parasite that lives in or on the hair and/or skin. They include leeches, insects, mites, and various other taxa (Johnson-Delaney, 2009; Libersat et al.,

2009). The vast majority of ectoparasites in NHPs are either arthropods, including ticks and mites, or insects, such as fleas and lice (Johnson-Delaney, 2009; Wallis & Lee, 1999; Whitney Jr, 1974). The study of these parasites in primates helps anthropologists better understand primate biology and has also aided in answering questions about when humans developed clothing (Wrangham, 2017).

Many ectoparasites can infect both humans and NHPs. There have been many cases where ectoparasites can be classified as either zoonoses or anthroponoses. One great example of this can be seen with the itch mite (*Sarcoptes scabiei*). Scabies, which is an infestation of itch mites under the skin, is highly contagious (Wallis & Lee, 1999). Scabies is often associated with humans and it is believed that chimpanzees likely acquired the parasite from them (Wallis & Lee, 1999). Although it is notably uncomfortable for humans, the infestation in chimpanzees causes considerably more serious clinical signs. These signs include itching, weight loss, tremors, and anorexia; there also have been reported cases of infant chimpanzees dying due to *Sarcoptes* (Wallis & Lee, 1999).

It is important to note that grooming behavior in NHPs, although serving a social function, also helps remove ectoparasites, such as fleas, from individuals (Dunbar, 1991). However, NHPs are still at risk of contracting ectoparasites (Johnson-Delaney, 2009; Dunbar, 1991). This is especially the case in captive primates, who can contract the common flea (*Ctenocephalides felis*) from other household pets and do not have other primates to assist in grooming behavior (Johnson-Delaney, 2009). Fleas have been reported to affect all captive NHP species and are also known to transmit diseases to their hosts, such as spotted fever, which is an infection of the bacteria *Rickettsia rickettsii* (Bitam et al, 2010; Johnson-Delaney, 2009). As such, the use of topical treatments for dogs and cats have been successfully used to treat flea

infestations in captive NHPs (Johnson-Delaney, 2009). The common flea, which can feed on a variety of hosts, including NHPs, is a vector for cat scratch disease (**CSD**), an infection of *Bartonella henselae* (Bitam et al., 2010; Johnson-Delaney, 2009). Fleas carrying CSD are both a concern to NHPs and people due to the clinical signs associated with the disease (Bitam et al., 2010). Nonhuman primates and other household pets infected with CSD from flea bites have the opportunity to transmit the disease to their owners via bites and scratches. For this reason, ectoparasites in NHPs, such as fleas, create a disease risk to humans and other primates around affected animals. However, they are not the only parasites that cause diseases in primates.

Non-GI Endoparasites

There are many examples of blood-borne parasites in NHPs such as nematode larvae (*Dipetalonema gracile*), plasmodiums (*Plasmodium* spp.), and blood flukes (*Schistosoma mansoni*) (Johnson-Delaney, 2009). However, plasmodiums are the most discussed due to the notoriety of malaria. While the infestation of plasmodiums is known as malaria, they themselves are parasitic eukaryotes that are transmitted to hosts via insect bites, usually mosquitoes (Cormier, 2011). There is much to discuss regarding plasmodiums and malaria; however, it is first important to talk about the host specificity debate. When discussing host specificity, it is easier to think of primates as a whole instead of individual species. This is because the plasmodiums that affect primates are specific to primates and do not affect other groups of animals such as canines, felines, and ungulates. Despite the fact that 60-68% of known NHP parasites affect multiple hosts (Pedersen et al., 2005; Cleaveland et al., 2001), not every primate plasmodium affects every primate species. For example, humans are hosts to four plasmodiums that can cause malaria: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* (Cormier, 2011). These fall into the four main groups of plasmodiums that infect all primates: falciparum type, vivax

type, ovale type, malariae type (Cormier, 2011). Not only are these four plasmodiums not closely related, they also lack the ability to infect all primate species (Quammen, 2013; Cormier, 2011). That is not to say that plasmodiums cannot infect multiple primate species. For example, *Plasmodium knowlesi* is an emerging species of malaria parasite found in macaques (*Macaca* spp.) that has recently been found to infect humans (Ahmed & Cox-Singh, 2015). This phenomenon is known as host-switching.

There are many case studies that focus on malaria in NHPs. Most of these have importance to conversations regarding either conservation or public health, especially if malaria is seen as zoonotic. One study looked at the relevance of NHP malaria models for humans. The idea of using NHPs as models for humans, especially in the medical industry, is well established. It was concluded that by comparing and contrasting human models to NHP models, it is possible to produce fruitful knowledge about malaria in general (Langhorne et al., 2011). Another study more specifically tied to malaria looked at infection rates of plasmodiums in the Amazon. The study focused on humans and several South American primate species. The authors of the work suggested that understanding the zoonotic potential of plasmodiums is necessary and that the prevalence of this parasite poses a threat to both public health and anti-malaria campaigns (Lourenço-de-Oliveira & Deane, 1995). Again, like the previous study and many more, the use of malaria models in NHPs has aided in an understanding about how malaria affects and spreads through humans. However, plasmodiums are not the only parasites used in comparative models. NHP gastrointestinal parasites are also used as comparative models for humans.

2.6 Introduction to GI Parasites

Research on NHP parasitology is largely focused on gastrointestinal parasites. Although many studies have extensively examined GI parasites in African monkeys, apes, and howler monkeys, the parasites of various other taxa remain relatively understudied (Gillespie, 2006). As previously noted, GI microbiomes are extremely important to the health of organisms (Clayton et al., 2018; Stumpf et al., 2016; Hollister et al., 2014; Yildirim et al., 2010). Parasites living within the GI tract have the ability to negatively affect these delicate microbiomes. As such, the study of GI parasites is important to primate health and conservation. Furthermore, it was found that more than half of helminths, GI nematodes (Pourrut et al., 2011; Pedersen et al., 2005), and approximately one third of protozoan parasites (Nunn et al., 2005) overlap between NHPs and humans. Therefore, NHP GI parasite studies are also important to public health.

GI parasites can be defined as any parasite that lives within the gastrointestinal system, which includes the stomach, small intestine, colon, and anus. GI parasites can be broken into two basic categories: microparasites and macroparasites (Nunn & Altizer, 2006). Although a significant amount of GI parasites take the form of small microorganisms called protozoa, the impact they have on the host can still be quite drastic (Cassady et al., 2018; Berrilli et al., 2011; Charles-Smith et al., 2010; Irwin & Raharison, 2009; Johnson-Delaney, 2009; Muriuki et al., 1998). Clinical signs include diarrhea, intestinal inflammation, behavioral changes, lethargy, and sometimes malnutrition and death (Cassady et al., 2018; Johnson-Delaney, 2009; Junge & Sauther, 2006).

The methods of GI parasite studies tend to be similar for all species of parasites and primates. Most of the parasite-containing samples are collected noninvasively, although, invasive methods are sometimes used. Necropsy is an ‘invasive’ method of collecting macro-GI parasite

data (Gillespie, 2006). There are far fewer ethical concerns with *opportunistic* collection of parasites during necropsy as opposed to intentionally euthanizing an animal for the collection of parasites. The most common method of collecting macro-GI parasite data is through the collection and sampling of fecal matter. There are two methods of obtaining fecal samples, one of which is relatively invasive. When using invasive methods of fecal sampling, anesthesia is administered to primates. After this, individuals are rectally stimulated in order to induce bowel movements (Pourrut et al., 2011). This method is more common in captive primates than wild primates. Noninvasive methods of collecting fecal matter requires primatologists to be relatively quick. Fecal samples must be collected immediately after natural defecation in order to preserve the sample and avoid anonymous samples (Pourrut et al., 2011; Gillespie, 2006). Avoiding anonymous samples is particularly important in captive situations. As captive populations are periodically dewormed, knowing which individuals are infected with parasites helps veterinarians with targeted deworming if necessary and prevents having to deworm the entire captive population.

Fecal samples can contain abundant information about macro-GI parasites. They can be used to identify parasites and measure prevalence and diversity. Fecal samples can contain adult parasites, which can be easily identified with or without magnification (Pourrut et al., 2011; Johnson-Delaney, 2009; Gillespie, 2006). Scientists use fecal flotations more commonly when looking for the eggs and larvae of parasites. Fecal flotation and sedimentation techniques can be used to separate the larvae and eggs from the fecal matter (Pourrut et al., 2011; Johnson-Delaney, 2009). Once separated, scientists can begin identifying species and life stages. Fecal samples also provide the opportunity to create thin smears of fecal material that can be looked at under a

microscope. This method allows for scientists to look at both helminths and protozoans as long as larvae and egg concentrations are high (Gillespie, 2006).

There are various biological and ecological factors that can impact the diversity of GI parasites present in NHPs. Factors such as geographic location, diet, body size, ranging behavior, and social behavior have been linked to GI parasite diversity and richness. Lindenfors *et al.* (2007) found that being further from the equator increases parasite prevalence and diversity although it is never speculated as to why this was the case. Diet studies have also produced interesting parasite diversity results. Folivorous species are noted to have more parasite diversity (Nunn & Altizer, 2006; Vitone et al., 2004; Nunn et al., 2003). Vitone *et al.* (2004) suggests this is because folivores exhibit a higher chance of consuming fecal matter, partly because they tend to be arboreal and defecate on the plant material that conspecifics may eat. Omnivores, due to their expansive diet, are also associated with high GI parasite diversity (Vitone et al., 2004; Guégan & Kennedy, 1993). It is also suggested that larger-bodied primates, which tend to live longer and be folivorous, exhibit higher parasite prevalence and diversity (Nunn & Altizer, 2006; Vitone et al., 2004; Nunn et al., 2003; Lafferty & Kuris, 2002). Although social behavior has been related to parasite diversity, the exchange of individuals between facilities and sometimes isolation experienced by captive NHPs makes this factor less reasonable to examine in captive populations. Since species' ranging behaviors may be altered by captive situations, this factor is also not appropriate for study in captive populations. Even though certain parasite taxa, such as *Strongylus* spp., are known to transmit to their hosts through soil contact (Viña et al., 2020), Nunn & Altizer (2006) suggest there is no relationship between terrestriality and risk of contracting parasites. While many studies have looked at parasite diversity in various primate

species, little to no published studies have compared parasite diversity between captive free-ranging lemur species, particularly in the United States.

2.7 Lemur GI Parasites

Wild populations of lemurs are known to carry over 20 species of nematodes and a variety of other GI parasite species (Irwin & Raharison, 2009; Junge & Sauther, 2006). It is important to note that parasite species can vary between captive and wild lemur species; however, the same genera of parasites can generally be found in both situations. Johnson-Delaney (2009) provides a list of known GI parasites that can be found in captive lemurs.

Although little to no published studies exist comparing the GI parasites and GI parasite diversity or prevalence between different captive free-ranging lemur species in the U.S., various studies have researched different lemur species and their parasite ecologies. However, GI parasite studies focused on strepsirrhines seem to be less common compared to parasite studies of other NHP taxa (Gillespie, 2006) and focus primarily on different populations within a singular species (Villers et al., 2008). This lack of literature is noteworthy due to the importance of information gained from comparative parasite studies. Villers *et al.* (2008) found that there were significant differences in GI flora between wild and captive *Lemur catta* populations. Of the parasite species found within wild populations, none were considered pathogenic. The same could not be said about captive populations. This study by Villers *et al.* is a great example of how comparative lemur parasite studies can help primatologists better understand lemur health, especially within captive situations.

Lemur parasite ecology studies can also be used to strengthen predictors for parasitic infection and parasite diversity. For example, significant numbers of pinworms were found in

two populations of silky sifaka (*Propithecus candidus*) in northeastern Madagascar. The lifecycle of pinworms is based on fecal-oral transmission (Loudon et al., 2017). Silky sifakas are a folivorous species and should, therefore, have greater parasite richness and diversity according to Vitone *et al.* (2004). The study found two genera of nematodes (*Lemurostrongylus* spp. and *Lemuricola* spp.), a tapeworm (*Bertiella* spp.) and an unknown species of oocyst (Loudon et al., 2017). The significant parasite richness and diversity represented in the species suggests that folivorous lemurs may be associated with higher parasite richness and diversity.

One parasite in particular that is considered problematic in both captive and wild lemurs is *Cryptosporidium*. *Cryptosporidium* is a genus of protozoan parasites with the capability to cause gastrointestinal diseases and inflammation in their hosts (Cassady et al., 2018; Rasambainarivo et al., 2013; Charles-Smith et al., 2010; Johnson-Delaney, 2009). Although several wild lemur species, such as the greater bamboo lemur (*Prolemur simus*), eastern rufous mouse lemur (*Microcebus rufus*) and ring-tailed lemur (*Lemur catta*), have been documented with the protozoa (Rasambainarivo et al., 2013; Villers et al., 2008), captive populations of Coquerel's sifaka (*Propithecus coquereli*) seem to suffer the most from *Cryptosporidium* (Cassady et al., 2018; Charles-Smith et al., 2010). While *Cryptosporidium* is noteworthy in captive *P. coquereli* populations, it is difficult to examine without special stains in microscopy or polymerase chain reaction (**PCR**) analysis.

Giardia, another genus of protozoa, which tends to be more widespread amongst different lemur species in captivity, can cause diarrhea in infected NHPs (Rasambainarivo et al., 2013; Berrilli et al., 2011; Johnson-Delaney, 2009). Studies have found *Giardia* spp. in captive *Lemur catta* (Berrilli et al., 2011; Martínez-Díaz et al., 2011; Levecke et al., 2009; Villers et al., 2008), *Varecia variegata* (Martínez-Díaz et al., 2011; Levecke et al., 2009), and *Varecia rubra*

(Martínez-Díaz et al., 2011), all of which are species of captive free-ranging lemurs that can be found at either the DLC or LCF. *Giardia* is a great example of common GI parasites that can infect various lemur species.

NHP parasite species are capable of spreading between different host species (Pedersen et al., 2005; Cleaveland et al., 2001). As such, it is reasonable to believe that if a parasite is affecting one lemur species, the close proximity of captive individuals could allow for the spread of parasites between lemur species (Rushmore et al., 2017; Nunn & Altizer, 2006). However, access to free-ranging enclosures in combination with the various biological and ecological differences provide the opportunity for variations to exist between the parasite diversities and parasite species of different lemur species at different facilities, such as LCF and DLC.

CHAPTER 3: HYPOTHESES & ETHICS

3.1 Hypotheses

The majority of NHP parasites are known to affect multiple host species. Therefore, I hypothesize that there will be overlap with some of the parasites found in different multiacre free-ranging lemur species at each facility. I also predict that there will be differences in parasite diversity due to biological, ecological, and geographical factors. More specifically, I hypothesize:

1. Species found at both LCF and DLC (*L. catta*, *E. mongoz*, and *V. rubra*) should have different parasite diversities and parasite prevalence between each facility.
2. There should be differences in parasite prevalence between lemur species.
    a. I predict that omnivorous species, e.g., *L. catta*, should have greater parasite prevalence than frugivores.
    b. I predict that larger-bodied frugivores, e.g., *V. rubra*, should have greater parasite prevalence than smaller-bodied frugivores, e.g., *E. mongoz*.
3. There should be differences in parasite diversity between lemur species.
    a. I predict that omnivorous species, e.g., *L. catta*, should have greater parasite diversity than frugivores.
    b. I predict that larger-bodied frugivores, e.g., *V. rubra*, should have greater parasite diversity than smaller-bodied frugivores, e.g., *E. mongoz*.

3.2 Ethics

The ethical use of animals in research projects is of the utmost importance. As such, I received Institutional Animal Care and Use Committee (**IACUC**) approval from both DLC and

LCF prior to my research. I also received approval from UNC Charlotte's IACUC board that I would not need additional IACUC approval given the approvals of both DLC and LCF. Fecal samples were also collected noninvasively to avoid stressing sampled lemurs. This study was also conducted during the COVID-19 pandemic and therefore extra precautions were taken. Wearing a mask and social distancing was mandatory when around lemurs and staff members at both facilities.

SECTION 4: METHODS

4.1 Study Sites & Species

Fecal collection took place at two separate facilities: the Duke Lemur Center and Lemur Conservation Foundation. As stated before, the Duke Lemur Center is an 85-acre facility located in Durham, North Carolina. It is home to the largest collection of lemurs outside of Madagascar. It is also one of the 33 facilities in the U.S. to house *P. coquereli* (Cassady et al., 2018). The Lemur Conservation Foundation is a 120-acre facility located in Myakka City, Florida. It is home to a variety of lemur species, including various species also found at DLC. Both facilities grant some of their lemurs access to free-ranging enclosures. This study focused on the following captive free-ranging lemur species found at the DLC and LCF: *Lemur catta*, *Varecia rubra*, and *Eulemur mongoz*.

4.2 Data Collection

Fecal Collection

Fecal samples were collected from all available free-ranging *L. catta*, *E. mongoz*, and *V. rubra* at DLC and LCF. Samples were also collected from *P. coquereli* at DLC but due to a lack of a comparative sample at LCF, these results are not included in the analysis, however, are represented in the appendices (TABLE 1-4). The use of personal protective equipment is essential due to the zoonotic potential of some lemur parasites. As such, I used examination gloves and tongue depressors to pick up fecal samples. Examination gloves and tongue depressors were replaced between each sample to prevent cross contamination. Fecal samples were collected noninvasively from adult free-ranging lemurs and were dependent on natural defecation. Fecal samples were collected as quickly as possible after defecation to prevent

ground contamination and anonymous samples (Gillespie, 2006). Samples were placed in plastic bags and labeled until they could be properly prepared and stored later. Each fecal sample was broken down into 2 g sections for fecal flotations. Remaining amounts of each sample were frozen for later PCR analyses.

Demographic Data Collection

Identification charts and information were used to identify which fecal sample came from which individual. This information was obtained from staff members at each location. Taking photographs of some lemurs was necessary to identify individuals later. In such cases, photographs were labeled with corresponding fecal samples. The facility, weight, age, sex, and deworming history of each individual sampled was collected along with parasite data. Demographic information also required some of the staff members' time to collect from DLC's and LCF's private databases.

4.3 Sample Analysis

I used a standard fecal flotation method (RUSVM 2020) with the individual steps as follows:

I mixed 2g of fecal matter from collected samples with 15mL of distilled water in a clean container and strained distilled water into a new container. The strained material transferred into a culture tube and labeled with a sample number. Tubes were centrifuged for 5 minutes at 500 G. Resultant supernatant was poured off. Zinc sulfate flotation solution (spg 1.25) was added and mixed with an applicator stick. Additional flotation solution was added to form a positive meniscus and then a cover slip was placed on the tube. The tube was centrifuged for 5 minutes at 500 G and then left to sit for

approximately 10 more minutes. The coverslip was then removed and placed on a slide with a drop of lugol's iodine and examined under a microscope at 100 to 400x magnification for parasite identification. Several references were used for the identification of organisms seen, including Haidar & De Jesus (2020), Issa (2014), Irwin & Raharison (2009), Scholz *et al.* (2001), and Duszynski *et al*. (1999).

4.4 Data Analysis

I compiled these data into tabulated tables using Microsoft Excel. I used these tables to organize my data for statistical analyses. I calculated percentages based on the number of parasite-positive versus parasite-negative samples and parasite diversity between lemur species and location. I used Fisher's exact tests on JMP Pro 15 to determine statistical significance between parasite-positive samples and species and parasite-positive samples and location. I chose Fisher's exact tests due to the categorical nature of the data and small sample size. Demographic information was used post-statistical analyses to explore the findings (TABLE 4).

CHAPTER 5: RESULTS

5.1 Percentages

I collected a total of 54 samples from all 42 available free-ranging individuals at both facilities. I collected 34 samples from 27 *L. catta*, 11 samples from 9 *V. rubra*, and 9 samples from 6 *E. mongoz* (TABLE 1). The study found that approximately 19% (8 of 42) of lemurs across both locations and all sampled lemur species tested positive for parasites. The Duke Lemur Center accounted for 87.5% (7/8) of parasite-positive lemurs, with the Lemur Conservation Foundation accounting for 12.5% (1/8). Of all the positive individuals, 62.5% (5/8) were *Lemur catta*, 25% (2/8) were *Eulemur mongoz*, and 12.5% (1/8) were *Varecia rubra* (TABLE 2).

5.2 Statistics

The proportion of lemurs hosting parasites differed across location (DLC 27% and LCF 6%), however, the difference was not statistically significant (Fisher's exact: $p \geq 0.127$). The proportion of lemurs hosting parasites differed between species (*L. catta* 18.5%, *E. mongoz* 33%, and *V. rubra* 11%), however, the difference was also not statistically significant (Fisher's exact: $p \geq 0.717$).

5.3 Parasite Identification

A total of six different parasite taxa were found in fecal samples: *Ascaris* spp., coccidia, *Entamoeba coli*, *Lemuricola* spp., *Lemurostrongylus* spp., and an unknown trematode species. Of the parasite-positive fecal samples, *Entamoeba coli* and *Lemurostrongylus* spp. occurred in 37.5% of samples. *Lemuricola* spp. occurred in 25% of samples. *Ascaris* spp., coccidia, and the

unknown trematode species only occurred in 12.5% of parasite-positive samples. Tentative differences were found in parasite diversity between locations. All 6 parasite taxa found in this study were found at DLC, while only 1 parasite species, *Entamoeba coli*, was found at LCF. Differences in diversity were also found between lemur species. *Lemur catta* was host for 5 of 6 parasite taxa found during this study. *Eulemur mongoz* was host for 2 parasite taxa, *Lemuricola* spp. and *Entamoeba coli*. Only *Ascaris* spp. was found in *Varecia rubra* during this study (TABLE 3).

Nematoda

An *Ascaris* spp. egg was found in the feces of a female *Varecia rubra*. Species is unknown, however, the size of 50x40 μm, round shape, and thick brown shell is consistent with several *Ascaris* species described by Irwin & Raharison (2009). However, the exact *Ascaris* species is unclear. Strongyle eggs were found in the feces of three female *L. catta*. The average measured size of the three ova was 68x32 μm, which is consistent with the size of *Lemurostrongylus* spp. described by Irwin & Raharison (2009) (FIGURE 1). It is also suggested that all strongyle ova found in lemur feces should be considered *Lemurostrongylus* spp. until a study proves otherwise (Irwin & Raharison, 2009). Species of the *Lemurostrongylus* ova could not be determined since identification is difficult without examination of the adults. *Lemuricola* spp. ova were only found in one male *E. mongoz* and one male *L. catta*. Pinworm eggs can be identified by their oblong and asymmetrical shape (Irwin & Raharison, 2009). Of the ova measured, the average size was found to be 59x26 μm, which is consistent with two species of *Lemuricola* described by Irwin & Raharison (2009) (FIGURE 1), *L. lemuris* and *L. bauchoti*. Without the presence of adults, *Lemuricola* species cannot be definitively identified.

Platyhelminthes

An unknown trematode egg was found in a female *L. catta*. The ovum was ellipsoid and operculated, as described by Irwin & Raharison (2009), however, measured only 20x13 µm. While not consistent with fluke ova sizes described by Irwin & Raharison (2009), Scholz *et al.* (2001) describes fluke ova sizes which are much more consistent with the one found in this study. The quality of the ovum and lack ability to collect the adult trematodes prevents a specific genera and species identification from being determined.

Protozoa

Entamoeba coli cysts were found in three different lemurs, one female *E. mongoz,* one female *L. catta,* and one male *L. catta*. *Entamoeba coli* can be identified by their circular shape and characteristic 8 nuclei (Haidar & De Jesus, 2020; Issa, 2014). The average diameter of the measured cysts was 16 µm, which is consistent with *Entamoeba coli* described by Haidar & De Jesus (2020) and Issa (2014) (FIGURE 1). A coccidian oocyst was found in a female *L. catta*. Although the genera and species could not be determined, the ellipsoid shape and 13x10 µm size is consistent various coccidian oocysts described by Duszynski *et al.* (1999) (FIGURE 1).

SECTION 6: DISCUSSION

This study aimed to examine differences in parasite prevalence and diversity between three lemur species as well as between two semi-free-ranging populations. It is important to note that single fecal samples only show parasites that are being actively shed and that can be found using the project's methods. As such, additional samples were taken and analyzed even if a sample had already been collected from an individual. Although duplicate samples were taken from some individuals, no individuals tested parasite-positive in multiple samples.

Only two sampled lemurs were dewormed close to the time of this study (2 *E. mongoz* at DLC). However, one of the *E. mongoz* still tested positive for pinworm eggs. Although Gillespie (2006) suggests not looking at eggs found in flotations for parasite intensity studies, this project focused on parasite diversity. Since diversity studies rely on the identification of different parasite taxa, eggs still provided useful information.

Plant nematodes, of all life stages, were commonly found in fecal samples at both facilities. This is an interesting find considering the prompt collection of samples after defecation. Since samples were collected quickly, it is likely that the plant nematodes found in the samples were consumed by the lemurs while feeding on fruits and other plants. The presence of plant nematodes also made the identification of lemur nematodes considerably difficult, specifically when the condition of a nematode made it difficult to determine morphology. This finding highlights the importance of collecting fresh fecal samples in order to avoid even greater plant and soil nematode contamination in fecal samples.

One interesting result of this study is that lemurs at DLC had a greater parasite diversity and prevalence than LCF. Although the relationship is not statistically significant, a larger sample size could theoretically prove significance. As such, it is appropriate to discuss the

differences in parasite prevalence between locations. This study hypothesized that there would be differences in parasites between locations. Although not convincingly statistically significant, more parasite-positive samples and a higher diversity of parasite taxa were found at DLC compared to LCF. This finding does not suggest that LCF cares better for their lemurs for several reasons. Firstly, not all parasites are bad, some are beneficial and/or are a normal part of the GI microbiome (Issa, 2014; Poulin, 1999). Secondly, there are various factors beyond the control of each facility that may contribute to differences in parasite prevalence or diversity. One factor to consider is differences in latitude of the facilities since the Lemur Conservation Foundation is in Florida and closer to the tropics. While various studies suggest there is no correlation between latitude and parasite diversity or prevalence, Lindenfors *et al.* (2007) suggests being further from the equator increases parasite prevalence and diversity within animals. This may suggest why there were more parasites and parasite taxa found at DLC. Another factor to consider is differences in diet. Although both facilities provision similar diets (a combination of Mazuri (manufacturer) primate chow, fruits, and vegetables), differences may be found in the diets of lemurs when foraging in their free-ranging enclosures. As such, a study looking at differences in forage diets between the two facilities could provide further insight. Another factor to consider is the original source of the lemurs. If a lemur that tested parasite-positive in this study came from another facility or is housed with a lemur that did, parasites could have been brought over from another facility.

This study found no statistical significance between the presence of parasites in fecal samples and lemur species. It is highly reasonable to suspect that the low number of samples in this study could have impacted the significance. When conducting a cross-species study focused on captive free-ranging lemur parasites, the sample size is reliant on the number of free-ranging

lemurs in captivity that are available to sample. The number of captive free-ranging *L. catta* in the study provided many fecal samples, however, *L. catta* significantly outnumbered *E. mongoz* and *V. rubra* at both facilities. Therefore, without more captive free-ranging *E. mongoz* and *V. rubra* it may be difficult to find statistical significance comparing lemur species and the presence of parasites. For example, while the proportion of samples containing parasites from *E. mongoz* was higher than that of *L. catta*, there was only one parasite-positive sample from *E. mongoz*. Collecting an equal number of samples from these species may reveal patterns not captured by the current study.

Exploratory data analyses revealed an interesting difference in prevalence based on sex. While not statistically significant, the study found that female lemurs tested parasite-positive more often than males (6 of 24 females and 2 of 18 males; Fisher's exact: $p \geq 0.431$). Although no literature suggests that sex differences dictate parasite prevalence or diversity in lemurs, a larger future study may help clarify these results.

The parasite diversity aspect of the study provided interesting results. It is important to reiterate that the number of sampled lemurs differed between species; however, it was found that *L. catta* was host to the largest diversity of parasite taxa with five. *E. mongoz* had the second highest diversity with two parasite taxa and *V. rubra* was only host to one parasite taxon. This study hypothesized that *L. catta*, an omnivore, would have a greater parasite diversity than the frugivorous species, *E. mongoz* and *V. rubra*. It has previously been suggested that omnivores are likely to have a higher GI parasite diversity due to their varied diet (Vitone et al., 2004; Guégan & Kennedy, 1993). The result of this study adds to the existing literature supporting this claim.

Another hypothesis of this study was centered around body size being a predictor for parasite prevalence and diversity. Previous studies have suggested that larger bodied species tend to have greater parasite prevalence and diversity due to their greater consumption of food (Lindenfors et al., 2007; Nunn & Altizer, 2006; Vitone et al., 2004; Nunn et al., 2003; Lafferty & Kuris, 2002). Contrary to my hypothesis, the smaller-bodied *E. mongoz* was found to host a greater diversity of parasite taxa (two) than the larger-bodied *V. rubra* (one). Aside from sample size, there are two factors that could have contributed to this finding. Firstly, while both species are considered frugivorous, levels of frugivory differ between *E. mongoz* and *V. rubra*. *V. rubra* is described as being highly frugivorous (Rushmore et al., 2012; Razafindratsima et al., 2012; Dutton et al., 2008; Overdorff et al., 2005; Vasey, 2004) whereas *E. mongoz* is noted as being frugivorous but often supplementing its diet with leaves and flowers (Curtis, 2004; Tattersall & Sussman, 1975). Although both species are considered frugivorous, the slightly varied diet of *E. mongoz* may explain its higher parasite diversity/prevalence in this study. It is important to consider that although the two lemur species differ in size, the difference may not be significant enough for *V. rubra* to realistically have a greater parasite diversity and higher parasite prevalence. Nonetheless, this study suggests that diet may be a factor that drives parasite diversity.

Entamoeba coli was the only parasite found that had its specific species determined. Therefore, it is the only parasite in this study that could definitively be defined as zoonotic or not. Although *Entamoeba coli* is considered non-pathogenic, both humans and NHPs are known hosts for the parasite (Issa, 2014; Irwin & Raharison, 2009). As such, *Entamoeba coli* should be considered zoonotic since it may be possible for humans and NHPs to share the parasite through fecal-oral transmission. Although the species of *Lemurostrongylus* and *Ascaris* could not be

determined, based on similarly related species, the parasites could be potentially zoonotic and/or pathogenic (Viña et al., 2020; Johnson-Delaney, 2009). Consistent with Irwin & Raharison (2009), lemurs infected with *Lemurostrongylus* presented no clinical signs based on general observations.

Since *Lemurostrongylus* was exclusively found in *L. catta* and was one of the most prevalent parasites in this study, further discussion is warranted. Although the life cycle of *Lemurostrongylus* is unknown, it likely that the parasite is transmitted to lemurs via fecal-oral transmission (Irwin & Raharison, 2009) and/or contact with the soil (Viña et al., 2020). Interestingly, *Lemurostrongylus* spp. eggs were only found in *L. catta* at DLC. Although it is difficult to speculate as to why *Lemurostrongylus* was only found at DLC, it is reasonable to discuss why eggs were exclusively found in *L. catta*. Although Irwin & Raharison (2009) suggests there is no relationship between terrestriality and risk of contracting parasites, the exclusive presence of *Lemurostrongylus* spp. in *L. catta* might suggest otherwise. *L. catta* is known to the most terrestrial lemur species (Sauther, 1989; Taylor & Sussman, 1985), as such, it is possible that they are at higher risk of being infected by *Lemurostrongylus*. However, a parasite study focused on terrestriality or geophagy and *Lemurostrongylus* spp. may clarify this finding.

SECTION 7: BROADER IMPACT

With 94% of lemur species considered threatened (Schwitzer et al., 2014), the conservation of these species is important given the impact they have on their ecosystems, such as frugivorous lemurs and their role as seed dispersers (Razafindratsima et al., 2012). Maintaining good health among primate populations is essential to their conservation, particularly in captive breeding situations such as those found at LCF and DLC. Studies focused on GI microbiomes have shown scientists the relationship between gastrointestinal communities and the health of their host organisms (Clayton et al., 2018; Stumpf et al., 2016; Hollister et al., 2014; Yildirim et al., 2010). This study is focused on comparing GI parasite prevalence and diversity between lemur species. Although not all parasites are detrimental to their lemur hosts, some even being part of the normal GI microbiome, parasites notably can affect their host's health and behavior (Cassady et al., 2018; Nguyen et al., 2015; Junge & Sauther, 2006; Nunn & Altizer, 2006). Some parasite species have been found to alter breeding behaviors and lactation, which can be problematic for endangered lemur species (Nguyen et al., 2015). Knowing which nonpathogenic (e.g., *Entamoeba coli*) versus pathogenic (e.g., *Cryptosporidium* in *P. coquereli*) parasites are normally found in specific lemur species is also important for their care and management in captive situations. All of these factors make parasite studies important to lemur conservation.

Although there are some studies that identify specific GI parasites in *captive* lemur populations, there are little to no published studies focused on comparing GI parasite diversity among different *captive free-ranging* lemur species. This gap in the literature is problematic due to the impact that parasites can have on the health of their host. Compared to traditional captive situations, multiacre free-ranging enclosures allow primates to have significantly more

interaction with the native ecosystem found at their facility's location. As such, these less restricted enclosures theoretically allow for a greater possibility of lemur-parasite interactions. A future study needs to be conducted between the lemurs restricted to smaller enclosures and free-ranging lemurs to confirm this.

Parasite studies are also important to public health, and more specifically, staff health. In captivity, staff members and lemurs are often in close proximity with each other. Close proximity has been proven to increase parasite transfer risk (Rushmore et al., 2017; Nunn & Altizer, 2006). Therefore, staff members working closely with lemurs at LCF and DLC should be cautious, as they can receive and transmit parasites to the lemurs and possibly between lemur species. This is especially true for NHPs due to the fact that 60-68% of NHP parasites have multiple primate hosts (Pedersen et al., 2005; Cleaveland et al., 2001). The ability for GI parasites to be shared between NHPs and humans is separately concerning since direct contact is not necessary for transmission. During the cleaning of enclosures, infected fecal matter may come in contact with uninfected individuals, thus creating the potential for parasite transmission. While not all parasites found in this study were considered pathogenic, this research could help inform staff members of potentially zoonotic parasites found in the lemurs under their care. This would allow staff members to practice increased precautions, if necessary, when working with certain individuals or species to protect themselves and other lemurs from becoming infected.

SECTION 8: CONCLUSION

A total of eight parasite-positive samples were found in 54 samples from 42 free-ranging individuals: 5 in *L. catta*, 2 in *E. mongoz*, and 1 in *V. rubra*. Six parasite taxa were found during the study: *Ascaris* spp., coccidia, *Entamoeba coli*, *Lemuricola* spp., *Lemurostrongylus* spp., and an unknown trematode species. Although no statistical significance was found regarding parasite prevalence between lemur species and locations, the parasite diversity results proved interesting. Putting aside the different sample population sizes between species, further investigating diet as a predictor for parasite prevalence and diversity seems to be a reasonable next step. A future comparative GI parasite study between different lemur species at separate facilities would benefit from having a larger sample population. However, given the limited number of different captive free-ranging lemur species, a future study may prove difficult. A comparative study may be more practical if only one species, *L. catta*, is sampled between localities. In such a study, St. Catherine's island may prove useful in increasing the study population. A future comparative study between different captive free-ranging lemur species does not seem practical at this time, considering more individuals for particular species are needed to successfully conduct such a study. Nonetheless, I hope this project will spark a conversation between DLC and LCF regarding lemur parasitology. The conservation and future of both wild and captive lemurs would benefit from a strengthened relationship between the two facilities.

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APPENDIX A: TABLES

TABLE 1: FECAL SAMPLES shows the sampled lemur species at the Lemur Conservation Foundation (**LCF**) and Duke Lemur Center (**DLC**), the number of each species sampled, and the number of samples collected/analyzed from each species. Some sample numbers are higher than the number of individuals, which indicates that multiple samples were opportunistically taken from some individuals. **Propithecus coquereli* was omitted from this study since they are only found at DLC.

	Lemur catta	***Eulemur mongoz***	***Varecia rubra***	****Propithecus coquereli***
Total Individuals	**27**	**6**	**9**	**14**
LCF	9	2	5	n/a
DLC	18	4	4	14
Total Samples Analyzed	**34**	**9**	**11**	**16**
LCF	15	5	7	n/a
DLC	19	4	4	16

TABLE 2: PRIMARY DEMOGRAPHIC DATA shows the sampled lemur species at Lemur Conservation Foundation (**LCF**) and Duke Lemur Center (**DLC)**, their respective diet type, their relative size compared to each other, and the total number of lemurs with parasites by location. The total number of parasite-positive lemurs from all locations is 8. Despite analyzing duplicate samples from singular lemurs, no lemur tested parasite-positive in multiple samples. **Propithecus coquereli* was omitted from this study since they are only found at DLC, however, no parasites were found in this species.

Lemur Species	**Diet**	**Relative Size**	**# of Parasite-positive lemurs (LCF)**	**# of Parasite-positive lemurs (DLC)**
Lemur catta	Frugivorous, Folivorous, & Omnivorous	Medium	0	5
Eulemur mongoz	Frugivorous	Small	1	1
Varecia rubra	Frugivorous	Large	0	1
**Propithecus coquereli*	Folivorous	Large	n/a	0
		Totals	1	7

TABLE 3: PARASITES lists all sampled lemur species: *Lemur catta*, *Eulemur mongoz*, and *Varecia rubra*. Each species is listed twice, once for the Lemur Conservation Foundation (**LCF**) and once for the Duke Lemur Center (**DLC).** Along the top of the chart are all the parasites found during the study. The numbers indicate how many individuals of that species' population at LCF or DLC tested positive for the respective parasite. Some individual lemurs were infected with multiple parasite taxa. **Propithecus coquereli* was omitted from this study since they are only found at DLC, however, no parasites were found in this species.

Location	**Lemur Species**	***Ascaris* spp.**	**Coccidia**	***Entamoeba coli***	**Fluke spp.**	***Lemuricola* spp.**	***Lemurostrongylus* spp.**
LCF	*L. catta*	-	-	-	-	-	-
	E. mongoz	-	-	(1 of 2)	-	-	-
	V. rubra	-	-	-	-	-	-
DLC	*L. catta*	-	(1 of 18)	(2 of 18)	(1 of 18)	(1 of 18)	(3 of 18)
	E. mongoz	-	-	-	-	(1 of 4)	-
	V. rubra	(1 of 4)	-	-	-	-	-
	**P. coquereli*	-	-	-	-	-	-

TABLE 4: ADDITIONAL DEMOGRAPHIC DATA shows the demographic and deworming data for all lemur species sampled at both the Lemur Conservation Foundation (**LCF**) and Duke Lemur Center (**DLC**). **Propithecus coquereli* was omitted from this study since they are only found at DLC.

Location	**Lemur Species**	**Average Weight (kg)**	**# of Males**	**# of Females**	**Age Range (years)**	**# of Individuals Dewormed in 2020**
LCF	*L. catta*	2.7	3	6	4 - 28	0
	E. mongoz	1.4	1	1	4 - 6	0
	V. rubra	3.4	4	1	2 - 20	0
DLC	*L. catta*	2.4	6	12	4 - 29	0
	E. mongoz	1.5	2	2	3 - 14	2
	V. rubra	3.5	2	2	2 - 32	0
	**P. coquereli*	3.7	6	8	.5 - 16	0

APPENDIX B: FIGURES

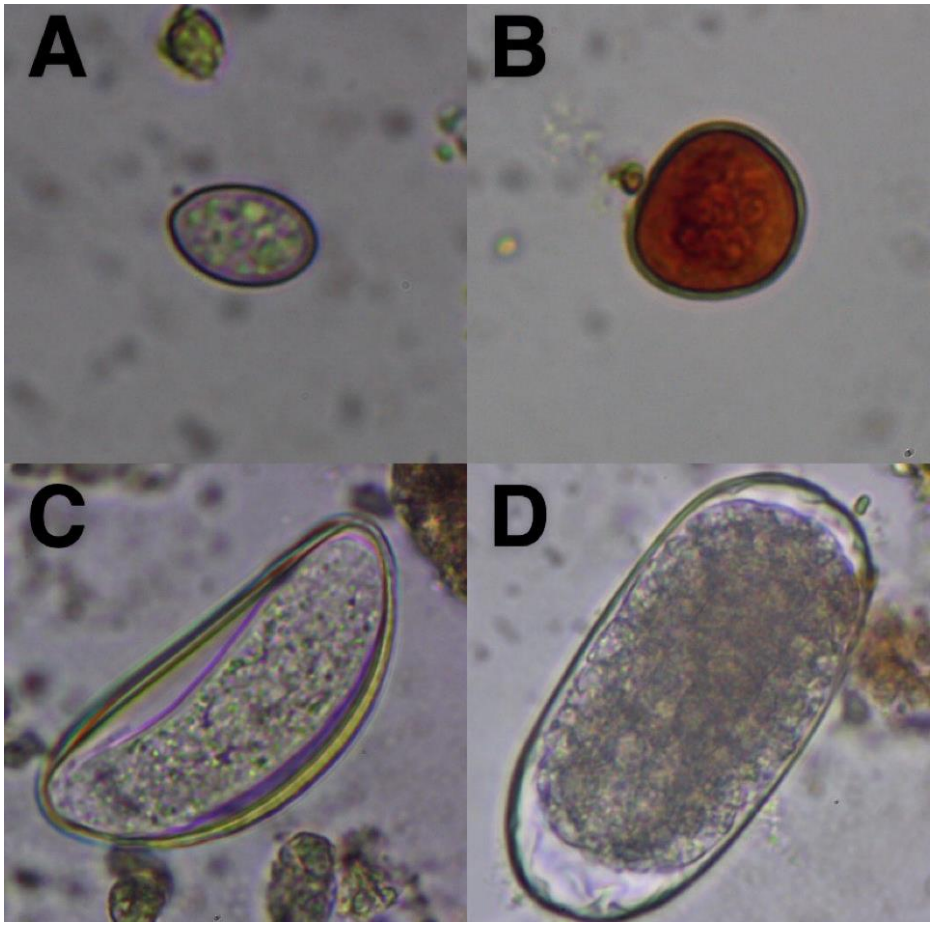

FIGURE 1: PARASITE PHOTOS shows a selection of four clear images of different parasite taxa found during the study. Images of parasites were taken during fecal flotations under 100x – 400x magnification and are stained using Lugol's iodine. Image A shows a coccidian oocyst (13x10 µm) found in a female *Lemur catta*. Image B shows an *Entamoeba coli* cyst (16 µm) found in a female *Lemur catta*. Image C shows a *Lemuricola* spp. ova (59x27 µm) found in a male *Eulemur mongoz*. Image D shows a *Lemurostrongylus* spp. ova (72x35 µm) found in a female *Lemur catta*.