**INTRODUCTION:**

One mechanism for the preservation of threatened species is the creation of sustainable captive populations that may act as a genetic repository that buffers against extinction.

The red-shanked douc (*Pygathrix nemaeus*) is a species of Asian colobine that inhabits east-central Lao People’s Democratic Republic (PDR), northern and central Vietnam, and northern Cambodia. It is an endangered primate that is of great concern to conservationists [(Nadler & Momberg 2008; Timmins & Duckworth 1999; Phiapalath et al. 2011; Heistermann et al. 2004)](https://paperpile.com/c/yngFXe/m9ve+7cqG+YVJo+huol+hIvw). Colobines are folivorous Old World monkeys that are anatomically, physiologically, and ecologically unique amongst the living primates [(Davies & Oates 1994)](https://paperpile.com/c/yngFXe/XQmK). They possess specialized GI systems similar to ruminants, including a sacculated stomach, allowing for the digestion and utilization of extremely high fiber diets [(Chivers 1994; Lambert 1998)](https://paperpile.com/c/yngFXe/X505+iHQu). As folivorous primates, they rely on a diet which is less nutrient-dense than that consumed by non-folivorous primates. Colobines are capable of metabolizing plant cell wall biomass due to the cellulolytic microorganisms that colonize the compartments of their GI tract. These mutualistic microbial populations are imperative to digestive processes such as the fermentation of polysaccharides and subsequent production of short-chain fatty acids, which are later assimilated and metabolized as an energy source [(Jablonski 1998; Nijboer et al. 2006)](https://paperpile.com/c/yngFXe/hIym+exDv).

*Alouatta palliata*, the mantled howler monkey, is the largest of the neotropical primates and is found across a wide range of habitats in Central and South America (Maldonado-Lopez et al., 2014). As frugivorous New World monkeys, they consume mainly fruits and flowers, but they can also consume leaves when seasonal shifts leads to decreases in fruit supply (Milton and McBee 1983; Maldonado-Lopez et al, 2014). Unlike colobines, *A. palliata* do not have a sacculated stomach, but instead have two large sections in their hindgut where mutualistic gut microbes catalyze similar digestive processes such as fermentation and energy metabolization (Nagy and Milton 1979; Milton and McBee 1983).

[paragraph or two on parasite-microbiome relationships in humans]

**The Gut Microbiome**

The intestine of any host is a rich and diverse ecosystem composed of host tissues and cells, enteric parasites, and commensal gut bacteria. These microbial bacteria exist in high density and diversity and come together to form a complex community called the gut microbiome (Zaiss and Harris 2015). The gut microbial community has different compositions and diversities based on host species and location within the intestinal system (Amato 2013; Kreisinger et al. 2015).

The gut microbiome is crucial in many biochemical processes, including the maintenance of homeostasis in the digestive system, the functioning of nutrition and metabolism, the stimulation of epithelial cells, the regulation of both the immune system and brain, and the protection against pathogens (Kinross et al. 2011; Partida-Rodriguez et al. 2017; Duarte et al. 2016). Human health has been linked to microbiome diversity, where a diverse and balanced microbial composition corresponds to a healthier individual (Zaiss and Harris 2015). Dysbiosis, or an imbalance in the abundance and diversity of populations of gut microbiota, can occur when there is a pathogen infection in the intestinal ecosystem (Partida-Rodriguez et al. 2017; Zaiss and Harris 2015).

**Microbiome-Pathogen Niche Interaction**

Since both intestinal parasites and non-pathogenic bacteria inhabit the same gut niche, they are likely to interact with each other (Zaiss and Harris 2015). The microbiome has a strong role in protecting the host from intestinal parasites and in reducing pathogenicity during infection (Costello et al. 2012; Berrilli et al. 2012). Microbes can either indirectly prevent parasite  colonization by creating an environment that is hostile to the parasite, or they can compete directly for niche opportunities with the parasite (Costello et al. 2012; Peachey et al. 2017). Dysbiosis of the microbiome can increase pathogen susceptibility and colonization, which then allows parasites to modify the composition and diversity of the microbiome and affect the outcome of parasitic infection (Costello et al. 2012; Partida-Rodriguez et al. 2017).

Each parasite-bacteria interaction is species-specific, creating unique host-parasite-microbe systems (Peachey et al. 2017; Zaiss and Harris 2015), as examples describe in Table 1. The microbial community can either protect the host against the intestinal parasite or favor the parasite by aiding in colonization against the host immune response. This decision can influence the composition and abundance of normal bacterial species in the gut to change parasite survival in either direction. Similarly, parasites can change the overall microbial diversity or abundance and/or composition of specific classes of bacteria through secretions and by-products to promote their own survival (Reynolds et al. 2014; Zaiss and Harris 2015). Some *Lactobacillus* species promote the clearing of Giardia cysts (i.e. *L. johnsonii, L. casei*, and *L. rhamnosus*) while others (i.e. *L. acidophilus* and *L. plantarum*) reduce the ability of the protozoan flagellate to latch onto the intestine (Partida-Rodriguez et al. 2017). The abundance of microbial species can vary based on the parasite species infecting the gut. For example, *Lachnospiraceae* populations increased during *Heligmosomoides polygyrus* infection but decreased during *Syphacia* spp. infection (Kreisinger et al. 2015). Most studies have shown a change in bacterial composition rather than a change in overall diversity (Kresinger et al. 2015). Pigs infected with *Trichuris suis* and wild mice infected with *H. polygyrus, Syphacia* spp., and *Hymenolepis* spp. did not exhibit a change in microbial diversity, but the composition of the bacterial communities was altered (Li et al. 2012; Wu et al. 2012; Kreisinger et al. 2015). Thus, niche interaction and its consequences are not unidirectional (Kreisinger et al. 2015).

The presence of a healthy, species-rich gut microbiome can protect against intestinal parasites and benefit the host against clinical manifestations of infection (Amato 2013; Lee et al. 2014). Jaenike et al. (2010) reported the presence of *Spiroplasma* bacteria in the fly species, *Drosophila neotestacea* protected the flies against sterilization caused by the helminth *Howardula aoronymphium*. Regardless of species, when compared to individuals with a healthy and diverse gut microbiome, individuals with no microbial community or a less diverse one were more prone to infection. Microbe-free mice were more susceptible to *Shigella*, *Bacillus*, and *Leishmania* infection (Smith et al. 2007). Bees with a normal gut microbial diversity had lower parasite abundances when compared to bees with no gut microbiota (Koch and Schmid-Hempel 2001), and pigs raised in environments with a high diversity of microbes were more resistant to parasitic colonization than pigs raised in sterile, microbe-less environments (Mulder et al. 2009).

However, many parasites require the presence of a gut microbiota to develop an infection and colonize subsequently (Kreisinger et al. 2015). In mice, *Trichuris muris* required the presence of gut microbes to establish infection (Hayes et al. 2010). Several studies have shown that the gut microbiota was necessary for colonization by protozoa *Entamoeba histolytica* (Phillips et al. 1955), *Blastocystis hominis* (Phillips and Zierdt 1976), and *Eimeria* spp. (Visco and Burns 1972; Owen 1975; Gouet et al. 1984). In some cases, the abundance of certain microbes increases during parasitic infection. In mice, *Lactobacillus* and *Bacteriodetes* bacteria are positively correlated with infection presence of *H. polygyrus* and the presence of tapeworms, respectively (Reynolds et al. 2014; Kreisinger et al. 2015). Since natural parasitic infections promote a diverse microbial community, which could lead to host health benefits, the parasite-bacteria relationship might be a key factor in a healthy intestinal homeostasis (Zaiss and Harris 2015; Kreisinger et al. 2015; Cooper et al. 2013; Lee et al. 2014; Cantacessi et al. 2014).

**Physiological Effect of Microbe-Parasite Interaction**

Interactions between microbiota and parasites within the intestinal ecosystem can lead to changes in the host’s immune response and metabolism (Peachey et al. 2017). As a part of the unique host-parasite-microbe systems, different parasites induce specific host immune responses. Parasites *H. polygyrus* and *Nippostrongylus brasiliensis* can inhibit the production of inflammatory cytokines but *T. muris* cannot (Maizels et al. 2009). The role of individual microbiota can also differ based on the parasite and host immune response. For example, an increased prevalence of *Blastocystis* has been linked to general compositional changes in the microbiome, corresponding to a decrease in helminth infection and morbidity in Mexico. On the other hand, *Toxoplasma gondii* infection led to a decrease in abundance of *Bacteriodetes* and *Firmicutes* and a temporary abundance of *Escherichia coli*, which has the potential to cause tissue damage from the host immune response (Partida-Rodriguez et al. 2017).

During interactions, parasites and microbes produce secretions and by-products that affect each other and impact the metabolism of the intestine ecosystem (Partida-Rodriguez et al. 2017; Zaiss and Harris 2015). Microbial products can interfere with the survival and persistence of parasite infection, and intestinal parasites can produce secretions that alter the composition of the microbial community (Partida-Rodriguez et al. 2017). Secretions during *Giardia intestinalis* infection can create abnormalities in the biofilm of gut microbes, a disruption that allows for pathogenic bacterial invasion (Partida-Rodriguez et al. 2017). This back-and-forth interaction of parasite and microbial secretions can result in both pathogenesis and dysbiosis and can activate host immune responses (Berrilli et al. 2012; Holm et al. 2015).

Parasitic infections have a role in maintaining the inflammatory and regulatory balance of the immune system (Holm et al. 2015). Specifically, intestinal helminths are known to be host immunomodulators (Kresiginer et al. 2015) and can change the immune properties of their host, which can lead to alterations in the mucosal and systemic immunity of commensal bacteria (Peachey et al. 2017). Helminths can create an anti-inflammatory environment and redirect hostile host immune responses away from themselves, thus influencing the composition and abundance of the gut microbiota (Kreisinger et al. 2015). Host immune responses can also be inhibited by gut microbiota immunoregulation, allowing parasites to colonize and manipulate the

immune system (Partida-Rodriguez et al. 2017).

**Evolutionary Relationship between Microbiota and Parasites**

Gut microbes and intestinal parasites might have a co-evolutionary relationship where they coordinate to promote the growth and colonization of both types of organisms within the host intestinal ecosystem (Zaiss and Harris 2015). In a study by Hayes et al. (2010), the decrease in abundance of gut microbes led to a reduction in hatched *Trichuris* eggs in the murine intestine. Helminths like *Trichuris* can act as a determinant when selecting interleukin genes to regulate immune response. This evolved T-cell response driven by helminths can counter infection and repair the intestinal damage done by secretions and products from microbe-parasite interactions (Hayes et al. 2010).

The  absence of intestinal parasites is an incomplete microbiome and has been associated with a higher prevalence of autoimmune disease and intestinal inflammation (Kreisinger et al. 2015; Broadhurst et al. 2012). As per the Hygiene Hypothesis, intestinal helminths play an important role in reducing and controlling allergies and autoimmune disease through mutualistic interactions between host immune responses and helminth infections (Berrilli et al. 2012; Broadhurst et al. 2012; Maizels et al. 2009). Prevalence of parasitic infection negatively correlates with the prevalence of autoimmune disease. An absence of worm infection in mice corresponded to an increased prevalence of immune-associated diseases, including inflammatory bowel disease, allergies, multiple sclerosis, rheumatoid arthritis, and type 1 diabetes (Holm et al. 2015). The protective effect of helminth infections as seen in mice is an avenue to be explored in humans as soon as possible (Maizels et al. 2009). Modern medicine is eradicating helminths and therefore, decreasing parasite diversity. This shift in the evolutionary balance might lead to higher occurrence of autoimmune disorders in the future (Zaiss and Harris 2015).

**Gut Microbiota-Parasite Interaction in Non-human Primates**

Captivity has a strong effect on gut microbial composition and diversity, so it is crucial to study wild animals on their own (Amato 2013; Clayton et al. 2016). Thus far, there has been a heavy emphasis on human-helminth and rodent-helminth models and not enough on wild animals (Peachey et al. 2017). Our study will illuminate the intestinal microbe-parasite relationships in two vastly different non-human primates (NHPs) as a significant contribution in this field.

Similar to other species, the very few and small studies done in non-human primates show that NHPs also display species-specific host-parasite-microbe interactions (McKenna et al. 2008). Intestinal helminth infections from *Trichuris* and *Necator* species in both humans and NHPs correlated to an increase in microbial diversity (Peachey et al. 2017). Although studied in captivity, rhesus monkeys treated with helminths were found to have improved symptoms after suffering chronic diarrhea. *Trichuris trichiura* reduced the host immune response and led mucosal repair and immunoregulation in the intestine (Broadhurst et al. 2012). Similarly, macaques with chronic diarrhea displayed reduced microbial diversity (Broadhurst et al. 2012). However, colitis-infected macaques have also demonstrated the association between disease and dysbiosis of the microbiome. Macaques with colitis exhibited an altered, imbalanced microbial composition with higher populations of *Campylobacter* bacteria when compared to healthy individuals (McKenna et al. 2008).

Monitoring the gut microbiota is a non-invasive method to study the effects of parasitic infections in wild primates (Moeller et a. 2015). Simian immunodeficiency virus (SIV) infection altered the gut microbial community in macaques and chimpanzees but did not affect the gorilla gut microbiome (Moeller et al. 2015; McKenna et al. 2008). Infection status was not associated with changes in the gut microbiome, leading to the hypothesis that there is an absence of SIV pathogenesis in gorillas (Moeller et al. 2015). All these studies reiterate that each host-parasite-microbe relationship seems to stand alone and should be studied across a wide range of host, parasite, and bacterial species. Certain combinations can prevent parasitic infection while others tend to favor parasite colonization in the intestine community.

**Gastrointestinal Parasites in *Alouatta palliata***

Intestinal parasitic infection has been documented in *A. palliata* in a multitude of studies, including prevalence of *Parabronema* sp., *Controrchis* sp. and *Controrchis biliophilus*, *Trypanoxyuris* sp. and *Trypanoxyuris minutus*, *Strongyloides* sp., *Entamoeba* sp., *Isospora* sp., *Ascaris* sp., *Coccidia*, *Physaloptera* sp., and other unidentified nematodes, trematodes, and flukes (Stuart et al., 1990, Stoner 1996, Trejos-Macia and Estrada 2012, Trejos-Macia et al., 2007, Maldonado-Lopez et al., 2014, Cristobal-Azkarate et al., 2010). Multiple studies have correlated seasonality and continuity of habitat as key factors in influencing parasitic infection and parasite richness, where higher rates of infection as well as increased diversity of gut parasites have been found during wet seasons and in fragmented habitats (Stoner 1996, Trejos-Macia and Estrada 2012, Cristobal-Azkarate et al., 2010).

**Gastrointestinal Parasites in *Pygathrix nemaeus***

The scientific literature on parasitic infection in *Pygathrix nemaeus* is very sparse and contains large gaps on parasite diversity and prevalence. The few and scattered cases of infection span across the Pygathrix genus, which includes *Pygathrix nemaeus*, *Pygathrix nigripes* (black-shanked douc langur), and *Pygathrix cinerea* (gray-shanked douc langur). A variety of pathogens have been detected in douc langurs, but small sample sizes and inconsistent analytic methods make it difficult to assess the true parasite burden in the species.

Many of these parasitic cases are random and low-risk as only few individuals are infected, and the pathogen is usually not seen in these primate hosts. In another study at Cuc Phuong National Park, there was a case of an *Echinococcus ortleppi* infection in one red-shanked douc female individual (Plesker et al. 2009). Although primates are susceptible to infection, *E. ortleppi* has a preferential host transmission between dogs (definitive host) and cattle (intermediate host). Since nonhuman primates are usually not involved in maintaining the transmission of *Echinococcus* genus parasites, this case can be considered singular and not an indication of general prevalence in the *Pygathrix nemaeus* population. Similarly, a case of bacterial *Mycobacterium avium* infection was found in two captive male red-shanked douc individuals in a Germany zoo. However, the risk of zoonotic transmission was determined to be low (Plesker et al. 2010), and infection in a captive setting is not indicative of infection patterns in the wild.

A study on the effect of orally applied ivermectin on gastrointestinal nematodes in *Pygathrix nemaeus* in Cuc Phuong National Park demonstrated a high parasite pressure in the species (Hartmann et al. 2015). Although there was a lack of demonstrated symptoms of disease, 73% of individuals remained positive for gastrointestinal nematodes after application of ivermectin. The study also detected the presence of *Trichuris* nematodes (24%) and *Strongyloides* spp. (49%) in the population. These individuals were studied in captivity, a factor that could have influenced transmission due to close proximity and high density of different species.

*Pygathrix nemaeus* is on the IUCN Red List of Threatened Species, so it is crucial to understand their risk for parasitic infection to shape the management and conservation of the species. Further, the close phylogeny between humans and other primates increases the potential for pathogen exchange, especially in anthropogenically-disturbed habitats (Gillespie 2006). Thus far, the baseline data on the gastrointestinal parasites of red-shanked doucs contains large gaps. Our study is the first comprehensive gastrointestinal parasite analysis in wild and free-ranging *Pygathrix nemaeus.*

NOTES

Shotgun sequencing: phylogeny and gene function of microbiota

Review paper: <https://genomemedicine.biomedcentral.com/articles/10.1186/gm228>

* Papers in the above review paper (all human):
  + Gill et al. 2006, <http://science.sciencemag.org/content/312/5778/1355>
  + Kurokawa et al. 2007, <https://academic.oup.com/dnaresearch/article/14/4/169/466883>
  + Qin et al. 2010, <https://www.nature.com/articles/nature08821>

Review papaer: Berrilli et al. 2012, Interactions between parasites and microbial communities in the human gut, <https://www.frontiersin.org/articles/10.3389/fcimb.2012.00141/full#B72>

Other papers to read:

Kreisinger et al. 2015, Interactions between multiple helminths and the gut microbiota in wild rodents  
Houlden et al. 2015, Chronic Trichuris muris Infection in C57BL/6 Mice Causes Significant Changes in Host Microbiota and Metabolome: Effects Reversed by Pathogen Clearance  
Hayes et al. 2010, Exploitation of the Intestinal Microflora by the Parasitic Nematode Trichuris muris  
Holm et al. 2015, Chronic Trichuris muris Infection Decreases Diversity of the Intestinal Microbiota and Concomitantly Increases the Abundance of Lactobacilli

It has been found that effect of host species (host phylogeny) is strong factor in influencing gut microbiota - also influenced by diet coming from morphological/anatomical differences

-- important to consider when comparing doucs and howlers

Via Sahana:

Additionally, I've been doing some research on how to statistically compare microbiome and parasite data. I found two papers with potential methodology we can use:

Slapeta et al., 2015, Differences in the faecal microbiome of non-diarrhoeic clinically healthy dogs and cats associated with Giardia duodenalis infection: impact of hookworms and coccidia

- This study used an ANOVA to compare Giardia-positive and negative groups. We can use something similar to compare parasite presence and absence.

Vlckova et al., 2018, Relationship between gastrointestinal parasite infections and the fecal microbiome in free-ranging Western Lowland Gorilla