# Prevalence of *Toxoplasma gondii* oocysts in and around the ring-tailed lemur exhibit at a zoo in the midwestern United States

**An Honors Thesis (HONR 499)**

**By**

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## ABSTRACT

*Toxoplasma gondii* is a parasite for which felids are the definitive hosts and the oocysts are shed in the felid's feces. Oocysts that are prevalent in the soil where ring-tailed lemurs (*Lemur catta*) live can be ingested by them and infect them with toxoplasmosis, which can lead to reproductive problems, anorexia, cellular necrosis, depression, and even death. Soil samples were taken from the inside and outside of the ring-tailed lemur exhibit at a zoo in the Midwest to determine the prevalence of *Toxoplasma gondii* oocysts there. Samples were taken through two separate treatments to isolate the oocysts from the soil. Both treatments of both inside samples and outside samples did not return any isolated oocysts. Even though *Toxoplasma gondii* oocysts were not found in the soil samples that were taken for this study, the ring-tailed lemurs at this zoo have still gotten toxoplasmosis, so more research is necessary to determine how this is happening. Recommendations for lowering the risk to the lemurs at this zoo include reevaluating the construction of their exhibit and taking other preventative measures to avoid this disease.

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Without support from the Josie and Geoff Fox Research Fund at Ball State University, this project would not have been possible to carry out. I also thank the Zoo in the Midwest that allowed me to conduct research involving their grounds and their animals.

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# PROCESS ANALYSIS STATEMENT

When it came time for me to have my thesis meeting with the honors college in early fall semester 2018, I knew I wanted to do a scientific research project that related to my field, but I wasn't sure exactly what yet. My initial idea was to do a comparative analysis of great ape enrichment between accredited zoos and non-accredited zoos in the midwestern United States. I was planning on doing interviews, visiting the institutions, and doing a write-up on the differences (if any) in quality of great ape enrichment between the two. My honors thesis advisor, Dr. Barb Stedman, thought that this was a good idea and recommended that I find a faculty advisor to talk it over with. However, upon picking Dr. Tim Carter as my advisor, he advised me to pick a different topic. He feared that I would have a hard time fitting it all into one semester, and said that in his experience, it takes a while to schedule interviews with busy zoo officials. I picked Dr. Carter as my main advisor because he is the mammalogist in the Biology Department. I have taken a couple of classes with him, Mammalogy and Wildlife Biology, and they have proven to be the most useful courses for my career in animal care that I have taken here at Ball State. Additionally, he has overseen both of my internships and helped me get college credit for completing them, and I am going on a field study to Costa Rica with him in the summer of 2019. I knew Dr. Carter well and I trusted that he would be an excellent advisor for my thesis project.

However, after that initial meeting with Dr. Carter, I will admit that I was feeling discouraged. I had no ideas about what to do for my thesis at this point, so I took a week or so to think about other possible projects. My honors thesis, "Prevalence of *Toxoplasma gondii* oocysts in and around the ring-tailed lemur exhibit at a zoo in the midwestern United States," originated

as an interest I had in *Toxoplasma gondii* upon learning about it in my parasitology class that week. I have always thought that parasites were fascinating, but this one stood out to me, as I had heard about it before from working in animal rescue, an animal hospital, and a zoo that I interned at. That day in class, I recalled talking to the primate zookeepers (during my internship over the summer) about a disease called Toxoplasmosis that had killed a few of the ring-tailed lemurs over the past several years. I didn't know what this was at the time, but I connected the dots and raised my hand in class to tell my professor, Dr. Randall Bernot, about what I had heard at the zoo. He was quite interested because there isn't a lot known about the parasite and the disease it causes, so it was interesting to him that it affected the lemurs. Lemurs are primates like humans, but they are considered prosimians, which are much more primitive. I began to wonder about *Toxoplasma gondii* after this, and questions went through my mind, including: how does it affect lemurs differently than it affects humans? How did the lemurs get the disease? Where is the parasite coming from? Is there anything the zoo can do to minimize its harmful effects on this endangered species?

I met with Dr. Bernot during his office hours to talk about my interest in *Toxoplasma gondii* a little bit more, and I brought up that I was trying to find a thesis idea and that this might have been something I could go off of. I really cared about how Toxoplasmosis was negatively affecting the lemurs, and I found myself wanting to help solve the problem. The honors thesis was a perfect opportunity to do just that. I met further with Dr. Bernot about how exactly I could go about analyzing how the lemurs were getting the disease. I learned that felids, or cats, are carriers of the parasite *Toxoplasma gondii* and that they shed their oocysts in their feces. When ingested, these oocysts can cause a range of problems depending on the organism that ingests them. I asked Dr. Bernot to be my co-advisor for my honors thesis because of his expertise in

parasitology and ecology. I took Invertebrate Zoology and Parasitology with him, so I was confident in my decision because it was clear that he had a lot of knowledge on the subject.

The process of carrying out this thesis came with some challenges right from the beginning. Completing my proposal to turn into Dr. Stedman was simple enough, but it was denied because it did not contain everything that was outlined in the Honors Thesis Guide. Initially, I had included those things, but omitted them after speaking to my faculty advisors about how a typical scientific research proposal is done. I was able to quickly correct my proposal and get it approved the second time. Contacting the zoo that I carried out my research with was simple enough, but when it came time to submit my proposal to them, it took three months for them to get back to me despite constant reminders. It wasn't until January 2019 that I received the go-ahead from the zoo. This made me increasingly nervous because if the zoo denied my research proposal, my project would simply turn into a literature review over *Toxoplasma gondii* in lemurs, and I wanted to take it further than that by doing the physical research myself. Once I was approved to take soil samples from the zoo's property, an additional obstacle became apparent: I had to wait until the soil was not frozen to be able to actually take a sample. Because of this, I had a few extra weeks to do research on my topic, but unfortunately that resulted in a few less weeks to actually do the lab work with the soil samples.

Communication issues did not stop with the zoo. Due to both of my advisors' busy schedules, it was difficult to work out meeting times and get responses to emails quickly. Waiting on permission from the zoo, edits of my proposal, and meetings with my advisors was something that was frustrating, but part of the overall process. It was a hard lesson to learn that not everything in life goes according to plan, and that is okay. Another challenge that I faced was struggling with time management in general. Waiting was difficult for me because I often felt

that I was at a complete standstill, when that was not true. However, while waiting on the weather to turn warm enough to go to the zoo, I applied for the Josie and Geoff Fox Research Fund Grant and got it. That grant allowed me to purchase a cooler and Ziploc baggies to use when collecting soil samples. It also paid me back for gas used to drive to the zoo and back. I also contacted Dr. Jessi Haeft in the Department of Natural Resources and she loaned me a soil auger to use. A soil auger is a type of probe that is used to remove a core of soil from the ground without having to dig it up. With all the supplies that I needed, I was eventually able to make my trip to the zoo and successfully take soil samples with the help of primate curator Peggy Hoppe.

The lab work was extremely daunting and extensive throughout my analysis because it was like I had to learn a brand new science. I had taken a Parasitology course, but it did not involve actually finding oocysts in soil, so the methods that I used were completely foreign to me. I figured it out with some help from Dr. Bernot, but he had never done this either, so it was definitely a learning process for both of us. It may be difficult to understand the methods section of this thesis, so I will explain what I did in the lab. I began by adding some soil from each sample to a test tube and adding 2% sulfuric acid to it, which I diluted myself in the lab. I then added deionized water to each, which is simply water with no positive or negative charge to it. Each sample was then taken through a process, Treatment A, where I filtered it through a fine mesh sieve, centrifuged it (spun it), vortexed it (mixed it), added a sugar-water solution, centrifuged it again, added regular water, centrifuged it again, then preserved what was left over to analyze later. I took each sample through another process, Treatment B, where I separated it into two tubes, added a sugar-water solution, centrifuged it, added regular water, centrifuged it again, mixed the two tubes together added water once more, and centrifuged it again. The result was then preserved to be analyzed along with the others. The analysis process involved preparing

a microscope slide with a small amount of sediment from each processed sample and inspecting it through a compound microscope to look for *Toxoplasma gondii* oocysts.

Although this project had its fair share of challenges, it also provided me with some great insights, as well. I realized through studying Toxoplasmosis that parasitology is something that I could see myself going to graduate school for. I never expected to like invertebrates so much, but the connections that I have made between parasites and mammals have intrigued me, and I hope to be able to research them more. My plans after graduation involve a full time position at a zoo, which is amazing, but this project has planted a seed about graduate school that has never been there before, so who knows where this will take me in the future. Additionally, I never expected that I would be able to help animals in this way. I have always known that I wanted to work with animals, and my time as a biology major at Ball State has directed me to the zoology path to work with exotic and endangered species. This project inspired me to never let anyone tell me that my work isn't important, because any effort to protect our Earth and the animals on it is not wasted. I didn't realize how much more I could be doing, however, with the species that I have worked with, until I began this project and became interested in epidemiology and parasitology. I now know that I can make an even bigger impact by paying attention to how these things affect animals.

Personally, this project means a lot to me. I have poured my heart and soul into it and I am proud of my work. To me, my thesis is meaningful because it is yet another way for me to use my skills to help endangered animals. I have struggled with the fact that I am only one person and have felt inadequate when facing the issue of endangered species conservation. However, through this project, I have come to realize that even though I may be one person, I have the skills and education to do more than I ever thought. Along with a way to help animals,

this project could mean that more research will be done on the subject that could lead to a decrease in toxoplasmosis in ring-tailed lemurs and other primates. Additionally, this project could lead to an improvement in captive life for primates susceptible to toxoplasmosis.

# PREVALENCE OF *TOXOPLASMA GONDII* OOCYSTS IN AND AROUND THE RING-TAILED LEMUR EXHIBIT AT A ZOO IN THE MIDWESTERN UNITED STATES

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## INTRODUCTION

***Toxoplasma gondii:*** *Toxoplasma gondii* is a parasite for which domestic or feral cats are the definitive hosts. The oocysts of the parasite are shed in cat's feces. They then mature in the soil until another animal comes in contact with them (Jones et al. 2009). The other animal, usually a rodent or livestock, ingests them and becomes infected. Though this parasite and its infection are common in both humans and other animals, its life cycle was unknown until approximately the 1970s (Dubey et al., 1970). *Toxoplasma gondii* prevalence was higher among feral cats than domestic cats according to a survey done in 2005 (Vollaire et al., 2005). Cats do not usually present with clinical problems from infection with toxoplasmosis, but with severe infections they can develop fever, pneumonia, and damages to the central nervous system. Prevalence of *Toxoplasma gondii* in cats is typically documented through directly finding oocysts in their feces or indirectly doing serological studies for antibodies (Pereira et al., 2018).

Humans can be infected by *Toxoplasma gondii* post-natally by consuming food or drink containing oocysts, ingesting tissue oocysts by eating undercooked meat, or accidently ingesting the oocysts from the environment (Dubey and Jones, 2008). Most humans infected post-natally

are asymptomatic, but some have developed mild diseases. More research needs to be done on whether or not chronic toxoplasmosis in humans has any effect on behavior, mental illness, reaction time, and more (Dubey and Jones, 2008). The biggest risk to human health from infection by *Toxoplasma gondii* is when a woman is infected during pregnancy. This leads to congenital toxoplasmosis which can lead to spontaneous abortion, still-birth, hydrocephalus or microcephalus in a live infant, and other developmental problems (Dubey and Jones, 2008).

The three main ways that *Toxoplasma gondii* is transmitted are congenital infection, ingestion of infected tissues, and ingestion of oocysts. There is no way to distinguish whether the infection was caused by ingesting infected meat or by ingesting oocysts, so most evidence of ingesting infected meat is found by doing prevalence studies with animals such as livestock, however, it is believed that most infections are caused by ingesting oocysts (Dubey and Jones, 2008). The life cycle of *Toxoplasma gondii* is reliant on oocysts staying in the environment, namely cat feces and soil. In the United States, there are an estimated total of about 150 million cats, with about half being domestic and about half being feral (Dubey and Jones, 2008). There is plenty of potential for transmission of these oocysts in rural settings in the United States, and the midwestern region is known for its rural landscape. Because of the extreme number of cats in the United States, environmental oocyst contamination is nearly impossible to avoid (Dubey and Jones, 2008). Typical cats only shed oocysts for about a week in their lifetimes, so several are shedding oocysts at any given time (Dubey and Jones, 2008). House cats are not the only species to shed oocysts, other felids such as bobcats, which live in the midwestern United States, also shed oocysts, as well as other big cats in other areas of the world (Dubey and Jones, 2008). Viable oocysts have not yet been detected in drinking water in the midwestern United States, but they have been directly found in animal feed and soil, so it is prevalent in that area. It is therefore

likely that oocysts are regularly transported by humans on shoes that have come into contact with contaminated matter in the environment (Dubey and Jones, 2008).

**Ring-tailed Lemurs:** Ring-tailed lemurs (*Lemur catta*) are prosimians native to the southern and southwestern regions of the island of Madagascar, which is the only area in which they are found in the wild. Their diet consists of mainly fruit and leaves. Females are dominant over males, which allows the females to choose a higher quality diet before the males can (Wilson and Hanlon, 2010). Ring-tailed lemurs, like other folivorous primates, add to their diet by eating soil, and it is hypothesized that this is because they are able to supplement their sodium intake by doing so (Ganzhorn, 1987). In captivity, they eat a larger range of foods which can decrease the fiber that would be found in their diet in their natural habitats (Ganzhorn, 1987). Lemurs obtain their food in part by foraging for it on the ground in both natural and captive habitats (Ganzhorn, 1987). Ring-tailed lemurs in captivity are also highly susceptible to diseases such as toxoplasmosis, coccidiomycosis, and hemosiderosis (Spenser et al., 2004).

***Toxoplasma gondii* in primates:** *Toxoplasma gondii* does not usually lead to death in humans, which are primates (Pearson et al. 2011). However, some species such as marsupials, new world monkeys, lemurs, and others can die of toxoplasmosis if severe enough, and lemurs and new world monkeys are at a high risk for contracting clinical toxoplasmosis (de Camps et al., 2008).

Seven risk factors were outlined in the study "Seroepidemiology of *Toxoplasma gondii* in zoo animals in selected zoos in the midwestern united states," and they are: sex, freezing meat temperature, washing vegetables thoroughly, frequency of feral cat sightings on zoo grounds, frequency of feral cat control programs, capability of feral cats to enter grain barn, and type of

exhibit (de Camps et al., 2008). These risk factors, if present, lead to increased susceptibility to *Toxoplasma gondii* infection in primates in captivity (de Camps et al., 2008).

One study detailed the investigation surrounding six squirrel monkeys, six ring-tailed lemurs, and two owl monkeys that died within two months of each other at a zoo in the Netherlands. It was found that the primates died of toxoplasmosis, and that the most likely source of the infection was from infected sheep meat which was fed to the primates a couple of weeks earlier (Borst and van Knapen, 1984).

In one study, 269 serum samples were collected from eight midwestern United States zoos from animals including felids, marsupials, prosimians, New World monkeys, and birds. The serum samples underwent a serological examination and the presence of the *Toxoplasma gondii* antibodies from each species at each zoo was recorded. Four species of lemurs were found to have seropositivity to the parasite, and all four species of lemurs tested were also found to have respective microscopic agglutination test (MAT) titers. None out of 36 species of New World monkeys were found to have antibodies to *Toxoplasma gondii*. (de Camps et al., 2008).

However, several squirrel monkeys, which are New World monkeys, at a zoo in Japan were found to be seropositive shortly before they died of toxoplasmosis. This article discusses that New World primates are particularly susceptible to infection of *Toxoplasma gondii*, but they are arboreal, so they have less contact with the ground (and therefore, soil) than other animals may. The study concluded that the source of the infection for these squirrel monkeys was likely from food or water contaminated with *Toxoplasma gondii* oocysts, as stray cats had been seen in the area around their enclosure (Nishimura et al. 2018).

Primates that have been studied in relation to toxoplasmosis have been known to give negative results even after deliberate experimental infection, which suggests that false negatives could skew the actual prevalence of toxoplasmosis in primates (Itakura and Nigi, 1968).

***Toxoplasma gondii* in lemurs:** At a zoo in Alabama, a three year old female ring-tailed lemur presented with symptoms of lethargy, anorexia, and dyspnea, and was transferred to an animal clinic where she died before being examined. A necropsy was performed and tachyzoites of *Toxoplasma gondii* were found on lung smears and in sections of the liver and the spleen. Interstitial pneumonia and cellular necrosis were also found. All of this combined with other findings from the necropsy confirms toxoplasmosis as the cause of death for this lemur. The most likely cause of the toxoplasmosis that killed these lemurs was the passage of the oocysts either by fruits and vegetables or by being carried on the feet of blackbirds flying from felid exhibits to the lemur exhibit (Spenser et al. 2004).

At one zoo in the Midwest, the Cincinnati Zoo, four ring-tailed lemurs died within two months of each other, with depression being the main symptom of all of them. Toxoplasmosis was determined to be the cause of death for all four lemurs, with their necropsies showing necrosis in the lymph nodes, livers, and spleens of all of them. *Toxoplasma gondii* tachyzoites were found in all of the aforementioned organs, as well (Dubey et al., 1985). The six lemurs at the Cincinnati Zoo were housed inside the primate building next to 20 cats of a variety of species and four ruffled lemurs (*Lemur L varigatus*). Fecal samples were collected from the cats that were housed near the lemurs, but all were negative for *Toxoplasma gondii* oocysts. The most probably cause of the lemur's infections was oocysts shed by the cats in the building. Even though their fecal samples were negative for oocysts, they had been fed raw meat routinely and

oocysts are only shed in feces for about a week after consuming infected meat. The feces could have been tracked from the cat exhibits to the lemur exhibit, as the same person was in charge of cleaning all exhibits in the building. The two surviving ring-tailed lemurs were not seropositive for *Toxoplasma gondii* (Dubey et al., 1985).

*Toxoplasma gondii* is known to cause reproductive issues such as spontaneous abortions and still-births when human women are infected during pregnancy (Dubey and Jones, 2008). A study done in Spain followed an adult female ring-tailed lemur in captivity that was pregnant with four fetuses. She fell ill and presented with a distended abdomen and lowered appetite. One week later, she delivered two still-births, one fetus was removed due to bad positioning, and the last fetus was delivered still-born as well. The lemur recovered from the births but died two weeks later after presenting with anorexia (Juan-Salles et al., 2011). At this zoo, feral cats are known to frequent the areas around the lemur exhibit and have even been seen sitting on top of the wire fence that constitutes the roof of the enclosure. Pregnancy has been found to be a factor in increasing vulnerability to *Toxoplasma gondii* infection (Thouvenin et al., 1997), and because of this, the authors of this study concluded that an apparent reactivation of toxoplasmosis in the mother is what caused her death as well as the deaths of her offspring (Juan-Salles et al., 2011). There is evidence of vertical transmission of *Toxoplasma gondii* because the mother's placentitis was followed by fetal infection. This also indicates that it may be possible for fetuses to survive and maintain toxoplasmosis infection through vertical transmission (Juan-Salles et al., 2011).

***Toxoplasma gondii* in lemurs in the Midwest in certain enclosures:** In the case of other nonhuman primates, namely the lemurs at a zoo in the midwestern United States, it has led to illness and a few cases of mortality. Per zoo employees, feral cats roam the zoo grounds by night.

Before the lemur exhibit was built, the plot of land that currently houses the lemur exhibit was used for a gibbon exhibit, and previous to that, it was an open grassy area.

According to one study, *Toxoplasma gondii* infection sources could not be exactly pinpointed, but animals in zoos in the midwestern United States are mainly becoming infected by oocyst ingestion, which happens by consuming stray cat feces containing shed oocysts or by transport of the shed oocysts to the enclosures through the clothes, shoes, or equipment of the zookeeper, or though other animals such as birds and insects. This study also explains that animals in open outdoor enclosures had a significantly high risk factor for *Toxoplasma gondii* infection (de Camps et al., 2008).

Another study points to *Toxoplasma gondii* oocysts being dropped into the lemur exhibit from the feet of blackbirds that had also been near the feline exhibits or by unwashed fruit or vegetables (Spenser et al., 2004). The construction and type of exhibit contributed to this, as the mesh covering it was large enough to allow droppings from birds to fall through.

## MATERIALS AND METHODS

**Preparation**: An extensive literature search and thorough analysis was conducted over the topics of parasite and mammal interaction, *Toxoplasma gondii*, ring-tailed lemurs, *Toxoplasma gondii* in ring-tailed lemurs, and common ring-tailed lemur enclosure designs in the Midwestern United States.

A grant from the Josie and Geoff Fox Research Fund at Ball State University was obtained for the amount of $258.80 to pay for gas and supplies for this research. An AMS one-piece gator

probe was obtained from Dr. Jessi Haeft from the Ball State Department of Natural Resources. A 60 quart cooler and several gallon-sized re-closable bags were purchased with money from the grant. Travel authorization was approved from Ball State University before travel to a zoo in the Midwest to obtain soil samples.

**Sampling:** Upon arrival at the zoo at 8:30am on March 21, 2019, access to the ring-tailed lemur enclosure was granted and ten soil samples were taken at random points within the exhibit. Samples were obtained by inserting the gator probe into the soil at a 90 degree angle and pushing it down until it was approximately six inches into the soil. The probe was then slowly removed from the soil and the samples were each placed in a separate bag that was labeled with the location and the date. After collecting the ten samples inside the exhibit, the gator probe was cleaned off with Lysol brand disinfecting wipes, and then dried. Next, ten soil samples were taken in random locations within the grassy area surrounding the ring-tailed lemur exhibit in the same way. All samples were then placed in the 60 quart cooler along with ice to keep them cool and transported back to Dr. Bernot's lab at Ball State University to be refrigerated.

**Analysis Preparation:** 50ml glass test tubes, 50ml falcon tubes, gauze, and a mesh sieve were obtained. A 2% solution of $H_2SO_4$ was made by diluting 95% sulfuric acid with distilled water. A sucrose solution with a specific gravity of 1.15 was made by adding 113.4 grams of table sugar to 946.4ml of distilled water (Lelu et al., 2011).

**Treatment A:** 5 grams of soil from each sample from inside the exhibit was added to its own 50ml test tube. 2.5ml of $H_2SO_4$ (2%) was added to each and was left to stand overnight. 10ml of

deionized water was added to each tube and they were each then vortexed for 1 minute. Each solution was filtered through a fine mesh sieve and then centrifuged for 5 minutes. The supernatant was discarded and then 2.5ml of sucrose solution (s.g. 1.15) was added. It was centrifuged for an additional 5 minutes. The supernatant (1.5ml) was then discarded and 7.5ml of water was added. This was then vortexed for 1 minute and centrifuged one last time for 5 minutes. The result was preserved. Repeat for each sample from outside of the exhibit (Lelu et al., 2011).

**Treatment B**: 5 grams of soil from each sample from inside the exhibit was added to its own 50ml test tube. 2.5ml of $H_2SO_4$ (2%) was added to each and was left to stand overnight. 10ml of deionized water was added to each tube and they were each then vortexed for 1 minute. The sample was then separated into two falcon tubes. A cold (4° C) sucrose solution (s.g. 1.15) was then placed under the soil mixture and centrifuged for 20 minutes. The interface was then discarded. 17.5ml of water was added, then it was vortexed for 1 minute and centrifuged again for 20 minutes. The two sediments (1ml each) were mixed together in one 50ml test tube. 17.5ml of water was added, then it was again vortexed for 1 minute and centrifuged for 20 minutes. 1ml of the result was preserved. Repeat for each sample from outside of the exhibit (Lelu et al., 2011).

**Analysis:** A vial of *Toxoplasma gondii* oocysts was obtained from Dr. Bernot's lab at Ball State University. A slide was prepared with the oocysts and was looked at through a compound microscope for comparison purposes for analyzing the soil samples obtained from the zoo in the Midwest for oocysts.

Methods were adapted from Lelu et al. (2011).

## RESULTS

None of the ten soil samples from inside the exhibit yielded *Toxoplasma gondii* oocysts with either treatment A or treatment B. Similarly, none of the ten soil samples from outside the exhibit yielded *Toxoplasma gondii* oocysts with either treatment A or treatment B.

## DISCUSSION

In the present study, no *Toxoplasma gondii* oocysts were found in any of the soil samples taken using either treatment A or B from inside or outside of the exhibit. While these results are disappointing, it does not mean that there are no oocysts in any of the soil surrounding the exhibit. The methods used were adapted from Lelu et al (2011) but were not followed exactly due to lack of chemicals. Only 18% of oocysts were recovered from that study even with more extensive methods (Lelu et al., 2011). It is not easy, technically speaking, to determine environmental oocyst contamination because cat feces can be found anywhere from buried in soil to on street pavement. Not much is known about the survival rate of oocysts that are exposed to the natural elements, either (Dubey and Jones, 2008). New techniques to find oocyst prevalence in soil are needed in order for studies to be more successful in finding the source of *Toxoplasma gondii* oocysts.

Other studies have found the source of *Toxoplasma gondii* oocysts to have originated from other sources. For example, the thorough washing of produce can still not remove all *Toxoplasma gondii* oocysts (Spenser et al., 2004), so infection may stem from dirty produce fed to lemurs. Additionally, undercooked meat served to felids is a large risk to captive lemurs, as well. One study done in Cincinnati suggests that for optimal prevention of *Toxoplasma gondii* infection in captive ring-tailed lemurs, all meat should be cooked to 66° C for 30 minutes. This is extremely important before being fed to felines, because one cat is able to shed millions of oocysts in the span of one week, and those oocysts are able to live in soil for months (Dubey et al., 1985). This study also suggests distancing the enclosures of primates and cats so that accidental contamination is less likely to occur when zookeepers enter them (Dubey et al., 1985). A study from Alabama suggests that zoos with captive lemurs should use cautious hygiene methods and also not feed raw meat to felids without first freezing it to rid any oocysts that may have persisted (Spenser et al., 2004).

Because ring-tailed lemurs have been known to eat soil to supplement their diets in the wild, there is reason to believe that they may do so in captivity, as well (Ganzhorn, 1987). This natural behavior could increase the risk of contracting toxoplasmosis in exhibits where the ground consists of soil, such as at the zoo at which this study was conducted. Outdoor lemur enclosures typically have a mesh "roof" to keep the animals contained, but soil or seeds dropped from the feet of birds can easily pass through most mesh sizes that may be used in exhibit construction (Spenser et al. 2004). Similarly, cats may transport *Toxoplasma gondii* oocysts by defecating into an exhibit through the mesh by sitting on top of it (Juan-Salles et al., 2011). Stillbirths, placentitis, and disseminated fetal infection could be a future problem for the zoo at which this

study was conducted (Juan-Salles et al., 2011), but more research should be done on whether ring-tailed lemurs can survive toxoplasmosis infection.

Although no *Toxoplasma gondii* oocysts were found in the soil collected for this study, the ring-tailed lemurs at this particular zoo are still susceptible to toxoplasmosis, and since it has been seen before at this zoo, the oocysts are coming from somewhere. Research on more efficient and successful methods for *Toxoplasma gondii* oocyst extraction from soil should be done. The ring-tailed lemur exhibit construction at this zoo should be reevaluated and altered to decrease the risk of toxoplasmosis infection to the lemurs. Meat fed to felids at the zoo should either be thoroughly cooked or contained where wild birds are not able to transport it to other exhibits. Care should also be taken when zookeepers travel between exhibits to not transfer potentially-contaminated soil to areas where the ring-tailed lemurs could ingest it.

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