COMPARING DIAGNOSTIC TECHNIQUES FOR DETECTING INTESTINAL PARASITES AND PHYLOGENETIC ANALYSIS OF *BABESIA* IN CAPTIVE BABOONS

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INTRODUCTION

Parasites and relationships with their hosts make up a vast and ever-changing field of science. Parasites thrive because they have been able to adapt to different hosts, such as humans, animals, plants and even other parasites. Due to findings from archaeological artifacts and preserved bodies, the long history of parasitic infections of humans and other animals is becoming better known (Cox, 2002). To describe man's long association with parasites Roberts and Janovy (2000) write "Humans have suffered greatly through the centuries. Fleas and bacteria conspired to destroy a third of the European population in the 17th century, and malaria, schistosomiasis, and African sleeping sickness have sent untold millions to their graves." It is uncertain when the first written records of parasites infecting humans came about, but Greek physicians between 800 and 300 BC described various diseases that could have been caused by parasites. Later in AD 850-1037 Arabic physicians detailed information about diseases that obviously arose from parasitic infections (Cox, 2002).

Although there are hundreds of parasites whose definitive host is man (Cox, 2002), there are also numerous zoonotic parasites that can be transmitted between animals and humans. Zoonotic parasites are transmitted to humans when their infective stages are ingested, introduced through vectors such as ticks for *Babesia* spp., or when larval stages penetrate the skin of the host such as *Ancylostoma* spp. This can occur either directly by human-animal contact, contaminated fecal or soil contact, or eating meat contaminated with the infective stages. Water and food can also be a source of infection, and in countries with poor animal and human waste removal systems, contamination of water sources and food products with parasite stages is a big issue (Slifko et al, 2000).

Wild caught and captive olive baboons (*Papio cynocephalus anubis*) are natural reservoirs for many parasites (Myers and Kuntz, 1965). Since baboons are natural reservoirs of some parasites that infect humans, they can be used as models for pathogenesis and drug efficacy. Baboons are rising in importance in human biomedical research (Rogers and Hixson, 1997). Because of this, diagnosing and treating parasites in these animals is important to provide quality animals for research and to maintain healthy baboon populations in the breeding colony. The present study focused on common parasites found in these research animals: *Trichuris trichiura*, *Entamoeba histolytica, E. dispar, E. coli*, and *Babesia* sp.

Trichuris trichiura, commonly known as the whipworm, embeds it's long, thin anterior region into the mucosa of the large intestines or cecum and feeds off the host's tissue. The life cycle is direct with transmission occurring when embryonated eggs are ingested. Once swallowed, the embryonated eggs pass through the stomach and small

intestines, stimulating the larval stages to emerge via the polar plugs of the egg, where they go on to embed themselves into the lining of the large intestines (Bundy and Cooper, 1989). The prepatent period, or the time from infection to detection of eggs in feces, has yet to be definitively determined but is thought to be 60 days (Bundy and Cooper, 1989). Once mature, female whipworms can produce 3,000 to 20,000 eggs per day (Bundy and Cooper, 1989). Since adult *T. trichiura* live for several years (Roberts and Janovy, 2000), an individual baboon can acquire a large worm burden. Once eggs are passed in the feces, it takes on average 21 days for them to embryonate and become infective to the next host.

Trichuris trichiura infects both man and non-human primates (Munene et. al., 1998). Both wild and captive baboons have been found infected with *T. trichiura* (Myers and Kuntz, 1965; Kuntz and Myers, 1967; Flynn, 1973; Munene et al, 1998; Murray et al, 2000; Hahn et al, 2003). Infections with low numbers of *T. trichiura* are usually subclinical but larger worm burdens can cause dysentery, anemia, and rectal prolapse in humans (Roberts and Janovy, 2000). Growth retardation and finger clubbing are also seen, particularly in children, in association of intense worm burdens (Roberts and Janovy, 2000). Intussusception in baboons can occur with heavy worm burdens and if not detected can lead to death (Hennessy et al, 1994). A study from Japan in 1984 reported the transmission of *T. trichiura* from non-human primates (*Macaca fuscata*) to humans (Horii, 1985). Horii (1985) had four human subjects ingest 30-50 infective *T. trichiura* eggs recovered from Japanese monkeys. The subjects started passing eggs on average 127 of days after ingestion.

Diagnosis of *T. trichiura* depends on detecting eggs in the feces of an infected host. Most commonly used in diagnostic laboratories is fecal flotation (Zajac and Conboy, 2006). There are a few different anthelmintics that are used to treat *T. trichiura* infections. It has been shown that fenbendazole is more efficacious than milbemycin oxime for treating whipworm infections (Reichard et al, 2007) in baboons. In humans, mebendazole and albendazole are effective in clearing infections of *T. trichiura* (Roberts and Janovy, 2000). Harlan-Teklad (Madison, WI) formulated fenbendazole into their 20% protein commercial primate diet in order to provide a cost and labor effective method to treat non-human primates infected with intestinal parasites including *T. trichiura*. It was shown, by significant reduction in fecal egg counts, that the fenbendazole-formulated diet is effective for treating specific pathogen free baboons infected with *T. trichiura* (Reichard et al, 2008).

There are several *Entamoeba* spp. that infect wild and captive baboons, including *Entamoeba histolytica*, *E. dispar*, and *E. coli*. *Entamoeba* spp. have direct life cycles and hosts become infected when they ingest infective cysts in contaminated food or water (Schuster and Visvesvara, 2004). Trophozoites of *Entamoeba* spp. live and feed in the intestines. As fecal matter passes through the intestines it becomes dehydrated, thereby stimulating the amoeba to form cysts that are passed with host feces (Roberts and Janovy, 2000).

Although most infections are asymptomatic, *E. histolytica* can be pathogenic causing amoebic dysentery and intestinal ulcers (Cox, 2002). Trophozoites of *E. histolytica* can invade the intestinal wall and become extra-intestinal, producing flask-shaped lesions in the liver, lungs, and brain. *E. histolytica* is the third most common

cause of parasitic death in the world in humans (Roberts and Janovy, 2000). *Entamoeba dispar*, on the other hand, is a nonpathogenic species that is morphologically identical to *E. histolytica* (Diamond and Clark, 1993). *Entamoeba dispar* is thought to be the causative agent of many asymptomatic infections in humans (Schuster and Visvesvara, 2004). Along with *E. histolytica* and *E. dispar*, *E. coli* is a third species found in baboons. *Entamoeba coli* is a commensal and usually feeds on bacteria, other protozoa, yeast, and the occasional red blood cell (Roberts and Janovy, 2000). *Entamoeba coli* is often found coexisting with *E. histolytica*.

Identification of cysts in feces from an infected individual is necessary for diagnosis of *Entamoeba* spp. infection. Fecal flotation the most commonly used to examine for cysts. Cysts of *E. histolytica* and *E. dispar* range in size from 5-20 um and mature cysts have 4 nuclei. *Entamoeba coli* cysts are larger in size (10-33um) and contain 8 nuclei. Since *E. histolytica* and *E. dispar* cysts are morphologically identical under the microscope, additional techniques are needed to differentiate the two. A polymerase chain reaction (PCR) technique has been developed to distinguish infections of *E. histolytica* and *E. dispar* (Verweij et al, 2000). Another quick and reliable method to differentiate the two species is the enzyme-linked immunosorbent assay (ELISA) which detect amoebae antigens in the feces (Fotedar et al, 2007).

There are several techniques that can be used when diagnosing intestinal parasites (Zajac and Conboy, 2006). Fecal flotations with centrifugation coupled with different flotation media can be very useful when quickly looking for intestinal parasite eggs and/or cysts. A flotation medium with a higher specific gravity (SG) than that of the parasite stage will cause the eggs and or cysts to float to the surface where they are

collected on a cover slip for microscopic examination. With the higher SG of the flotation medium, the more parasite stages will be floated. However, with increasing SG the amount of fecal matter and debris that will float also increases (Zajac and Conboy, 2006). Fecal centrifugation flotations with sugar solution (SG=1.27) is effective when floating common parasite eggs but often distorts small protozoan cysts like *Giardia* and *Entamoeba* (Dryden et al, 2006). Zinc sulfate flotation medium (SG=1.18), because of its lower SG, is more useful when looking for smaller protozoan cysts but will not float many heavier parasite stages.

Centrifugal sedimentation is a concentration technique used to isolate parasite stages that wouldn't normally float in flotation solutions. The formalin ethyl-acetate method is used to concentrate eggs and cysts of parasites in feces into the bottom of a centrifuge tube where they can be easily collected and identified. Sedimentations are often easier to read than fecal flotations because the method removes a lot of fecal matter and debris (Zajac and Conboy, 2006). Trichrome staining of fixed fecal smears can be used to identify protozoan trophozoites or other stages that are too delicate to float (Zajac and Conboy, 2006). Even though trichrome staining can be useful, it is not a concentration technique and some parasite infections can be easily missed.

There is an enzyme-linked immunosorbent assay (ELISA), *E. histolytica* II (TechLab, Blacksburg, VA), that has been developed to rapidly detect infections of *E. histolytica* in humans and upon consultation with TechLab, non-human primates. This ELISA is useful when wanting to distinguish between *E. histolytica* and *E. dispar* (Fotedar et al, 2007).

The final parasite of captive baboons that was involved in the present study is *Babesia* sp. *Babesia* spp. are tick-borne apicomplexans that infect red blood cells of their vertebrate hosts (Bronsdon et al, 1999). A vertebrate host becomes infected with *Babesia* spp. when an infected tick releases sporozoites, the infective stage, while taking a blood meal. Asexual reproduction occurs within erythrocytes in the vertebrate host yielding piroplasms. Ticks become infected when they ingest piroplasms with their blood meal. Within infected ticks, gamogony produces gametes which then fuse to form a zygote and eventually sporozoites in the salivary glands (Roberts and Janovy, 2000). Different modes of transmission have been examined for *Babesia* spp. including blood-to-blood contact and transplacental transmission. Jefferies et al (2007) found that blood-to blood contact through dog fighting is another method of transmission of *B. gibsoni*. It has been shown that *B. gibsoni* can be passed from mother to offspring by transplacental transmission (Fukumoto, 2005), but all pups infected through this route died before reaching 60 days of age.

Babesia sp. was first documented in monkeys and baboons in 1905 (Ross, 1905). Originally, *Babesia* sp. in nonhuman primates was classified as *Entopolypoides macaci* (Bronsdon et al, 1999). Recent phylogenetic studies have suggested that *Entopolypoides* and *Babesia* sp. are synonymous (Bronsdon et al, 1999). *Babesia microti* was documented in a baboon that was from the University of Oklahoma (OU) baboon breeding colony (Ezzelarab et al, 2007) as a complication of a heart transplantation study.

Babesia microti is the species that infects humans in the northeastern and north central regions of the United States (Rodgers and Mather, 2007). In the wild, vertebrate hosts for *B. microti* include voles and other rodents (Roberts and Janovy, 2000). The tick

Ixodes scapularis transmits infection of *B. microti* among rodents as well as from rodents to humans (Roberts and Janovy, 2000). Preliminary studies suggested that the *Babesia* found in captive baboons in the U.S. is *B. microti* since it is the piroplasm found in humans in the U.S. and they were phylogenetically similar (Bronsdon et al, 1999)

Most healthy individuals infected with *Babesia* spp. do not have clinical signs (Bowman, 2003). However immunocompromised individuals infected with *Babesia* spp. can exhibit hemolytic anemia, anorexia, splenomegaly, lethargy, and weight loss (Bowman, 2003). Diagnosis of *Babesia* spp. can be made by observing piroplasms in red blood cells on stained blood films or by PCR (Bronsdon et al, 1999).

The purpose of the present study was four-fold. First, to compare zinc sulfate centrifugation flotation, sugar centrifugation flotation, trichrome staining, and formalin-ethyl acetate sedimentation for identifying intestinal parasites of captive baboons. Second, to determine the efficacy of ELISA for distinguishing between *E. histolytica* and *E. dispar*. Third, to determine how many baboons in the OU colony were infected with *Babesia* sp. Finally, to phylogenetically compare *Babesia* sp. 18s rDNA sequences from infected baboons to orthologous sequences published in GenBank.

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CHAPTER II

COMPARING DIAGNOSTIC TECHNIQUES FOR DETECTING INTESTINAL PARASITES OF CAPTIVE BABOONS

Introduction

Baboons are naturally infected with several types of parasites including *Entamoeba histolytica, E. coli, E. dispar* and *Trichuris trichiura* (Schuster et al, 2004; Reichard et al, 2008). *Entamoeba histolytica* is a protozoan parasite of the colon and cecum that can cause amebic dysentery and in severe cases liver, lung, and brain abscesses in humans (Roberts and Janovy, 2000). It has been well documented that baboons are naturally infected with *E. histolytica* causing different levels of tissue damage and sickness in the animals (Munene et al, 1998). Cysts are the transmissible stage and a host becomes infected through ingestion of cysts. *Entamoeba histolytica* causes of 34-50 million cases of human amoebiasis worldwide each year and leads to 40-100 thousand deaths annually (DiMiceli, 2004). In 1993, *E. dispar* was described as genetically distinct yet morphologically indistinguishable from *E. histolytica* (Rivera, 1998). *Entamoeba dispar*, unlike *E. histolytica*, is noninvasive and thought to be the basis for large numbers of asymptomatic infections in humans (Schuster et al, 2004).

Entamoeba coli is another amebae found in baboons and often coexists with *E. histolytica* and *E. dispar* infections.

Entamoeba coli cysts are distinguished from *E. histolytica* and *E. dispar* cysts microscopically by their larger size (10-35um to 10-20 um) and having eight nuclei compared to four nuclei of the smaller amoeba. *Entamoeba coli* is commensal and feeds on bacteria, other protozoa, and the occasional blood cell. Infection of *E. coli* is not considered pathogenic to baboons.

Trichuris trichiura, also known as the whipworm due to its bullwhip appearance, is a parasitic nematode that attaches itself to the epithelium of the cecum and large intestines and feeds off the lining. *Trichuris trichiura* is zoonotic and can be passed between humans and baboons (Horii et al, 1985). In baboons, *T. trichiura* has been known to cause severe diarrhea, lethargy, loss of appetite, and weight loss, as well as intussusception of the small intestines (Hennesy et al, 1993).

Accurate diagnosis, proper treatment, and effective control strategies for these intestinal parasites within captive baboon populations are important to provide quality animals for biomedical research as well as to maintain healthy baboon colonies. For captive baboons, fecal examination techniques can be employed to detect infection of intestinal parasites. Fecal flotation using centrifugation coupled with different flotation solutions is useful to quickly and economically detect parasite eggs and/or cysts in fecal samples. Sedimentation, which removes much of the fecal matter and debris, can be used to identify smaller parasite stages as well as heavier parasites stages that won't float readily. Trichrome staining of fixed fecal smears can also be used to detect parasites stages, yet the small amount of fecal sample used makes this an unreliable diagnostic method. Stained fecal smears allow visualization of the trophozoite stages of *Entamoeba* spp., where as other diagnostic methods don't. The purpose of the present study was to

compare zinc sulfate centrifugation flotation, sugar centrifugation flotation, trichrome staining, and formalin-ethyl acetate sedimentation for identifying intestinal parasites of captive baboons. As well as to determine the efficacy an enzyme-linked immunosorbent assay (ELISA) to detect *E. histolytica* and distinguish it from *E. dispar*.

Materials and Methods

Experimental design. Adult olive baboons (*Papio cynocephalus anubis*) naturally infected with intestinal parasites were used in the present study. Zinc sulfate and sugar flotations, formalin ethyl-acetate sedimentations, ELISA, and trichrome stain, were performed on each sample. The fecal flotation as well as the sedimentation techniques were measured quantitatively by counting numbers of *T. trichiura* eggs as well as cysts of *E. histolytica*, *E. dispar*, and *E. coli* per gram of feces, and compared to one another. The number of positives according to the *E. histolytica* ELISA test kit was determined by a positive color change when compared to negative and positive controls. Trichrome stained fecal smears were examined under oil immersion covering the entire area under the slide cover slip, any eggs or cysts were recorded.

Animal housing and husbandry. Baboons were housed in accordance to the guidelines from the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). Baboons in the University of Oklahoma (OU) breeding colony were housed in outdoor/indoor corrals. Baboons were fed Harlan primate diet 2055 as well as fresh fruit, vegetables, trail-mix, and dry cereal (Reichard et al, 2007). Animals used in this study were housed separately at OU Health Sciences Center annex but originated from the breeding colony. Cages used in the annex are designed so feces and urine pass through the bottom. Rooms housing the animals were hosed down every day.

Specimens. Fecal samples were collected from individual adult olive baboons housed at the OU Health Sciences Center Annex. Samples were uniquely identified and transported to Oklahoma State University for testing. All fecal samples were stored in individual containers in a refrigerator (4 °C) and processed within 2-3 days of being collected.

Centrifugation Flotation. Centrifugal fecal flotation procedures as described by Zajac and Conboy (2006) were followed. Briefly, five grams of feces were weighed and put into a small (100 ml) beaker, 22 ml of water was added, and the two were mixed together and strained through a tea strainer into a clean beaker. The material in the strainer was mashed until nearly dry. Water (8 ml) was then added to the original beaker to remove any material clinging to the sides. This excess fecal material plus the 8 ml of water was then strained into the beaker containing the 22 ml and material in the strainer again mashed until nearly dry. The 30 ml mixture was then stirred and immediately poured into two 15 ml centrifuge tubes. The mixture was centrifuged at 550 x g for 10 min. The supernatant was decanted and each tube filled with 7.5 ml of either Sheather's sugar solution (SG 1.27) or zinc sulfate solution (SG 1.18-1.2). The sediment and flotation solution was mixed with an applicator stick and the tubes filled the rest of the way with the respective flotation solutions until a meniscus was formed. A cover slip was added to each tube and centrifuged again at 550 x g for 10 min. Both cover slips for each sample were placed onto a labeled microscope slide with a drop of iodine. Slides were observed under the microscope at 100X magnification. To determine the number of eggs (Figure 1) or cysts (Figure 2) per gram of feces (EPG/CPG) the amount of eggs or cysts found under coverslip 1 was added to the amount of eggs/cysts observed under coverslip 2 and

then divided by the total number of grams of feces used [EPG/CPG = (coverslip 1 + coverslip 2) / grams of feces used].

Formalin-ethyl acetate sedimentation. The centrifugal formalin-ethyl acetate technique described by Zajac and Conboy (2006) was used. Briefly**,** five grams of feces were weighed and mixed with 10 ml of 10% formalin in a beaker and the mixture was strained through a tea strainer into another beaker. The solution was then put into a 15 ml centrifuge tube and spun at 600 x g for 2 min. The supernatant was discarded and the sediment resuspended in another 10 ml of formalin, and the centrifugation was repeated for 2 min. This procedure was repeated until the supernatant was clear. Once clear, the sediment was again resuspended in 10 ml of formalin, 3 ml of ethyl acetate was then added, the tube capped, and shaken vigorously for about 10 sec. The formalin ethyl acetate sample mixture was centrifuged one last time for 1 min at 600 x g, the supernatant was decanted, the sediment transferred to a microscope slide with a drop of lugol's iodine, and covered with a cover slip. All of the sediment was observed for parasite stages. Slides were observed under the microscope at 100X magnification and the EPG/CPG for each sample was determined.

Trichrome staining. A small amount of feces (approximately 0.01 grams) from each individual animal was fixed in polyvinyl alcohol (PVA) and smeared on slides thin enough to be able to read newsprint through. PVA-fixed preparations were allowed to dry overnight at room temperature and then stained using the Wheatly's Modification of Gomori Trichrome stain as described by Pritchard (1982). Briefly, fixed slides were placed in 70% ethanol for 5 min and then transferred to 70% ethanol plus iodine for 5-10 min. Next the slides were placed in 70% ethanol for 5 min and then again for 3 min.

After that the slides were placed in trichrome stain for 10 min then dipped in 90% ethanol plus acetic acid for 3 seconds. Slides were rinsed by dipping several times in 100% ethanol and then placed in 2 different washes of 100% ethanol for 3 min each. Next the stained slides were put into xylene for 10 min and then put into another change of xylene for 10 more min. Once dry, a cover slip was fixed on the slide using cytoseal (Electron Microscopy Sciences, Fort Washington, PA), and were allowed to dry overnight. Slides were examined at 100X magnification.

Enzyme-Linked Immunosorbent Assay (ELISA). The procedure for *Entamoeba histolytica* II Kit (Techlab, Blacksburg, VA) was followed according to manufacturer's instructions using 0.2 gram fresh fecal samples. Tests were conducted within 12 hours of sample collection. A dilution tube (2.5 ml microcentrifuge tube) was set up for each sample, into it was put the sample and 400 ul of diluent. To homogenize the fecal samples to ensure adequate sampling, tubes were vortexed thoroughly for 1 minute or until the fecal material had formed a homogenous mixture. Two control test wells were used, one for a positive control and one for a negative control, and two wells for each sample was allotted. One drop of conjugate was added to all wells and then one drop of positive control reagent was added to the positive control well and 100 ul of diluent was added to the negative control well. 200 ul of each diluted specimen was transferred to their respective wells. The 96 well-plate was then covered with parafilm and allowed to incubate at room temperature for 2 hrs. After incubation, the contents of the wells were shaken out into a discard pan and all wells were washed with the wash solution, directing the stream to the bottom of each well. The wells were shaken out and blotted on a paper towel to remove excess wash solution. This wash process was repeated a total of 4 times.

After washing, the wells were dried by again shaking out all liquid and inverted and blotted on paper towels, 2 drops of substrate were added to each well, the wells tapped, and then let sit for 10 min at room temperature with a light tap half way through. Finally, 1 drop of stop solution was added to all wells and wells were tapped gently to mix contents. After 2 min results were read with a yellow color change showing a positive sample.

Statistical analysis. An ANOVA was used to determine differences in fecal egg counts among the diagnostic techniques (Sokal and Rohlf, 1997). A chi-square test was used to determine difference among techniques used for determining prevalence of parasite infections among baboons tested. Analyses were performed using SigmaStat 3.1 statistical software package (Systat Software, Point Richmond, CA). Repeat samples were counted only once when determining prevalence, but when comparing EPG, each repeat sample was counted individually.

Results

Overall, infected individual baboons showed high variation of the numbers of eggs of *T. trichiura* and/or cysts of *Entamoeba* spp. (Table 1). Samples were collected from 43 individual baboons, and when available, repeat samples were taken from some individual baboons. Nine of the 43 baboons tested had repeat samples collected from them one month after the initial samples were collected, and one individual baboon had repeat samples taken 1month and 2 months after the first collection.

Zinc sulfate centrifugation readily floated cysts of *Entamoeba* spp (Figure 2). For samples that were positive for *E. histolytica/E. dispar*, zinc sulfate floated higher ($H = 28.6$, 3 df, $P \leq 0.001$) numbers of cysts per gram of feces (61.2 ± 24.2) than Sheather's

sugar centrifugation flotations (0.2 $\pm$ 0.02) and sedimentation procedures (8.9 $\pm$ 3.6). The prevalence of *E. histolytica/E. dispar* infection (53.5%, 20 of 43) was higher ($X^2 = 30.0$, 2 df, $P < 0.001$) when zinc sulfate was used than other techniques. The prevalence of *E. coli* infection (72.1%, 31 of 43) and the number of cysts (136.5 $\pm$ 38.01) were higher ($X^2 = 27.8$, 2 df, $P < 0.001$; $H = 33.7$, 3 df, $P \leq 0.001$) when zinc sulfate was used compared to the other techniques.

Conversely, Sheather's sugar centrifugation floated more *T. trichiura* eggs (Figure 1) per gram of feces (158.3 $\pm$ 43.0) than the other methods ($H = 25.9$, 4 df, $P \leq 0.001$). Statistically, there was no difference ($X^2 = 3.2$, 2 df, $P \geq 0.30$) among the zinc sulfate (67.4%, 29 of 43), Sheather's sugar solution (67.4%, 29 of 43), and sedimentation (51.2%, 22 of 43) techniques when comparing the prevalence of *T. trichiura* infection within the captive baboons.

Even though the stages were easier to recognize because most of the fecal matter and debris was removed, sedimentation recovered fewer eggs and cysts than compared to the flotation techniques. Trichrome staining of fixed fecal smears was not a concentration technique, and only one cyst of *E. histolytica*/*E. dispar* and 2 eggs of *T. trichiura* were found out off all slides examined. According to the *E. histolytica* ELISA, the prevalence of *E. histolytica* infection was 6.9% (3 of 43) (Table 2).

Discussions

The most commonly used diagnostic technique for detecting infections of intestinal parasites in animals are fecal flotation and occasionally sedimentations (Zajac and Conboy, 2006). Zinc sulfate with centrifugation flotation detected more cysts and gave a higher prevalence of *Entamoeba* spp. infections. However, sugar with

centrifugation flotation detected more eggs of *T. trichiura*. Zinc sulfate and Sheather's sugar flotation were equally effective for determining the prevalence of *T. trichiura* infections within the captive baboon population.

Flotation solutions with higher SG will float more of a variety of parasite stages, yet, as the SG increases the amount of debris that floats will also increase and some parasite stages will lyse (Zajac and Conboy, 2006). Because of this, when looking for the less dense *Entamoeba* spp. cysts the lower SG of zinc sulfate solution is preferred. Zinc sulfate (SG 1.18-1.2) readily floated large numbers of small *Entamoeba* spp. cysts as compared to Sheather's sugar solution (SG 1.27). Previous research has shown that sugar solutions will distort cysts of *Giardia* spp. and other protozoal cysts compared to zinc sulfate (Broussard, 2003). Even though zinc sulfate with centrifugation detected more cysts of *Entamoeba* spp, the high salinity can be harsh on helminth eggs and about one quarter of the *T. trichiura* eggs observed were distorted. Helminth eggs, such as *T. trichiura*, are dense and need a solution with a higher SG than most salt flotation solutions. Sheather's sugar solution allows for heavier parasite stages to float, but because of its viscous nature, more sensitive results are gained when using a centrifuge (Dryden et al, 2005).

An ELISA, originally developed to rapidly detect *E. histolytica* infections in humans (TechLab, Blacksburg, VA), was tested to determine its efficacy for identifying *E. histolytica* infections in baboons. Because the ELISA used in the present study showed such a large difference in prevalence of *E. histolytica* than compared to the flotation procedures (53.5% from zinc sulfate flotations and 6.9% by ELISA) (Table 2), we believe that most cysts were actually *E. dispar*. Another method that has been used to

differentiate between *E. histolytica* and *E. dispar* cysts is the DNA- based diagnostic technique PCR. Using cysts recovered from stool samples, genomic DNA can be amplified by PCR and a distinction made between *E. histolytica* and *E. dispar* (Rivera et al, 1996). Due to time constraints in the present study, we did not perform PCR to differentiate between *E. histolytica* and *E. dispar*.

Results from this present study coincide with what has been reported for similar parasites in other hosts. A study of diagnostic techniques, including direct smears, zinc sulfate, and sugar table top and centrifugation flotation (Dryden et al, 2005) showed that Sheather's sugar solution with centrifugation was able to float a larger number of *T. vulpis* eggs as compared to zinc sulfate solutions. Dryden et al (2005) also showed that different SG and flotation solutions produced varying results for different parasites. They reported that two different zinc sulfate solutions with slightly different SG (1.1 and 1.2) could give different results. The zinc sulfate solution with the lower SG readily floated eggs of *Ancylostoma caninum*, which are less dense than other parasite eggs (*Toxocara canis* and *T. vulpis*) the study focused on, yet the solution with the higher SG readily floated all in question.

Baboons are increasing in importance in biomedical research because of their physiological similarities to humans (Rogers and Hixson, 1997), and because of this there is a need to quickly and accurately detect parasites that could confound results of future research. In order to quickly and most effectively detect intestinal parasites in these animals it is important to know which diagnostic test to use to detect certain parasites. While there are different flotation solutions that best float/detect different parasites, to be quick and timely, the investigator most likely would want to run just one fecal test per

sample. In the present study, zinc sulfate centrifugation flotations would be most effective when determining the prevalence of *Entamoeba* spp. within a population of captive baboons, but both sugar and zinc sulfate were equally effective when looking for the prevalence of *T. trichiura*. Yet when one wants to quantitate a certain parasite in a fecal sample, the best results would be using zinc sulfate for *Entamoeba* spp, and Sheather's sugar solution for *T. trichiura* eggs. Not only is it important to be able to quickly and efficiently detect intestinal parasites in laboratory animals to provide quality research animals, but also to reduce the risk of zoonosis to animal handlers and investigators.

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Table 1. Comparison of diagnostic techniques showing egg/cyst per gram of feces and prevalence of *Entamoeba histolytica/E. dispar, E. coli,* and *Trichuris trichiura* infections within baboons.

	Sample size	Zinc sulfate Centrifugation		Sheather's Sugar Centrifugation		Sedimentation	
		EPG /CPG[a]	Prevalence	EPG/CPG[a]	Prevalence	EPG /CPG[a]	Prevalence
E.histolytica/E. dispar	43	61.2 ± 24.2	53.5%	0.2 ± 0.02	2.3%	8.9 ± 3.6	48.8%
E. coli	43	136.5 ± 38.0	72.1%	1.1 ± 0.7	16.3%	29.2 ± 7.8	53.5%
T. trichiura	43	47.8 ± 15.7	67.4%	158.3 ± 43.0	67.4%	2.1 ± 0.6	51.2%

a. Average egg/cyst per gram of feces ± standard error.

Table 2. Zinc sulfate centrifugation vs. ELISA for detection of *E. histolytica*

	Number positive	**Number negative**	**Prevalence**
Zinc sulfate	20	23	53.5%
ELISA	3	40	6.9%

Figure 1. *Trichuris trichiura* eggs with bipolar plugs recovered using sugar flotation with centrifugation, stained with iodine. 400X magnification.

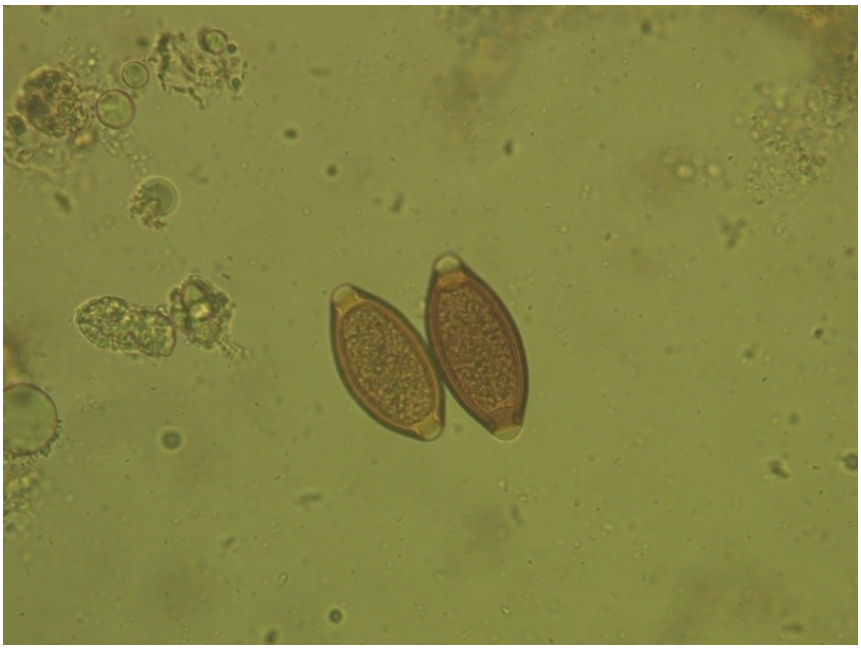
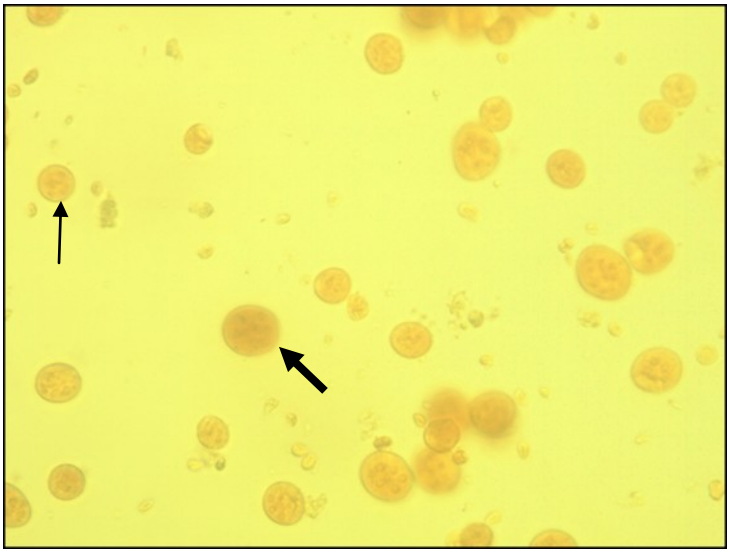


Figure 2. *Entamoeba* spp. cysts recovered with zinc sulfate flotation with centrifugation and stained with iodine. 100X magnification. Thin arrow points to a cyst of *E. histolytica/E. dispar* and the thick arrow points to a cyst of *E. coli*.



CHAPTER III

PREVALENCE OF INFECTION AND PHYLOGENETIC ANALYSIS OF *BABESIA* IN CAPTIVE BABOONS

Introduction

Babesia spp. are tick-borne apicomplexans of mammalian red blood cells. Babesiosis can range from subclinical to hemolytic anemia, persistent fever, and lethargy in vertebrate hosts. In baboons, clinical infections of *Babesia* spp. are most often seen as complications in immunocompromised individuals (Bronsdon et al, 1999). In a xenotransplantation study (Ezzelarab et al, 2007), a baboon (*Papio cynocephalus anubis*) obtained from the breeding colony at the University of Oklahoma (OU) received a pig heart. After a course of immunosuppressive therapy and approximately 5 weeks after the transplant, the baboon became lethargic, developed a high fever (102.9°), white blood cell counts reached 39,000/$mm^{3,}$ and became anemic with hematocrit levels dropping down to around 20%. Based on the morphology of the piroplasm and preliminary DNA sequencing, the baboon was diagnosed as being infected with *Babesia microti* (Ezzelarab et al, 2007).

Bronsdon et al (1999) surveyed a total of 65 baboons that had either originated from Africa or were born and raised in two different breeding colonies (Regional Primate Research Center at University of Washington, Seattle, Washington and Southwest Foundation for Biomedical Research, San Antonio, Texas) in the United States. They deduced through sequencing of a 500 bp 5' portion of the nuclear single strand rDNA and phylogenetic analysis that a *Babesia* sp. found in one of their baboons was most similar to *B. microti* (97.9% sequence similarity). A prevalence of 31% was found among all 65 baboons tested by Bronsdon et al (1999).

Babesia microti is most prevalent in the northeastern and north central regions of the U.S. (Vannier et al, 2008). There have not been any documented cases of babesiosis in Oklahoma. *Babesia microti* infects rodents and is transmitted to humans by *Ixodes scapularis* ticks and is often associated with Lyme disease since *I. scapularis* transmits both pathogens. In the past few years, it has been documented that *B. microti* is an increasing problem in humans as a result of increased contact with ticks and reservoir hosts (Rodgers and Mather, 2007).

Baboons have an immune system similar to that of humans. This is useful when using the baboon as organ transplant models because tolerance and the immune response to the transplanted organ can be documented and new therapeutic drugs can be tested (Haustein et al, 2008). Baboons are also physiologically similar to humans, such as older baboons exhibit a natural menopause. They also exhibit the same physiological characteristics that are critical to common diseases in humans; it is these physiological similarities to humans that make the baboon model valuable to biomedical research (Rogers and Hixson, 1997). Because of the baboon's rising importance and use in

biomedical research, it is important to be able to supply researchers with pathogen free animals. The purpose of the current study was to determine how many baboons in the OU colony were infected with *Babesia* sp. and to phylogenetically compare the 18s rDNA sequences from infected baboons to orthologous sequences published in GenBank.

Materials and Methods

Experimental design. Captive adult and juvenile olive baboons were used in the present study. Polymerase chain reaction (PCR) using primers specific for the 18s rRNA gene of *Babesia* spp. was used to determine the prevalence of *Babesia* spp. infection within the population of baboons in the breeding colony and among SPF baboons. 18s rDNA PCR product from baboons were sequenced and compared to orthologous sequences of *Babesia* spp. published in GenBank.

Animal housing and husbandry. Baboons were housed in accordance to the guidelines from the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Baboons in the OU breeding colony are housed in corrals, with approximately 100 animals per corral. Each corral has an open air outdoor pen as well as an indoor area. Baboons are fed Harlan primate diet 2055 as well as fresh fruit, vegetables, trail-mix, and dry cereal (Reichard et al, 2007). Animals housed at OU Health Sciences Center annex originated from the breeding colony and are housed separately in cages. Cages are designed so feces and urine can pass through the bottom. Rooms where the animals are housed are hosed down every day. Specific pathogen free (SPF) baboons were all born in the OU breeding colony and brought to the OU annex within the first day of being born where they are kept isolated from other non SPF baboons. SPF baboons are housed in

individual cages until 3 months of age when they are moved to gang housing with other SPF infants.

Specimens. Blood samples were collected from individual adult and juvenile olive baboons housed at the OU Health Sciences Center breeding colony and SPF baboons housed at the OU Health Sciences Annex. Baboons were anesthetized using Ketamine (Fort Dodge, Fort Dodge, IA) intramuscular injection (5-7.5 mg/kg) and blood was drawn from either the femoral region or the forearm. Blood was collected every 6 months when baboons received a health check and tuberculosis test (fall and spring blood draws) from spring 2007 through spring 2008. Blood was collected once from the SPF baboons during summer 2008. Samples were transported back to Oklahoma State University, processed within 1-2 days, and stored at -20 °C.

DNA Extractions. After thin blood smears were made from each blood sample, DNA was extracted from the blood samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Briefly, 20 µl of proteinase K was added to 200 ul of anticoagulated blood, 200 ul of Buffer AL added to each sample, the tubes were mixed thoroughly by vortexing, and incubated at 56°C for 10 min. After incubation, 200 ul of 100% ethanol was added and samples were vortexed. This mixture was pipetted into DNeasy mini spin columns placed in 2 ml collection tubes, centrifuged at 6000 x g for 1 min, and the collection tubes were discarded. Each mini spin column was then placed in a new 2 ml collection tube, 500 ul of buffer AW1 was added, and centrifuged again at 6000 x g for 1 min. The mini spin column was again placed in a new collection tube, 500 ul of buffer AW2 added, and centrifuged for 3 min at 20,000 x g. The mini spin columns were then placed in clean 1.5 ml microcentrifuge

tubes and 200 ul of PCR grade water warmed to 56°C was added directly to the membrane. The samples were incubated at 56°C for 1 min and centrifuged at 6000 x g for 1 min to elute DNA. The elution step was repeated with another 200 ul of warm PCR grade water for maximum DNA yield**.** DNA was then stored at -20 °C until analyzed via PCR.

Polymerase Chain Reaction. An approximate 1700 bp product of *Babesia* 18s rDNA region was amplified by PCR using primers BabAF and BabAR (Table 1). Amplifications were preformed in 25 ul volumes containing 0.25 ul of U Taq polymerase (Promega, Madison, Wisconsin), 2.4 ul 10X Taq buffer (Promega), 1.5 ul of 25 mM $MgCl_2$, 2 ul of 10 mM deoxynucleoside triphosphate mixture (Promega), 0.5 ul of 40 uM of each primer, and 5 ul of template DNA. For the primary reaction, there was an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation for 1 min at 94°C, 1 min for annealing at 56.6°C, and extension for 2 min at 72°C. To make sure all reactions had gone to completion, a final extension cycle was run at 72°C for 5 min. A nested PCR reaction was run with 1ul of the primary PCR product (all other concentrations same as used in the primary reaction) and a 460-520 bp fragment was amplified using primers RLBF and RLBR (Table 1). The nested protocol consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension 72°C for 2 min, followed by the final extension cycle of 72°C for 5 min. Primers used were previously described by Medlin et al. (1988) and Gubbels et al. (1999). PCR was carried out in an Eppendorff thermocycler (Eppendorf, Westburg, NY). Amplified products (10ul) were separated on 1.5% agarose gels stained with ethidium bromide and observed under ultraviolet light.

Purification and Sequencing. Nested PCR products were purified using the Wizard SV Gel and PCR Clean Up System (Promega, Madison, Wisconsin) and sequenced at the Oklahoma State University Recombinant/DNA Protein Research Facility (Stillwater, Oklahoma) using a 373 automated DNA sequencer (Applied Biosystems, Foster City, California). Three sets of forward and reverse sequencing primers (Table 1) were used to get overlapping sequences on both strands of the 18s rDNA.

Phylogenetics analysis. Sequences were aligned using ClustalX (Thompson et al, 1997). For visual inspection and to determine hypervariable regions of the multiple sequence alignment that potentially violated the assumption of positional homology, aligned sequences were imported into MacClade (Maddison and Maddison, 2000). To evaluate phylogenetic affinities of *Babesia* sp. obtained from baboons to other known sequences of *Babesia* spp. and orthologous sequences of related genera (Table 2), we performed maximum likelihood and Bayesian phylogenetic analyses using PAUP (Swafford, 2000). With PAUP, maximum parsimony, bootstrap, maximum likelihood, and maximum distance tests were run on the sample *Babesia* sp. sequences.

Statistics. Chi Square tests were performed to determine differences in the prevalence of *Babesia* sp. infection among age groups ($\leq$ 4 years of age, 5-10 years of age, 11-20 years of age, and $\geq$ 21 years of age), sex, and housing corrals (Sokal and Rohlf, 1997). Analyses were performed using SigmaStat 3.1 statistical software package (Systat Software, Point Richmond, CA).

Results

Prevalence of *Babesia* infection. Overall, the prevalence of infection with *Babesia* sp. (Figure 1) within the baboon breeding colony was 8.8% (73 of 830). Whereas the prevalence of *Babesia* sp. infection in SPF baboons was 0.0% (0 of 26).

Spring 2007, the first sample period, showed the highest prevalence (12.6%, 34 of 269) of *Babesia* sp. infection among the breeding population (Table 3), compared to fall 2007 (8.2%, 23 of 281) and spring 2008 (5.7%, 16 of 280) ($X^2 = 8.31$, 2 df, $P = 0.01$). There was no difference in the prevalence of *Babesia* sp. infection between males and females during any of the collection periods (Table 3). However, there was a significant difference ($X^2 = 23.21$, 4 df, $P < 0.0001$) among age groups for all three test periods (Table 4). Adult baboons 11 years to 20 years showed the highest prevalence of infection with *Babesia* (Table 4). When broken down into housing areas, the northeast corral had the highest prevalence of the 4 different corrals ($X^2 = 17.95$, 3 df, $P < 0.001$) in the spring 2007, yet in fall 2007 the northwest corral had the higher prevalence ($X^2 = 14.2$, 3 df, $P = 0.01$); in spring 2008 there was no significant difference among the four housing corrals. Sexually immature baboons ($\leq$ 4 years old) were not infected.

Repeat sampling. Over the one year time in which the present study took place, samples were collected for each animal 3 times. There were 7 individual baboons that remained positive for *Babesia* sp. over the course of the study. Eight baboons that tested positive in the spring of 2007 tested negative in fall 2007 and spring 2008. One sample was positive for *Babesia* sp. during the spring 2007 sampling, but that same sample tested negative six months later in the fall of 2007. Six months later in spring 2008, the sample again tested positive. When comparing the prevalence of *Babesia* sp. infection during the

3 sampling periods (Table 3), it appears that the prevalence of infection was decreasing. However 3 baboons were negative for *Babesia* sp. infection during the first test sampling, but were positive during fall 2007 of spring 2008.

Phylogenetic analysis. Most of the 18 rDNA was sequenced from 2 independent DNA extractions from baboons numbered 37-6 and 1201. Sequences blasted most similar to *B. leo* (97-99%) as compared to 92-96% for *B. microti*. Fourteen related sequences (Table 2) were phylogenetically compared to the sequences obtained from baboons 37-6 and 1201. Neighbor-joining and branch-and-bound parsimony tests suggest that the *Babesia* sp. of the captive baboons is a novel species. Both individual sequences of *Babesia* sp. from colony baboons mapped out into a sister group most closely related to *Babesia leo*. Bootstrap analysis (Fig. 2) supports the conclusion that *Babesia* sp. in captive baboons is an as yet an undescribed species of *Babesia* most closely related to *B. leo* rather than to *B. microti.* Genetic divergence values were determined to be 2% divergent from *B. microti* and only 0.5% divergent from *B. leo*. Sequences of the novel *Babesia* sp. from baboons 37-6 and 1201 were submitted to GenBank and given accession numbers of FJ897741 and GQ225744, respectively.

Discussions

Previous research reported that *Babesia* sp. found in baboons was *B. microti* because that is the species that infects humans in the United States (Bronsdon et al, 1999). In 1999 *Babesia* sp. was found in 2 baboons that had undergone an experimental stem cell transplant (Bronsdon et al, 1999). This led to the phylogenetic analysis of the *Babesia* found in the baboons and it was reported that their *Babesia* was most closely related to *B. microti*. *Babesia* sp. from baboons in the present study was shown to be a

novel species most closely related to *B. leo* found in african lions (Penzhorn et al, 2001). Prevalence of *Babesia* sp. within the baboon breeding colony averaged 8.8% with no significant difference between male and female baboons. There was a difference in prevalence of *Babesia* sp. infection among age groups, with baboons 11-20 years old being most likely to be infected with *Babesia* sp.

Babesia spp. are widespread parasites found throughout the world and are usually transmitted to vertebrate hosts by ixodid ticks (Bronsdon et al, 1999). The environmental conditions (sandy ground, no live foliage) in which the baboons live at the breeding colony, as well as the social grooming behavior among baboons, are not conducive to maintaining tick populations. It has been suggested that some *Babesia* spp. can be transmitted without an ixodid vector by blood-to-blood contact through fighting among individuals (Jefferies et al, 2007). In the breeding colony, there are constant fights to determine and maintain dominance. We speculate that *Babesia* sp. can be transmitted within the colony through the infected blood during a fight.

While the phylogenetics of piroplasmids have been studied in depth (Criado-Fornelio et al, 2004), little is known regarding *Babesia* sp. found in captive or wild baboons. Previous reports of *Babesia* spp. of captive baboons indicated that the piroplasms were most closely related to *B. microti* (Ezzelarab et al, 2007; Bronsdon et al, 1999). One study performed by Bronsdon et al. (1999) suggested *Babesia* sp. found in baboons was most closely related to *B. microti*, by evidence of 97.9% sequence similarity, and neighbor-joining analysis. The present study reports a novel species of *Babesia* found in colony reared baboons. The 18s rDNA *Babesia* sequences we obtained from 2 captive baboons were blasted into GenBank, and had the highest sequence

similarity to *B. leo,* not *B. microti*. Within the baboon breeding colony there are wild caught baboons that were imported from Africa. We speculate that some of these wild caught baboons were infected with a novel *Babesia* sp. when they were captured. Baboon number 37-6 that yielded one *Babesia* sp. sequence, was a wild caught 14 year old female baboon from Africa, and baboon number 1201 was a 7 year old male that was born in the OU breeding colony. Since baboons are fastidious groomers and due to the fact that they are housed under conditions that do not support natural populations of ticks, we speculate that *Babesia* sp. is being maintained in colony baboons through the transfer of contaminated blood during a fight.

Throughout the 3 sampling periods over 1 year (every 6 months in spring and fall) most of the baboons that were infected maintained their infection and were still infected a year later. There were 8 baboons that initially tested positive which tested negative on the next 2 samplings. It is possible that the baboons cleared their infection, or they were false positives which could be due to contamination during sample collection, DNA extraction, or PCR procedures. There were 3 baboons that tested positive for *Babesia* sp. in the second and third sampling periods and were negative for *Babesia* sp during the first sampling. All the SPF baboons tested negative for infection with *Babesia* sp. Information obtained from testing SPF baboons for *Babesia* sp. infection could be important when modes of transmission are looked at since it has been shown in dogs that vertical transmission can occur (Fukumoto et al, 2005).

The prevalence of *Babesia* sp. within the breeding colony at the OU facility was relatively low, averaging about 8% with baboons aged 11-20 years being the most likely to be infected. We report a novel species of *Babesia* that is most closely related to a *B.*

leo from african lions. Baboons are increasing in importance in biomedical research because of their physiological similarities to humans (Rogers and Hixson, 1997), because of this, there is a desire to recognize any potential confounding variables for future studies. The overall health of the baboons is also important in order to maintain a healthy breeding population.

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Table 1. Oligonucleotides used to amplify and sequence the 18s rRNA gene of *Babesia* sp. from baboons.

Primer	Primer Sequence (5'->3')	Reference
BABAF	CCGAATTCGTCGACAACCTGGTTGATCCTGCCAGT	Medlin et al., 1988
BABAR	CCCGGATCCAAGCTTGATCCTTCTGCAGGTTCACCTAC	Medlin et al., 1988
RLB-F	GAGGTAGTGACAAGAAATAACAATA	Gubbels et al., 1999
RLB-R	TCTTCGATCCCCTAACTTTC	Gubbels et al., 1999
BSP1F	TGGCTTATTCGGATTCGTCGCTCT	Present study
BSP1R	CGCGCAAATTACCCAATCCAGACA	Present study
BSP2F	ATGGCCGTTCTTAGTTGGTGGAGT	Present study
BSP2R	CATCCTTGGCAAATGCTTTCGCAG	Present study
BSP3F	AAGCGCTGTGAACCCTATCACTCT	Present study
BSP3R	TGGCTTATTCGGATTCGTCGCTCT	Present study

Table 2. Known sequences of *Babesia* species and orthologous sequences of related genera from GenBank.

Sequence	**GenBank Identification**
Cytauxzoon manul	AY485690
Babesia bigemina	AY603402
B. canis canis	AY072926
B. felis	AF244912
B. gibsoni	AF175300
B. leo	AF244911
B. microti	AY89075
B. spp. *Raccoon*	AB197940
B. rodhaini	AB049999
B. spIORK/HM101	AB070506
Babesia sp.	AF244913
Theileria annulata	AY508472
Theileria annae	AY534602
Piroplasmida gen sp.	AF158707

Table 3. Prevalence of *Babesia* sp. according to sex and housing corral of baboons. Numbers in parenthesis are number of baboons which tested positive for *Babesia*.

	Total Number of Baboons	Sex	Northeast corral	Northwest corral	Southeast corral	Southwest corral	Prevalence
Sp 2007	269 (34)	Male	15 (4)	20 (3)	11 (1)	16 (0)	12.60%
		Female	58 (11)	50 (11)	49 (4)	50 (0)	
Fall 2007	281 (23)	Male	15 (1)	20 (4)	11 (1)	16 (0)	8.20%
		Female	60 (3)	52 (8)	51 (5)	56 (1)	
Sp 2008	280 (16)	Male	9 (0)	14 (2)	6 (0)	15 (0)	5.70%
		Female	56 (3)	45 (5)	54 (6)	65 (0)	

Table 4. Number of baboons tested and infected with *Babesia* sp. within the Oklahoma breeding colony according to age and housing corral of baboons. Numbers in parenthesis are number of baboons positive for *Babesia*. Age is broken down by less than or equal to 4 years, 5 to 10 years, 11 to 20 years, and greater than or equal to 21 years old.

	Total Number of Baboons	**Age in years**	**Northeast corral**	**Northwest corral**	**Southeast corral**	**Southwest corral**
Sp 2007	269 (35)	≤4	35 (1)	27 (0)	26 (1)	20 (0)
		5 to 10	19 (7)	28 (6)	18 (1)	18 (0)
		11 to 20	5 (3)	9 (5)	8 (2)	22 (0)
		≥21	1 (1)	1 (1)	2 (0)	0
		unk	5 (3)	8(3)	8 (1)	9 (0)
Fall 2007	281 (23)	≤4	40(0)	28(1)	28(0)	22(0)
		5 to 10	19(3)	26(3)	18(1)	18(0)
		11 to 20	4(1)	7(6)	10(4)	22(1)
		≥21	1(0)	1(1)	2(0)	0
		unk	5(0)	8(2)	7(0)	9(0)
Sp 2008	280 (16)	≤4	34(0)	28(0)	29(0)	26(0)
		5 to 10	21(2)	25(3)	21(2)	21(0)
		11 to 20	12(1)	7(3)	11(4)	24(0)
		≥21	1(0)	2(0)	2(0)	0
		unk	0	4(1)	3(0)	9(0)

Figure 1. Blood smear from a baboon infected with *Babesia* sp.

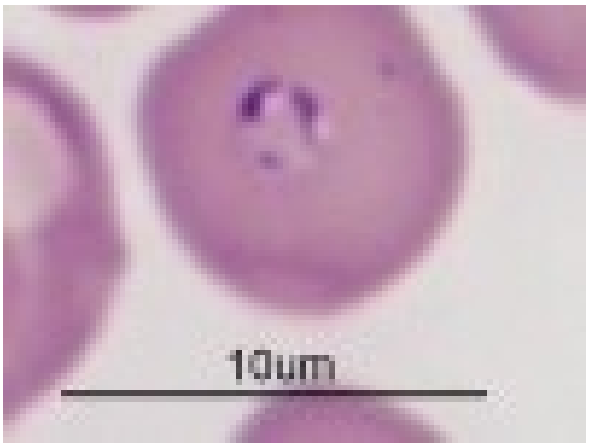
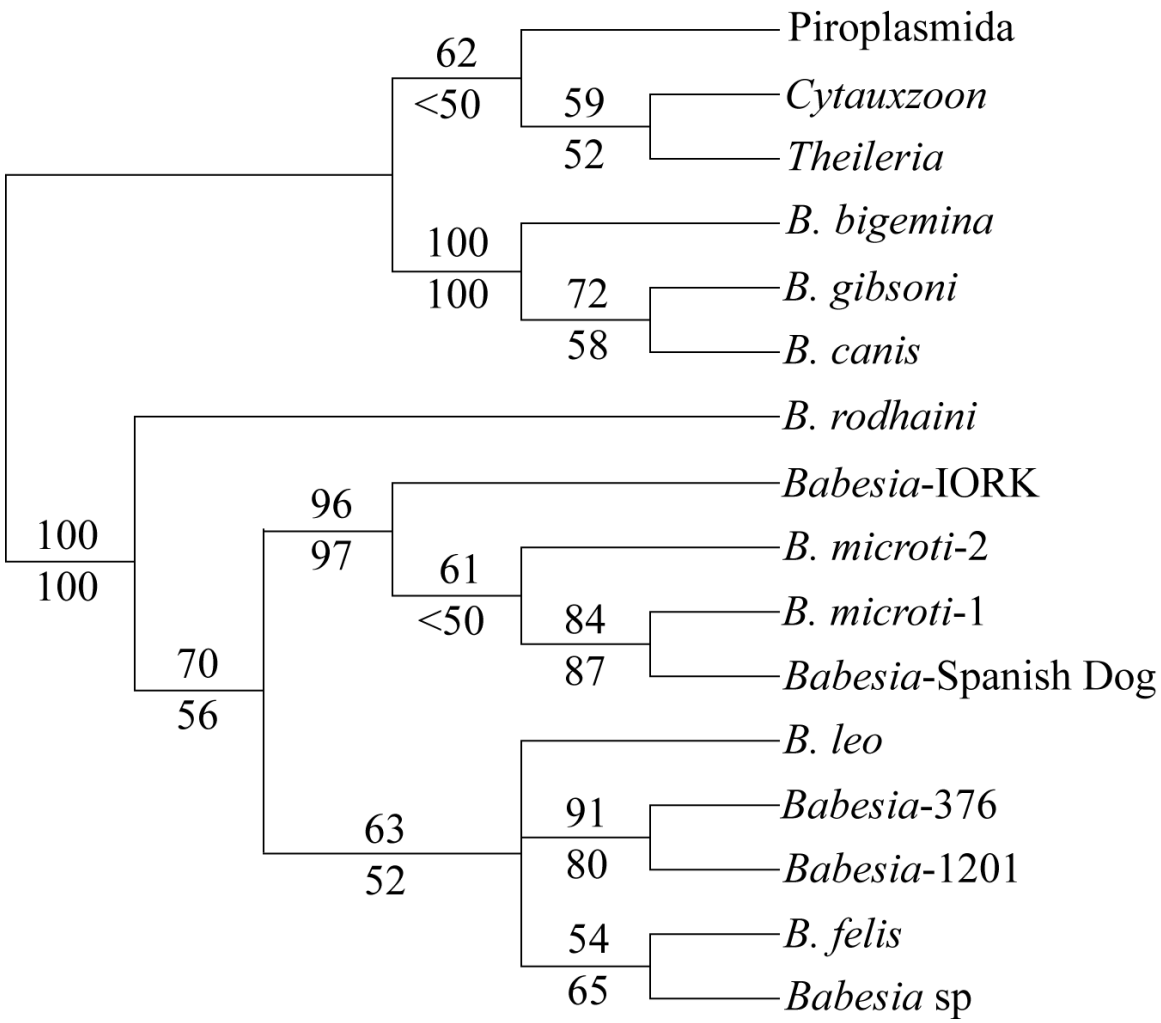


Figure 2. Phylogenetic relationships among species of *Babesia*. Numbers above clades are maximum likelihood percent bootstrap support values whereas numbers below clades are maximum parsimony percent bootstrap support values.



VITA

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Master of Science of Veterinary Pathobiology

Thesis: COMPARING DIAGNOSTIC TECHNIQUES FOR DETECTING INTESTINAL PARASITES AND PHYLOGENETIC ANALYSIS OF *BABESIA* IN CAPTIVE BABOONS

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Scope and Method of Study:
Baboons are important as models in biomedical research. The diagnosis and control of parasite infections in baboon research colonies provides quality animals for biomedical research. Fecal samples were collected and tested for parasites from baboons at the University of Oklahoma Health Science Center. DNA was extracted from blood of *Babesia* positive baboons and the 18s rRNA gene was amplified and sequenced.

Findings and Conclusions:
Cysts of *Entamoeba histolytica/E. dispar*, *E. coli* and eggs of *Trichuris trichiura* were detected. Our results indicated that zinc sulfate flotation and formalin ethyl-acetate sedimentation were more effective for detecting cysts of *Entamoeba* species whereas sugar flotation was for recovering eggs of *T. trichiura*. Overall, the prevalence of infection of *Babesia* sp. within the baboon population was 8.8% (73 of 830). Phylogenetic analysis of the sequenced *Babesia* DNA from 2 individual baboons revealed that *Babesia* sp. found in baboons is a novel species most closely related to *B. leo*.

ADVISER'S APPROVAL: Dr. Mason Reichard