PARASITE INFECTION IN HOWLER MONKEYS, *ALOUATTA PALLIATA,* AT WILDSUN RESCUE IN COSTA RICA

A Report of a Senior Study

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ABSTRACT

Wildlife rehabilitation centers attempt to provide captive primates with an environment that closely resembles their natural habitat. Due to limited space and resources, captive species are exposed to numerous changes related to their diet, their mental wellbeing, and the physical environment. These changes can increase the prevalence of health issues such as parasite infection, changes in their immune system, and increased stress. This study examined parasite infections in captive howler monkeys, *Alouatta palliata*, through fecal analysis. Behaviors were observed and blood collected, and fecal results were compared to general behavior and hematology. Treatment with Tinidazole was effective in limiting *Chilomastix mesneli, Giardia, Stronglyoides stercoralis* and *Ancylostoma duodenale.* Other parasites like *Pentatrichomanas hominis and Blastocystis hominis* remained prevalent despite treatment. Heavy infestation with parasites was associated with increased stress and higher leucocytes and neutrophil counts. It is recommended that wildlife centers with captive enclosures limit human interaction with primates as well as improve enclosure design to limit parasite infections.

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CHAPTER I

INTRODUCTION

Throughout the world, different biomes have developed due to differences in wildlife populations that inhabit that specific area. Some biomes provide nutrients to a diverse population while others may be limited in what resources they can provide. Differences in climate, nutrient availability, and space can impact the survival and reproduction rates of a species. These differences also may affect the overall health of a species by influencing the prevalence of different parasites and spread of infectious diseases. Changes in climate and nutrient availability that may affect the overall health of a species can also impact their genetic variability and survival. While these changes can occur in the natural environment, animals held in captivity can also be affected in different ways. In some cases, captive primates often have reduced gut microbial diversity and differences in the abundance of beneficial and pathogenic taxa when compared to healthy primates in the wild (Amato et al. 2016). Intestinal microbiota can be analyzed through fecal samples and have often varied in captive populations due to site conditions and diet diversity (Gonzalez et all 2021). Analysis of the microbiota can provide information regarding the causes of gastrointestinal illness in species and can help sanctuaries prevent it from occurring in other animals. Research performed on nutrition, disease, and genetic variability can help wildlife veterinarians, sanctuaries, and rescues provide effective care to injured and sick wildlife as well as protect endangered species.

Nutrition in Tropical and Temperate Wildlife

Tropical and terrestrial wildlife differ in many aspects including rainfall, temperature, and seasonality. Tropical rainforests are the most diverse biome with temperatures ranging from 20-34 degrees Celsius and rainfall ranging from 250-450 centimeters a year (Neal 2019). Temperate forests have temperature ranges between -30-31 degrees Celsius with average rainfall falling between 75 and 150 centimeters (Neal 2019). Additionally, tropical habitats are characterized by thermal constancy, whereas temperate environments experience seasonality in temperature (Neal 2019). The climate differences allow for different plant and animal species to live in these different environments. This results in different food availability and food type which can impact the overall health of different animals.

The nutritional requirements of tropical wildlife around the world are being studied to understand what these animals may need for growth, reproduction, immunity, and productivity. This understanding is particularly important when wildlife is brought into captivity, as most commonly fed captive diets are deficient in one or more essential nutrients (Koustos et al. 2001). By fully understanding how tropical species digestive systems work as well as what types of food they eat, veterinarians and wildlife rescues can create more well-balanced diets for tropical wildlife that are in captivity or require health assistance. In the tropics of Trinidad and Tobago, wild agouti consumed roots of ferns, tubers, bark, fruits, berries, seeds, nuts, and succulent plants (Lall et al., 2018). For instance, based on the analysis of native agouti, it was determined that captivate agouti could be fed coconut, bread, and cooked rice in order to remain healthy and they have the ability to digest higher levels of protein (Lall et al., 2018). While this diet has helped tropical birds, it is not a universal diet for all species. Biologists must consider differences between organisms that live in different ecosystems.

The nutritional requirements of temperate forest animals differ from the requirements of tropical wildlife, as the vegetation available for herbivores can change throughout the seasons. In a temperate environment, wildlife often must rely on low quality vegetation sources like bark, needles, and grasses throughout the winter months. Throughout the warmer months, non-carnivorous wildlife feed on leaves, flowers, and fruit. Consumption of needles by ruminants in a temperate forest during winter months increased by 40% and sometimes made up 90% of some species diets (Kamler and Homolka 2011). This large increase in consumption of needles just in the winter months indicates that temperate herbivores have adapted to consume a variety of vegetation in order to survive when other food isn't available. By analyzing the diet of ruminants, the nutritional content of the different vegetation can be evaluated and used to help feed domesticated or captive animals. Spruce needles are commonly consumed throughout winter and are known for contributing little metabolic energy when compared to broad leaved plants, however their crude protein content is comparable to broad leaved plants (Kamler and Homolka 2011). Different species that live in a temperate environment can also have a difference in the overall quality of their diet. Dietary nitrogen, and metabolized energy were higher in two species of deer during the vegetation season when compared to winter however the content of fecal nitrogen was higher in roe deer than red deer (Kamler and Homolka 2005). In summary, temperate wildlife must have the ability or adaptation to survive on vegetation with lower nutritional quality in the winter months.

Based on the analysis of temperate wildlife diets in their natural habitat, rescues can compare and see the differences between what is fed in captivity and what the animals eat in the wild. For temperate species of deer held in captivity, the foods fed was high in protein and polysaccharide contents which was different than the food consumed in the natural environment which was rich in fiber. The differences in microbiota found in the captive and wild deer may be strongly associated with the dietary differences (Li et al. 2017). Their nutritional requirements are different than tropical animals who live in a more constant environment. Based on these differences, it is important to provide captive animals with the nutrients they need so that they are able to recover and return to the wild.

While nutritional deficiencies can affect many different species, captive tropical birds are commonly fed deficient diets. Domesticated terrestrial birds are found to primarily feed on seeds, and bugs in grass areas. Tropical birds have to rely on food sources found in moist areas such as tree fruits, seeds, and flowers. Some tropical birds like parrots have been domesticated by humans and are fed a seed-based diet that doesn’t always contribute to healthy growth. Crop contents were sampled from scarlet macaw chicks at a research center. A diet analysis performed on scarlet macaw chicks acknowledged some nutrients like protein levels, fat content, magnesium, iron and calcium levels were higher than the recommended and phosphorus was lower (Brightsmith et al. 2010). Other captive diets were primarily seed based causing them to lack vitamins A, D, E, and K, biotin, and water-soluble vitamins since seeds are low in vitamin A. Physical characteristics of tropical birds can change because of their nutrient deficient captive diets. In one example a blue macaw had feathers that grew in tattered and unsymmetrical due to his diet of pasta, crackers, and pellets (Harrison and

McDonald 2020). To prevent such from occurring, future research needs to examine proper diets for captive animals.

Ultimately, by analyzing how diet impacts a species' overall health, veterinarians can also prevent certain diseases from developing. In the avian medical journal, nutritional imbalance such as high iron displayed differences in parrots and frugivores. Parrots fed high iron diets often experience sudden death where insectivores and frugivores had dyspnea and a swollen coelomic cavity. Treatment was similar in that these birds were fed a low iron diet while also avoiding vitamin C and excess vitamin A (Harrison and McDonald 2020). Other nutritional imbalances led to metaplasia of the respiratory epithelium. Parrots experienced clinical signs including rhinal discharge, wheezing, sinusitis, tracheal obstruction, panting, etc. (Harrison and McDonald 2020). Treatment for this side effect included flushing of the sinus area, drainage, nebulization, diet correction, and making sure the birds were in a healthy environment with avoidance of aerosols. Fatty liver disease in birds was also commonly associated with an unbalanced diet. Pesticides, unnecessary force feeding, or feeding improper formulated diets may all contribute to the development of fatty liver disease. Severe illness and disease can develop in both tropical and terrestrial animals that are not treated for nutritional deficiencies.

In order to effectively analyze an animal's diet, different techniques are used to measure the nutrient content. Plasma biochemistry is one technique that can be used to evaluate what nutrients are high or low. For an example, by looking at plasma lipid concentrations in tropical birds can be used to mark differences in plasma cholesterol concentrations between different parrot species (Bavelaar 2005). Another example of a technique used is to gather fresh crop and food samples and boil the samples with a nitric acid-perchloric acid mixture in order to perform mass spectrometry as well as measure moisture in the soil and available food resources (Brightsmith et al. 2010). Practicing frequent diet analysis of a species can be beneficial in preventing disease and illness from developing as well as it allows captive animals to recover faster if they are given the adequate amount of nutrients.

Common Diseases and Illnesses in Tropical and Terrestrial Wildlife

Captive wildlife can be at high risk for infectious disease which can lead to catastrophic effects like epidemics which result in almost complete decimation of a population. For instance, avian malaria in Hawaiian avifauna and rinderpest in African bovids were two epidemics that completely reduced population size by 90% (Ballou 1993). Other common diseases that affect both tropical and terrestrial animals can be found in Table 1. For such cases, veterinarians and rescues must work quickly to stop the spread as well as treat infected animals. Some infectious diseases may lead to other issues in a population that can be detrimental to the survival of a species. These effects may include reduced reproduction, increased risk for predation, or increased susceptibility to environmental stress. All of these effects can lead to severe population reduction if the spread isn't stopped or prevented from occurring in other locations. Tropical birds can also be presented to veterinarians for cases of dehydration. In these cases, it is important for Veterinarians to have a suitable fluid to use in fluid therapy which is a common treatment for animals that are severely dehydrated (Beaufrere 2011). Treatment should be both efficient and effective with the goal of returning injured or sick wildlife back to their natural environment.

Some animals that are severely injured or sick are brought into sanctuaries or rescues until they can go back into their natural habitat. While this is helpful in treating some disease and injuries, individuals can become exposed to diseases that aren't found in their natural habitat. These animals will lack natural immunity and also may be more prone to getting other infections while their bodies are trying to fight off other illness. Rescues and rehabilitation centers may then have to deal with outbreaks of disease that develop while animals are captive. In the tropics, there have been cases of rat mites infecting endangered Amargosa voles and this is suspected to have occurred due to the straw bedding used (Mantovani 2018). The infected voles developed skin lesions that required treatment with tropical selamectin due to infestation of mice. Ulcerated lesions typically had dozens of mites, however once the selamectin was applied, the lesions resolved within 1-2 weeks (Mantovani 2018). Besides being exposed to non-natural harmful agents, captive species also spend less time processing food, as animals being treated are given food to eat and aren't required to catch their own food. Previously captive Orangutans that were released started to feed on more fruits than flowers and invertebrates that wild orangutans did. It was predicted that their diet during rehabilitation and captivity caused them to be drawn towards the foods they were given by humans (Chappell and Thorpe 2021). Similar effects happen when temperate forest animals are held in captivity. Common diseases found in forest musk deer include gastrointestinal diseases, which have the highest mortality rate of 30%, as well as pneumonia, abscesses, parasitic diseases, and rumen impaction (Li et al. 2017). For these reasons it is important that rescues attempt to treat illnesses and injuries as effectively and efficiently as possible so that wildlife can return to their natural habitat and still survive on the food that is available. If a species doesn't go back to their normal food in the natural environment, they may become nutrient deficient or may develop other illness associated with eating new food their body is incapable of processing the same way.

In order to help prevent disease and injuries, rescues are working to develop conservation action plans before a population becomes too small. These plans should avoid keeping animals in captivity long term since captive species like the black footed ferrets have lost 12% of their genetic diversity since there is a smaller population size available for mating (Ballou 1993). Populations that are in the natural environment can freely mate with a larger population. Human's affect genetic diversity when they breed for specific and common traits which leads to a decrease in the overall genetic diversity of a breed or species as a whole.

Table 1: Diseases and injuries commonly found in tropical species.

Disease or injury	Species	Study performed	References
Intestinal and blood parasites	Scarlet macaw and great green macaw	- Fecal samples obtained from birds at four locations and were sent to a parasitology lab - Used qualitative examination by flotation technique in saturated sugar solution and examined using a microscope - Blood samples taken from the cutaneous ulnar vein or right jugular vein (blood smears were made and examined under a microscope to look for hemiparasites) - Location 2 and 3 of rehabilitation centers, had higher rates of direct transmission and frequent movement of birds between cages.	Dieckmann et al. 2020
Fatal Fungal dermatitis	Eastern Mississauga	- Healthy survey and disease investigation	Allender et al. 2011

		conducted in Carlyle Illinois - Used the polymerase chain reaction assay to analyze swabs taken from the snakes - Obtained hematologic and plasma biochemical data, and toxicological data -	
Leptospira spp. In captive animals	Vertebrates from zoos and rehabilitation centers	- Leprospiros is the most common zoonic disease and is more prevalent in humid and tropical climates. - Samples were obtained from different mammals like the Mexican Spider monkey, the Yucatan spider monkey, white tailed deer and reptiles like the Morelet crocodile - Collected 3 ml of peripheral blood from the jugular, saphenous, or femoral veins at different rehabilitation centers	Pérez-Brígido et al. 2020

Prevalence and distribution of *Vibrio spp*. in wild aquatic birds	Migratory aquatic birds in Venezuela	- Collected fecal samples of resident and migratory birds at two different coastal sites. - DNA was amplified by PCR - Found nontoxigenic *Vibrio cholerae* in one wildlife refuge had a higher prevalence in the resident birds - Saw that different samples related to different species of *Vibrio*	Delgado et al. 2016
Avian tuberculosis	Macaws in tropical rainforests	- Sampled Mycobacterium avium strains in feces which commonly causes avian tuberculosis	Washko et al. 1998

Genetic Variability in Tropical Wildlife

Tropical rainforests are known for being diverse biomes for many species of plants and animals. However, due to habit loss, wildlife trade, and hunting, species like the wild scarlet macaws have now been declared an endangered species in Central America (Dieckmann 2020). Different programs have started reintroducing these species to different areas they once inhabited. Reintroduction programs are complex and must meet specific requirements before approval. It is important to screen for disease, evaluate population viability, habitat quality, potential food availability, and consider potential predation. Following release of a species into an area, genetic analysis is often used to monitor the birds by identifying their sex, health status, as well as determine genetic variability within the population. By reintroducing animals into large populations risks of inbreeding and genetic variation can be limited (Estrada 2014).

Some species of birds, like parrots and macaws, lack sexual dimorphism which creates an obstacle in the identification of sexes as well as the establishment of breeding programs. PCR can be used to genotype the CHD gene found in macaws through invasive and noninvasive sampling (Gutierrez 2017). Invasive sampling can be used by drawing blood or using plucked feathers where noninvasive sampling includes using fecal samples or freshly-shedded feathers.

Genetic diversity can be evaluated in a species through extraction and genotyping of DNA which can be used to provide data for estimating allelic variation and heterozygosity levels (Ortiz et al. 2020). The use of mitochondrial sequence data can also provide information regarding phylogenetic relationships between two species. Biologists can make comparisons between a species that lives in one area and compare to another species that may be found in a different environment. For instance, two different species of macaws, military macaws and great green macaws, are closely related however they are classified separately due to differences in size, bill color, plumage, vocalization, and location (Eberhard 2015). Biologists can better understand why these differences exist by extracting DNA samples and performing genetic analysis. Genetic analysis has provided explanations for genetic diversity and the impact of geographical conditions on diversity, such as how mountain ranges have separated different species (Eberhard 2015). Reintroduction of a species to a new environment requires time and maintenance including close monitoring for parasites and infection that may affect the health of the entire ecosystem.

Parasite Loads

Fecal and blood samples can be used to monitor any parasites that may have infected reintroduced species. Birds that do not undergo proper health screening prior to release can expose wild bird populations to different parasites and diseases. Certain species like the Psttacidae family are known for carrying parasites like the *Haemoproteus* and Plasmodium which have affected 12.4% of Costa Rican macaw species (Dieckmann et al. 2020). Intestinal parasites can be detected in fecal samples from birds in both the wild and rehabilitation centers. It is common for some intestinal parasites like *Ascardia galli* and *Giardia duodenalis* to infect birds that are located at these centers and because of transmission, many birds can become infected quickly (Dieckmann et al. 2020). Parasites can cause many different clinical signs such as diarrhea, weight loss, anorexia, and liver lipidic degeneration, oedematose lungs, hydropericardium and death in untreated or severe cases (Alarcon et al. 2015). It is also possible for parasite infection to lead to other diseases as shown in table 1.

Parasite infection commonly develop while birds are at rehabilitation centers but can also occur in their natural environment. Some scarlet macaws have ingested gastropods, that are an intermediate host, inside the cages during rainy and humid seasons (Alarcon et al. 2015). Animals kept in a relatively small area are more likely to become exposed to other animals with parasite infection. For these reasons, keeping captive animals in clean environments with access to good nutrition and clean water can prevent parasitic infection from occurring (Doneley 2009).

Ensuring a species is healthy before releasing is important in keeping the other wild populations healthy. If a reintroduced species carries parasites, the wild population can experience environmental stress, susceptibility to predators, and it can reduce reproduction (Patino et al. 2018). Biologists will often take fecal samples from birds located in different regions to determine the infection rate of the species. Amplification of the 16S region of ribosomal RNA can determine presence of different avian pathogens (Patino et al. 2018). By examining infection, biologists can then determine what parasites are more prevalent in the area and how that environment has contributed to avian infection.

Purpose

Injured and sick tropical wildlife are commonly brought into clinics and sanctuaries in order to provide adequate treatment. Protection of these species is important, however more gastrointestinal illness challenges have recently been associated with housing wild animals. By further studying tropical wildlife, biologists and veterinarians can work together to analyze if their facilities are effectively treating illness and preventing other health issues from occurring. As mentioned previously, nutrition and spread of parasites can greatly impact the health and recovery of sick organisms.

The purpose of this study is to evaluate parasite infection in tropical, rehabilitating howler monkeys to analyze whether animals in captivity at treatment centers are experiencing changes within their gastrointestinal system or immune system. The impact of these changes was examined through fecal, behavioral, and hematology analysis. In addition, this study sought to determine whether treatment with Tinidazole and Fenbendazole was effective at eliminating infections.

CHAPTER II

MATERIALS AND METHODS

Sixteen howler monkeys, *Alouatta palliata,* were studied at Wild Sun Rescue in Cabuya Costa Rica (9.5973 N, 85.0994 W). Handling and procedures were approved by the Environmental Ministry of Costa Rica (See Appendix 1). Whereas all animals were named at the Rescue, for this study the individuals were numbered based on age, from youngest to oldest. Howler monkeys 9,11, 14, and 15 were being treated and closely monitored in the Intensive care unit and were kept in small kennels. The other monkeys were in outdoor enclosures in a more isolated area of the rescue campus. The enclosures were set up so that they were beside one another so the monkeys had social interaction with one another. Wild primate species would often pass through the area. Fecal samples were collected from howler monkey enclosures, located on site, on June 17th, 2022, and June 28th, 2022, and again after treatment on August 16th and September 13th, 2022. A sterile swab was used to add the sample to fecal containers which were stored in the refrigerator. Next wet mount slides were prepared using a saline solution and another sterile swab. A cover slip was placed over the sample on a microscope slide. The samples were examined under a light microscope at 400X magnification.

Presence of parasites was recorded and identified for each monkey using criteria outlined by Rondon et al. (2017; see Appendix 2) and Helenbrook et al. (2015, See Appendix 3). Fecal samples were then sent to Jackson Laboratory clinic in Santa Teressa, Costa Rica. Fecal samples were tested at the lab for protozoans, nematodes, and cestodes. A small sample was taken from the tubes and placed into a container with a saline solution. This will allow the eggs and material to float to the top where it will stick to a cover slip. The coverslip is placed on a microscope slide and examined under a microscope. Howler monkeys that were positive for parasite infection were given a dose of Tinidazole (50mg/kg) on day 1 and 4 post diagnosis. Howler monkeys that were positive for roundworms (*Stronglyoides stercoralis)* were also given a dose of Fenbendazole (50mg/kg) once a day for five consecutive days. Retesting for parasite infection was performed on August 16^{th} and September 13^{th}, 2022.

Blood samples were drawn from howler monkeys being treated in the Intensive Care Unit at Wild Sun Rescue from June 17^{th}-July 9^{th} Using a 21-gauge needle and syringe, blood was drawn and put into Greiner Bio-One tubes and labeled. The blood samples were sent to Jackson Laboratory clinic in Santa Teressa, Costa Rica. Hematology data was also collected from the blood samples including leukocyte concentration, erythrocyte concentration, hemoglobin concentration, and hematocrit percentage.

The behaviors of all the howler monkeys were observed. Normal behavior for young howler monkeys included being social, playful, and active. Depression symptoms included behavior of inactivity, a weak appetite, and unwillingness to socialize with other monkeys. Most howlers became stressed during handling by humans, however symptoms of extreme stress was observed as being extremely fearful, loose bowel movements, poor appetite, repetitive scratching, and making crying noises.

CHAPTER III

RESULTS

Behaviors

The general behavior of the howler monkeys was observed on both June 17th and June 28th, 2022. Howler monkeys 1, 2, 5, 6, 7, 8, 12, 14, 15, and 16 appeared to be normal. They were alert, responsive, and social with the other monkeys in their enclosure. All were eating well and appeared to be healthy. Howler monkey 11 recently underwent an amputation surgery prior to fecal sample collection and seemed alert and responsive however psychologically appeared depressed, as she had little motivation towards any sort of activity. Howler monkey 9's overall appearance was poor, she was lethargic, dehydrated, underweight, and stressed. She had a very weak appetite, loose bowel movements, and was very unsocial with the other monkeys. She lacked social skills and the ability to defend herself against dominate individuals. Both 3 and 4 were recently rescued as orphans and appeared to be stressed, and slightly dehydrated. Howler monkeys 10 and 13 were also observed to be stressed, with loose bowel movements, and were underweight.

Fecal samples

Fecal samples were examined under a light microscope at 400X magnification. Parasites were observed and identified for comparisons between individual howler monkeys. Frequent parasites observed included *Chilomastix mesneli, Giardia,* and *Pentatrichomanas hominis*

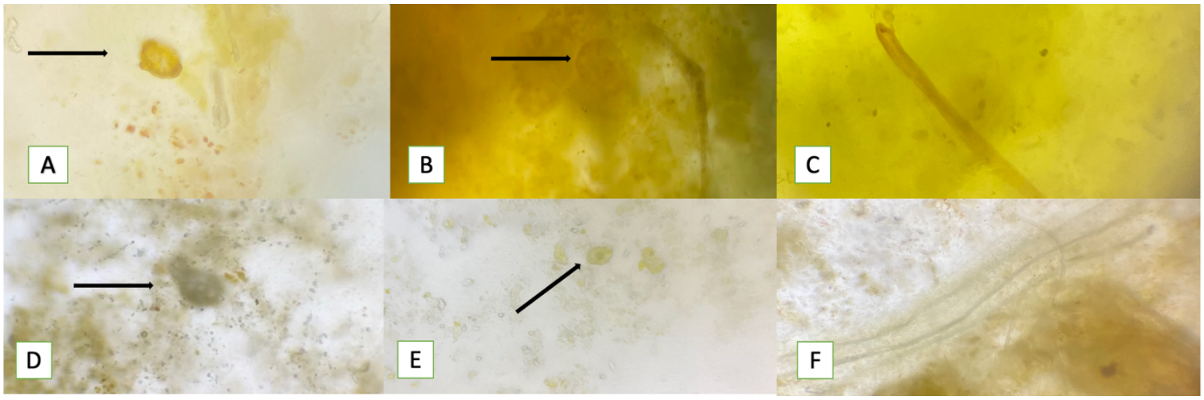

Figure 1: Parasites identified in Howler Monkey fecal samples collected on June 17th and June 28th, 2022 under 400X magnification. A. *Blastocystis hominis,* B. *Chilomastix mesneli,* C. *Stronglyoides stercoralis,* D. *Pentatrichomanas hominis,* E. *Giardia,* F. *Ancylostoma duodenal*e.

however some howler monkeys also had *Ancylostoma duodenal*e, *Stronglyoides stercoralis* and *Blastocystis hominis* as seen in Figure 1.

The fecal samples were sent to Jackson laboratory to confirm presence of identified parasites in each howler monkey as seen in Table 2 below. Jackson Lab performed numerous fecal flotations to examine the specimens under a light microscope for identification. Using both analyses, howler monkeys that were positive for certain parasites could be treated.

Fecal samples were analyzed again on August 16th and September 13th, 2022 in order to determine any changes following treatment. The total percentage of howler monkeys positive for *Chilomastix mesneli* decreased from 56.3% to 43.8%, the total for *Giardia* decreased from 37.5% to 6.3%, and 0% of howlers were positive for *Stronglyoides stercoralis* and *Ancylostoma duodenale* after treatment with Tinidazole and fenbendazole. The total percentage of howlers positive for *Pentatrichomanas hominis* increased after treatment from 31.3% to 43.8%, and the percentage of howler's with *Blastocystis hominis* remained the same.

Table 2: Parasites identified in fecal samples from Howler monkeys at Wild Sun Rescue before and after treatment with Tinidazole and Fenbendazole

Monkey	Age	Parasites (before treatment)	Parasites (after treatment)
1	5 months	none	none
2	7 months	*Giardia*	none
3	8 months	*Chilomastix mesneli* *Giardia* *Pentatrichomanas hominis* *Stronglyoides stercoralis*	*Chilomastix mesneli* *Pentatrichomanas hominis* (Small amount)
4	1	*Chilomastix mesneli* *Pentatrichomanas hominis* *Giardia*	*Chilomastix mesneli* *Pentatrichomanas hominis* (Small amount)
5	1	none	*Chilomastix mesneli* *Pentatrichomanas hominis* *Giardia*
6	1	none	none
7	1	*Giardia*	none
8	2	*Chilomastix mesneli*	none
9	2	*Chilomastix mesneli* *Pentatrichomanas hominis* *Giardia* *Stronglyoides stercoralis*	*Chilomastix mesneli* *Pentatrichomanas hominis*
10	2	*Blastocystis hominis* *Chilomastix mesneli*	*Chilomastix mesneli* *Pentatrichomanas hominis*
11	2	*Chilomastix mesneli*	none
12	2	none	none
13	2.5	*Chilomastix mesneli* *giardia* *Pentatrichomanas hominis* *Ancylostoma duodenale*	*Chilomastix mesneli* *Pentatrichomanas hominis*
14	3.5	*Chilomastix mesneli*	none
15	3.5	*Chilomastix mesneli* *Pentatrichomanas hominis*	none
16	4	*Chilomastix mesneli* *Pentatrichomanas hominis*	*Chilomastix mesneli* *Pentatrichomanas hominis* *Blastocystis hominis*

Hematology

Hematology samples were collected from the four howler monkeys being treated in the Intensive Care Unit (ICU) from June 17th- July 9th 2022. Samples were stored in Greiner Bio-one tubes and sent to Jackson Laboratory for hematology analysis. Hematology values were calculated and listed in Table 3.

Based on the hematology values, monkey 9 was the only howler to have significantly high values of leukocytes, neutrophils, and lymphocytes. Out of the other howlers in the Intensive Care Unit, she was the only individual positive for *Giardia* and *Stronglyoides stercoralis* (roundworms). Monkey 9 was also very underweight and stressed when compared to the other monkeys. Monkey 9 had diarrhea, frequently refused to eat, and often would pace in her kennel.

Table 3: Number of Leukocytes, Erythrocytes, neutrophils, and lymphocytes found in hematology samples analyzed at Jackson Laboratory clinic in Santa Teresa, Costa Rica. (+) represents values significantly above reference values. (-) Represents values significantly below reference values

Monkey	Leukocytes	Erythrocytes	Neutrophils	Lymphocytes
9	24,300 +	4,320,000	11,178 +	11,907 +
11	3,700	2,450,000	1,443	2,109
14	10,000	4,710,000	6,700	2,500
15	8,500	4,280,000	6,120	1,785

CHAPTER IV

DISCUSSION

Habitat Disturbances Effect on Parasite Infection

In the wild, howler monkeys are an arboreal species and spend most of their lives in the forest canopy. Howler monkeys and other primate species in captivity are kept in much smaller enclosures that often only have a small number of objects for them to use to climb during rehabilitation. Wildlife rescues like Wild Sun have attempted to provide these primates with an area that resembles their natural habitat; however, despite these efforts, these monkeys still are more likely to eat food off the ground and because of this, are at high risk of parasite infection. Human and habitat disturbances may increase the likely hood of these individuals being exposed to zoonotic transmission of gastrointestinal parasites including protozoans, nematodes, and platyhelminths (Helenbrook et al. 2015).

The howler monkeys at Wild Sun rescue were exposed to numerous habitat disturbances, including being in contact with wild primates nearby, human exposure, and were fed a mixed diet consisting of fruit, vegetables, and leaves from the cecropia tree. Diets that are deficient in nutrients or may include macro or micronutrients that aren't apart of the natural diet of howler monkeys can account for higher infection rates. Confined space can also lead to an increase in parasite infection since there is more crowding and a restricted host distribution (Trejo-Macías and Estrada 2012). These factors likely led to the 75% infection rate of the population. These results are consistent with the data collected by Rondón et al. which saw a rate of 72.9% of individuals in the species, *Alouatta seniculus, Ateles hybridus,* and *Cebus versicolor* being positive for parasites. Parasites can be transmitted between individuals quickly through direct and indirect exposure, and the four most common parasites are three protozoans (*Chilomastix mesneli, Trichomonas and Giardia) and a nematode (Stronglyoides stercoralis).*

Parasite Prevalence and Treatment

The protozoan *Chilomastix mesneli* is often found in the gastrointestinal microflora of primates. It is considered nonpathogenic but is often associated with parasitic infection (Helenbrook et al 2015). This protozoan was found in most of the fecal samples collected and often was found with other bacteria like *Pentatrichomanas hominis, Giardia,* and *Stronglyoides stercoralis.* The medication Tinidazole was administered to howler monkeys at Wild Sun rescue that were positive with this protozoan. Tinidazole slightly decreased the infection rate. In other species of primates, treatment with metronidazole was highly effective in eradicating *Chilomastix mesneli* (Kramer et al 2009).

Intestinal *Trichomonas* infection has been reported in captive howler monkeys which often present as asymptomatic (Pastor-Nieto 2014). This protozoan inhabits the cecum and will penetrate the mucosal epithelial layer in the body (Pastor-Nieto 2014). Some individuals that didn't show any behavioral, or hematology changes were positive for this parasite. Tinidazole, a synthetic nitroimidazole, was used for treatment against this parasite. Tinidazole's mechanism of action against this parasite may be secondary to generation of free nitro radicals produced by this protozoan (Kliegman 2020). In this experiment, treatment with Tinidazole appeared to be ineffective as almost all individuals were positive post treatment. Infection resistant to tinidazole may respond to metronidazole. The reasons that tinidazole was only moderately effective for both *Chilomastix* and *Trichomonas* could be that the individuals needed a longer course of treatment. Previous research found thar using smaller quantities over a 5-day period was more effective than a singular large dose (Armstrong and Wilson 2009). Metronidazole was used and successfully treated gastrointestinal trichomonas in squirrel monkeys (Brady et al. 1999).

Captive primates that are in close contact with humans are more susceptible to infection with *Giardia*. *Giardia* enters the body through ingestion of contaminated food or water, where it then attached to the intestinal mucosa and damages microvilli (Pastor-Nieto 2014). This parasite can cause a variety of symptoms including diarrhea, gas, cramps, anorexia, vomiting, and fever (Pastor-Nieto 2014). Treatment options for *Giardia* infection vary but usually involve administration of a medication like metronidazole or tinidazole. Many veterinary clinics use metronidazole, however, this specific medication requires 5-8 days of treatment, where tinidazole is administered as a single dose (Kramer et al. 2009). The infection rate with *Giardia* at Wild Sun Rescue did decrease after treatment using tinidazole. A combination of medication administration and sanitation practices most likely prevented other monkeys from becoming infected with this highly contagious parasite. Tinidazole proves itself to be effective in long term prevention in other species of primates, were 11 out of 13 individuals remained negative for giardia a year after treatment (Kramer et al 2009).

The nematode *Stronglyoides stercoralis* is more relevant in troops that are in areas near humans. Human presence causes an increase in the individuals stress levels which then impairs their immune response against this parasite (Camber 2020). Environments that are warm and humid can also increase the survival rate of species like *Stronglyoides* (Rondón et al. 2017). Howler monkeys positive for this parasite were given fenbendazole (50 mg/kg) for 5 consecutive days which was effective in eliminating the parasite completely.

Effect of Parasite Infection

Many parasites are harmful to their host species and can lead to other extreme health issues including psychological changes, nutritional disturbances, weakness, and increased susceptibility to secondary infection (Rondón et al. 2017). In this study, howler monkeys 9, 3, 4, and 13 all were infected with multiple parasite species including *Chilomastix mesneli, Pentatrichomanas hominis, Giardia* as well as some additionally had *Stronglyoides stercoralis* and *Ancylostoma duodenale.* The development of heavy parasite loads is often associated with captivity stress, chronic illnesses, and under nutrition (Pastor-Nieto 2014). When these four individuals were observed, their psychological behavior seemed to be poor, and they also were more dehydrated and malnourished. Their high parasite infection rate may have led to these specific nutritional disturbances and psychological problems. Hematology results from patients in the ICU indicated high levels of white blood cells, like leukocytes, in howler monkey 9 who was heavily infested with parasites. Howler monkey 9 came to Wild Sun rescue as an orphan and has been in and out of the ICU since her arrival due to repeated infections and health issues. She has been a patient at Wild Sun rescue for over a year and it is common for captive species to have higher white blood cell counts than free ranging individuals (Vié et al. 1998). Due to high infection with parasites, it is common to see changes in the body's immune system as it elicits a response to infection. Neutrophils are also key limiters of bacterial and parasitic inflammation as the immune system responds to foreign material in the body (Brinkworth et al. 2019). Howler monkey 9 was heavily infested with multiple parasites with high neutrophil counts when compared to monkeys 11, 14, and 15 who had normal levels of neutrophils and much lower infection rates.

Captive and rehabilitated animals experience many behavioral changes depending on their time spent in captivity and their new environment. These animals rely on humans to provide them with food, predation is not an issue, and group living is different. Wild howler individuals travel in group, known as a troop, with anywhere from 4-20 members consisting of mainly females, juveniles, and very few males. In wildlife rescues, howler monkeys may be put into enclosures based on age and thus individuals aren't necessarily exposed to the same group living they would have in the wild. Captivity provides a static environment and often results in reduced interest, decreased problem solving skills, and reduced attention (Pastor-Nieto 2014).

Recommendations

The results of this study suggest that several steps can be taken to lower the parasite loads of captive howler monkeys. To minimize the prevalence of parasite infection in howler monkeys in captivity, the monkeys should have very minimal human interaction. It is estimated that 60% of primate species are threatened with extinction due to human activity (Estrada et al. 2017). Enclosures should be built in areas with the least amount of exposure to humans. High stress levels seem to contribute to increased susceptibility to parasite infection, as stressed animals are often immunosuppressed (Cowl 2019). Veterinarians can use new methods to limit direct contact with these animals such as sedating the animals before exams so that they don't become comfortable with human interaction. The use of noninvasive sampling can also prevent the animal from being exposed to stress with handling. Previous research found that fenbendazole formulated in a commercial primate diet was effective in stopping the shedding of parasite eggs (Reichard et al 2008). By putting medications in food, direct human interaction with primate species is limited, reducing stress levels, and exposure to new pathogens or parasites from humans. Enclosures should also be built in areas that closely resemble their natural habitat. Using elevated feeding trays and perches can limit infection for these arboreal primates. Arboreal primates in rehabilitation or individuals living at rescue centers often experience abnormal behaviors such as moving on the ground. For captive primates, social group dynamics should also be considered. Primates at rescue centers may be put into unnatural social groups with large male or female populations. Unnatural social groups can lead to escalated aggression, abnormal behavior, and may increase stress (Cowl 2019). Based on the results from this study, wildlife rescue centers should focus on creating a environment, for captive animals, that resembles their natural habitat and social dynamic structure. These changes can contribute to decreasing the prevalence and spread of parasites in individuals.

Future Studies

Future research could potentially examine how environmental changes and land changes affect wild species of primates in terms of parasite infection. Understanding other environmental changes, like seasonal changes, and how it may affect the population dynamics of zoonotic parasites can help researchers understand the epidemiology (Rondón et al 2017). Veterinarians and wildlife centers can better prepare by giving antiparasitic medications during times of increased susceptibility. These data can also provide a better understanding of the health status of wild primate populations and how parasite infection differs between each population. It may also be beneficial to examine how parasite infection differs between species, such as capuchins, who have more frequent contact with the soil (Rondón et al. 2017).

Besides crowding and abnormal foraging, other variances from normal could affect parasite infection. Future research should examine whether the sex of the individual correlates with a higher infection rate. Sex hormones can potentially affect parasite infection as estrogen can produce antibodies against parasite antigens (Aguiliar Diaz et al 2015).

Parasite infection is just one of many problems seen in captive primates. The gut microbiome of primates is also extremely susceptible to issues due to the enclosure design, captive diets, and stress. Addressing and treating for these changes early on can help primates, as the gut microbiome provides their host with 70% of their daily needs including the facilitation of nutrient absorption and prevention of accumulation of toxic metabolic products (Amato 2016). More research in this area can greatly improve conditions for primates and species in captivity and prevent further health problems from developing.

APPENDICES

APPENDIX 1: Animal Handling documentation for howler monkeys at Wild Sun rescue in Costa Rica

WILD SUN

November 15th, 2022

Cóbano, Costa Rica

To Whom it may concern:

The current letter is to confirm that due to the nature of the work done at Wild Sun Rescue Center, animal handling must be done under certain circumstances. Such as moving the animals from enclosure, when physical veterinarian medical checks and sampling (such as blood sampling) is required, during the income of an animal or before its transportation due to its released. Handling at Wild Sun Rescue in done only by trained personal. During the Veterinarian Medicine Internships, handling of certain animals is approved when doing some of the procedures mentioned before.

If you require any further information, please do not hesitate to contact me.

Andres Perez, DVM.

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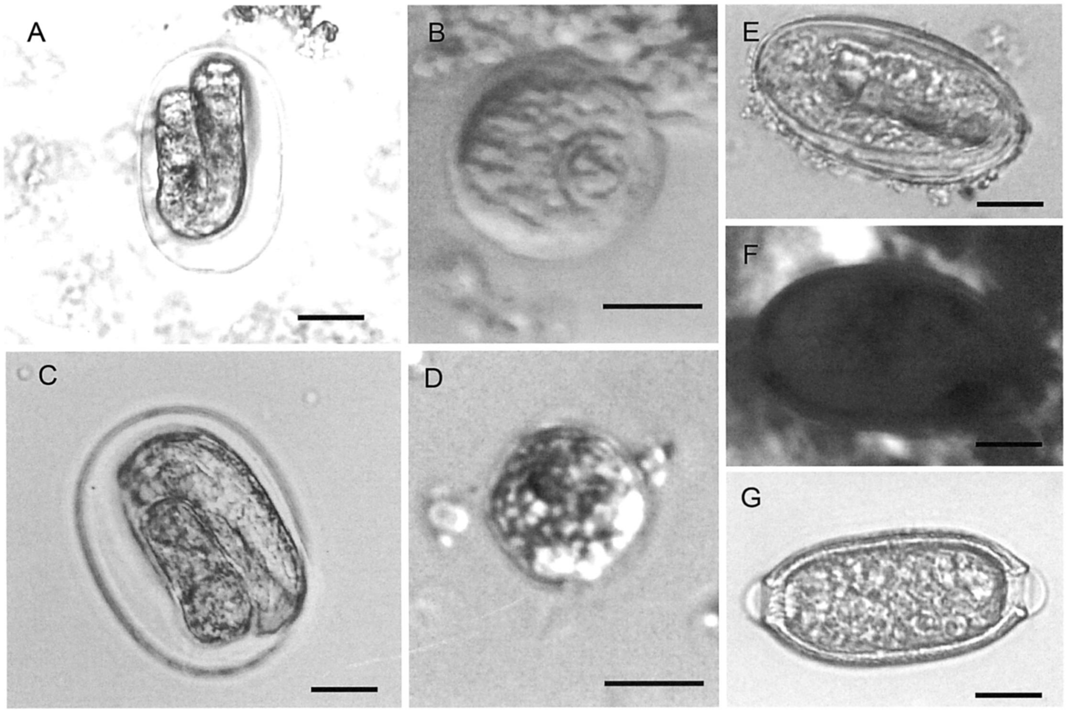
APPENDIX 2: Nematodes and protozoans located in fecal samples from Primates in Colombia.

This diagram was used to identify parasites seen in fecal samples at Wild Sun Rescue

A. *Trichuris sp*., B. *Oxyuridae*, C. *Ancylostomatidae*, D. *Stronglyoides*, E. *Ascarididae*, F. *Gnathostomatidae*, G. *Trichostronglyidae*, H-I *Trematodes*, J. *Entamoeba*, K. *Ancanthocephala*, L. *Balantidiidae*

APPENDIX 3: Parasites and parasite eggs found in howler monkeys in Ecuador.



This diagram was used to identify parasites seen in fecal samples at Wild Sun Rescue. A. *Stronglyoides*, B. *Entamoeba*, C. *Stronglyoids*, D. *Entamoeba*, E. *Trypanoxyuris*, F. *Enterobius*, G. *Capillaria*.

WORKS CITED

Aguilar-Díaz H, Nava Castro KE., Cerbón_Cervantes MA., Meneses-Ruiz DM, Ponce-Regalado MD., Morales-Montor J. 2015. Endocrine immune interactions in the host parsite relationship:steroid hormones as immune regulators in parasite infections. J. steroids horm sci. 6(3):1-12

Alarcon A, Morales J.A, Veneziano V., Santoro M. 2015. Fatal paratanasia bragai infection in scarlet macaws in costa rica. Jour of Acta Parasit. 60(3):548-552.

Allender MC, Dreslik M, Wylie S, Phillips C, Wylie DB, Maddox C, Delaney MA, Kinsel MJ. 2011. Chrysosporium sp infection in eastern massasauga rattlesnakes. Emerg Infect Dis. 17(12):2383-2384.

Amato KR, Metcalf JL, Song S, Hale VL, Clayton J, Ackermann G, Humphery G, Niu K, Cui D, Zhao H, Schrenzel MD, Tan CL, Knight R, Braun J. 2016. Using the gut microbiota as a tool for examining colobine primate GI health. Global eco conserve. 7:225-237.

Armstrong NR., Wilson JD. 2009. Tinidazole in the treatment of bacterial vaginosis. Int J Women's Health. 1: 59-65.

Ballou JD. 1993. Assessing the risks of infectious diseases in captive breeding and reintroduction programs. J of Zoo Wildli Med. 24 (3): 327-335

Bavelaar F, Kuilen J, Hovenier R, Lemmens A, Beyen A. 2005. Plasma lipids and fatty acid composition in parrots in relation to the intake of alpha-linolenic acid from two feed mixtures. J *Anim phys animal nut*. 89(9-10): 359–366

Beaufrere H., Acierno M, Mitchell M., Guzman D.S, Bryant H, Tully T.N. 2011. Plasma osmolarity reference values in african grey parrots (Psittacus Erithacus), hispaniola amazon parrots, and red fronted macaws. J Avian Med Surg. 25(2): 91-96.

Brady AG., Gardner WA., Culberson D., Scimeca J. Pindak FF., Abee C. 1999. Experimental genital trichomoniasis in squirrel monkey. Genitourinary med. 63(3):188-191.

Brightsmith D, McDonald D, Matsafugi D, Bailey C. 2010. Nutritional content of the diets of free-living scarlet macaw chicks in southeastern peru. J Avian Med Surg. 24 (1):9–23

Brinkworth J.F., Etten KV, Bhatt P., McClure K., Valizadegan N., Woo M., Gunasekera S., Suarez Y., Aldridge B. 2019. Functional comparison of human and non human primate neutrophil responses. J Immunol. 202 (1):73

Camber AM. 2020. Natural occurrence of Strongloyoides spp. in vervet monkeys (Chlorocebus pygerythrus) in Kuti Wildlife reserve, Malawi {thesis}. Uppsala: Swedish University of Agricultural Sciences.

Chappell J, Thorpe S. 2021. The role of great ape behavior ecology in one health: implications for captive welfare and re-habilitation success. Ameri J Primatology. 84(4-5): 23328

Cowl VB. 2019. The effects of captive management on primate social behavior and aggression {thesis]. United Kingdom; University of Manchester.

Dieckmann H, Jimenez-Soto M, Jumenez-Rocha A, Rojas E, Conrad P. 2020. Intestinal and blood parasites in saclet and great green macaws in wildlife rehabilitation centers in costa Rica. J Zoo Wild Med. 51(2): 385-390.

Doneley Rj. 2009. Bacterial and parasitic diseases of parrots. Vet clin of NA: exotic animal Practice.37 (1):21-35

Eberhard J.R, Elias E, Hoeflich E, Cun E.P. 2015. Phytogeography of the military macaw and the great green macaw based on MTDNA sequence data. Wilson J Orinth. 127(4): 661-669.

Estrada A. 2014. Reintroduction of the scarlet macaw (Ara Macao cyamoptera) in the tropical rainforests of Palenque, Mexico: project design and first year progress. Trop conser sci. 7(3): 342-364.

Gonzalez CQ, Cardenas LA, Ramierez M, Reyes A, Gonzalez C, Stevenson PR. 2021. Monitoring the variation in gut microbiota of captive woolly monkeys related to changes in diet during a reintroduction process. Scien repts. 11. https://doi.org/10.1038/s41598-021-85990-0

Gutierrez L, Cardona J, Calderon I. 2017. Sex identification of neotropical macaws from invasive and noninvasive samples. Ornit Colomb. 16:1-7

Harrison G., Lightfoot T., McDonald D. 2006. Clinical Avian Medicine; Nutritional Considerations - Section II: Nutritional Disorders. Spix Publishing.

Helenbrook WD., Wade SE., Shields WM., Stehman ST., Whipps CM. 2015. Gastrointestinal parasites of Ecuadorian mantled howler monkeys (Alouatta Palliata Aquetoralis) based on fecal analysis. Jour of Parsitol. 101 (3):341-350.

Kamler J, Homolka M. 2005. Faecal nitrogen: a potential indicator of red and roe deer diet quality in forest habitats. Folia Zool. 54: 89-98.

Kamler J, Homolka M. 2011. Needles in faeces: an index of quality of wild ungulate winter diet. Folia Zoolo. 60(1):63-69.

Kleigman MD. 2020. Principles of Antiparasitic therapy. Nelson Textbook of Pediatrics. 1(21): 1812-1829.

Koutsos E, Matson KD, Klasing KC. 2001. Nutrition of birds in the order psittaciformes: a review. *J* Avian Med Surg. 15(4): 257-275

Kramer JA., Hachey AM., Wachtman LM., Mansfield KG. 2009. Treatment of giardiasis in common marmosets (Callithrix jacchus) with tinidazole. Comp Med. 59(2): 174-179.

Lall K, Jones K, Garcia G. 2018. Nutrition of six selected neo-tropical mammals in Trinidad and tobago with the potential for domestication. Vet scien. 5(2):52 doi: 10.3390/vetsci5020052

Li Y, Hu X, Yang S, Zhou J, Zhang T, Qi L, Sun X, Fan M, Xu S, Cha M, Zhang M, Lin S, Liu S, Hu D. 2017. Comparative analysis of the gut microbiota composition between captive and wild forest musk deer. Front Microbiol. 8: 1705. Doi: 10.3389/f.micrb.2017.01705.

Mantovani S, Allan. N, Pesapane R, Brignolo L, Foley J. 2018. Eradication of a tropical rat mite (Ornithonyssus Bacoti) infestation from a captive colony of endangered amargosa voles. J Zoo wildlife med. 49(2);475-479.

Neal E. 2019. Difference between the temperate forest and rainforest [Internet]. Sciencing.com, https://sciencing.com/difference-between-the-temperate-forest-rainforest-12507773.html. (Accessed November 2022)

Ortiz F.A, Solorzano S., Arizmendi M.C, Aranda P.D, Oyama K. 2020. Genetic diversity and structure of the military macaw (ara militaris) in mexico: implications for conservation. Trop Conserv Sci. 10(1). Doi.org/10.1177/1940082916684346

Pastor-Nieto R. 2014. Health and welfare of howler monkeys in captivity. Devlop in primitaol:progress and prosp. 313-355. Doi.org/10.1007/978-1-4939-1960-4_12

Parasitol IJ. 2019. Detection of *Chilomastix mesneli* in common marmoset (*Callithrix jacchus*) and treatment with metronidazole. Iran J Parasitol. 14(2):334-339.

Patino LC, Monge O, Suzan G., Espeleta G, Chaves A.2018. Molecular detection of mycobacterium avium and mycobacterium Genevese in feces of free living scarlet macaws in costa rica. J wild disease. 54(2): 357-361.

Rondón S., Ortiz M., León C., Galvis N., Link A., González C. 2017. Seasonality, richness, and prevalence of intestinal parasites of three neotropical primates (*Aloutta seniculus, Ateles* *hybridus* and *Cebus versicolor)* in a fragmented forest in Colombia. Interna Jour for Parasit: parasites and wild. 6(3):202-208.

Reichard MV., Wolf RF., Clingenpeel LC., Doan SK., Jones AN., Gray KM. 2008. Efficacy of fenbendazole formukared in commercial primate diet for treatinf specific pathogen free baboons infected with Trichuris trichiura. J Am Assoc Lab Anim Sci. 47 (6): 51-55.

Trejo-Macías G., Estrada A. 2012. Risk factors connected to gastrointestinal parsites in mantled Aloutta palliata Mexicana and black howler monkeys Alouatta pigra kiving in continuous and in fragmented rainforests in Mexico. Current Zoolo. 58(3):375-383.

Vié JC., Moreau B., Thoisy B., Fournier P., Genty C. 1998. Hematology and serum biochemistry values of free ranging red howler monkeys from French Guiana. Jour of Zoo and Wild Med. 29(2): 142-149.

Washko RM, Hoefer H, Kiehn TE, Armstrong D, Dorsinville G, Frieden TR. 1998. Mycobacterium tuberculosis infection in green winged macaw(Ara chloroptera): report with public health implications. J clinical microbiology. 36(4):1101-1102.