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The Molecular Analysis of Hemoparasite Burdens of Free-Ranging Lions (Panthera leo) in the Kruger National Park, South Africa

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Abstract

Hemoparasites, or parasites of the blood transmitted by arthropod vectors, are commonly found in domestic animals and wildlife. Although they can cause serious illness in domestic species, they often persist at low levels in wildlife without compromising health. Current reports on wildlife parasitemia, specifically in lions (*Panthera leo*) are obtained from zoological parks and managed game reserves. However, few studies have examined parasitic burdens of free ranging lions. This study aims to semi quantitatively evaluate the presence of four hemoparasites including *Babesia sp.*, *Theileria sp.*, *Cytauxzoon sp.*, *and Hepatozoon sp* in free ranging lions of South Africa. DNA from 39 lions in the Kruger National Park, South Africa were analyzed for the presence of these four piroplasms by polymerase chain reaction. Total amplification of hemoparasite DNA was quantified using a standard DNA Mass ladder and AplaImager IS-2200. Results were examined for patterns of infection based on age, sex, locality, and habitat or land system. Male lions carried higher parasite burdens compared to females at all six localities and in both land systems. Furthermore, trends of infection were not found for age or land system. The findings suggest that gender is the only factor that affects disease susceptibility in lions. It can be conclude that monitoring parasitic burdens of free ranging lions is an effective strategy for maintaining the health of lion populations and assessing the threat they pose to domestic species. Understanding the factors that contribute to infection is necessary for the enlightened management of hemoparasite propagation in the Kruger National Park, South Africa.

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Acknowledgements

This thesis is the culmination of two years of field and laboratory work in the area of wildlife disease ecology. I was introduced to this field of study while studying abroad with the Organization for Tropical Studies in the Kruger National Park South Africa. While abroad I had the opportunity to work directly with South African National Parks' veterinarians and scientists. I attended SANParks lectures, took part in routine game captures and toured laboratory facilities. In doing so my enthusiasm for wildlife research blossomed, which led me to pursue an interesting and beneficial project for my undergraduate thesis. After two months of field research in the Kruger National Park funded by the Renee Crown Honors program, I returned to Syracuse University and joined the laboratory of Dr. Ramesh Raina. The combination of work in the field and the lab has helped me to gain valuable lessons in collaboration, work ethic, and autonomy. These experiences have inspired me to follow my passions by pursuing a career in wildlife research as a faculty member of an accredited university of government agency. The planning and work towards the completion of this thesis would not have been possible without the assistance of several people. I would like to take this time to thank these people for their advice and support, especially through the many obstacles and uncertainties associated with this project.

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Introduction

Most reports of African Lion (*Panthera leo)* parisitemia originate from zoological parks and managed game reserves without consideration of the lion's origins, diets, and natural habitats. Few studies have investigated parasite loads, specifically hemoparasites, of free ranging lions, (Bjork *et al.* 2000). Hemoparasites are typically hemoflagellates or filarid worms found in animal blood. The following hemoparasites, *Babesia*, *Theileria*, *Cytauxzoon*, and *Hepatozoons*, are the most common piroplasms observed in free ranging lions in sub-Saharan Africa (Munson *et al.* 2008). In isolation these piroplasms do not appear to cause serious illness, however when combined with other infections, or found in immuno-compromised individuals clinical symptoms can appear (Sherding 1994). In 1990, Averbeck *et al* found that the prevalence of parasitic infection of free ranging lions in the Ngorongoro Crater and Serengeti National Park, Tanzania varied significantly based on habitat, but not with sex or age.

Babesia, family *Babesidae*, is a tick-borne, family *Ixodidae*, hemoparasite that was first described by Babes in 1892 in cattle (Brown *et al.* 1991). This discovery was the first demonstration of the transmission of a protozoan parasite by an arthropod intermediate host. Today, *Babesia* is a widely distributed disease occurring from the tropics to the Arctic Circle. It is an intraerythrocytic sporozon parasite that reproduces by binary fission in the red blood cells of mammalian hosts. Ticks are capable of transovarial transmission of the parasite through the ova to subsequent generations. Sporozites are located in the salivary glands of the

arthropod host from where they are injected into the vertebrate host upon attachment. Once in the blood stream, the piroplasms invade and destroy red blood cells resulting in the release of hemoglobin. *Babesia* is not a serious disease in most wild species, however, it can cause problems when it spreads from wildlife to domestic animals (Howe 1971). This hemoparasite often infects lions at low levels without compromising their health (Munson *et al.* 2008). There are currently no reports of clinical babesiosis in free ranging lions in South Africa.

Theileria, genus *Theileria* and *Cytauxzoon*, genus *Cytauxzoon* are non-contagious infectious diseases found in a wide variety of animals, but most prevalent in ruminants. The family *Theirleriidae* was first described in 1907 in members of the deer family. These piroplasms occur most frequently in east, central and south Africa, but can also be seen in parts of Europe, Russia, Japan, India, Australia, and the United States. Disease propagation requires an arthropod as an intermediate host such as ticks. Therefore, *Theileria* and *Cytauxzoon* are most common where in climates favorable to large tick populations (Howe 1971). In South Africa, *Rhipicephalus appendiculatus* is the primary vector. *Theileria* and *Cytauxzoon* differ based on the location where asexual reproduction occurs. In *Theileria*, binary fission of the sporozytes occurs in the lymphocytes as opposed to the histiocytes, which is demonstrated by *Cytauxzoon* (Simpson *et al.* 1985). Clinical disease is characterized by high fever, leukopenia, anemia, and diarrhea. For both of these hemoparasites, transovarial transmission does not occur. There

have been few reports of clinical signs of these hemoparasites in wildlife including free ranging lions in South Africa (Howe 1971).

Hepatozoons, family *Hepatozoidae*, are protozoa first described in 1908 by G. Miller. There are over 300 species of intraerythrocytic parasites that are found in a wide range of vertebrates and arthropods. Cases have been reported primarily in the Middle East and Africa, however the disease has also been seen in parts of Europe and the United States. Arthropods, or ticks, are the primary vector of this disease. Sexual reproduction occurs in the haemocoel, a fluid filled cavity, of the invertebrate host. This disease is unique because it is results in infection from direct transmission. The vector must be ingested by the vertebrate host at which point sporozites are taken up by the blood stream and invade the erythrocytes. Sporozites are not located in the salivary glands and are therefore not transmitted by tick bites. Most *Hepatozoon* infections do not cause serious illness, however, hosts with concurrent disease such as babesiosis or compromised immune systems can demonstrate signs of serious disease (Sherding 1994).

Babesia, *Theileria*, *Cytauxzoon*, and *Hepatozoon* have all been observed in the blood smears of free ranging lions in South Africa. The Kruger National Park, South Africa, is home to approximately 1,600 free ranging lions (Funston and Ferreira 2006). Although it is believed that most hemoparasites can be seen dynamically across lion populations within the park, it has not been scientifically confirmed. The purpose of this study is to report hemoparasite burdens of free

ranging lions in various localities of the Kruger National Park. The four piroplasms that will be investigated in this study include, *Babesia sp.*, *Theileria sp.*, *Cytauxzoon sp.*, and *Hepatozoon sp*. The hemoparasite DNA values obtained will be used to detect trends in parasitic infection according to age, sex, locality, and land system or habitat.

Diseases caused by hemoparasites are best controlled by eliminating the intermediate host, however more attention is normally given to the spread of the disease from wildlife to domestic animals. It is important to understand hemoparasite burdens in wildlife, including free ranging lions, for the general health of these animals and to determine the threat their infections pose for domestic species. Enlightened wildlife managers coupled with thorough wildlife disease investigations would be beneficial to resolve epizootiologic problems early and to prevent the needless sacrifice of wildlife populations in emergency situations.

For this study blood samples were collected from 39 free ranging lions in 2006 across six localities in the Kruger National Park, South Africa. DNA was extracted from the blood samples and quantified using spectrophotometry. Gene-specific primers were used to amplify hemoparasite DNA by Polymerase Chain Reaction, which was later semi-quantitatively quantified using name of the software to asses parasitic burdens. These results were finally analyzed for basic trends in habitat, sex, and age.

Materials and Methods

Study Site

In order to assess hemoparasite burdens a total of 39 lion blood samples were collected from six sites within the Kruger National Park, South Africa from August to September 2007. The Kruger National Park is one of the largest game reserves in Africa, covering 18,989 km^2 (Mabunda *et al.* 2003). Thirty-two of the samples were collected from five localities in the Crocodile Bridge section located in the southern region of the park. These localities included, Crocodile Bridge, Duke Windmill, Gomondwani, Mpanamane, and Nlangenzwane Dam. The remaining seven samples were collected from Dzuweni Spruit in the Punda Maria section of the far northern region of the park. Each locality was classified into land systems based on geology, soils, topography, vegetation, and rainfall.

Crocodile Bridge, Duke Windmill, Mpanamane, and Nlangenzwane Dam are all located in the Satara land system. The Satara land system is characterized by mafic volcanic rocks, or basalts known for being nutrient rich soils. The flat plains observed in this system are typically covered in lush grasses and fine-leaved trees such as acacias. Satara occupies approximately 14.2% of the park's area and receives between 500-650 mm of rainfall per year (Venter *et al.* 2003).

Gomondwani is part of the Vutome land system, which is classified by two groups of Karoo sedimentary rocks, either sandstone or shale. Sandstone soils support mostly broad-leaved bushveld such as silver clusterleaf (*Terminalia*

sericea) whereas shale soils are dominated by fine-leaved woodlands, or acacias. These sodic soils are nutrient poor and characterized into flat or slightly undulating plains. The average annual rainfall in the Vutome systems is approximately 500-650 mm per year (Venter *et al.* 2003).

Dzuweni Spruit is located in the Bulweni land system, which is very similar to the Vutome land system. The flat to moderately undulating plains of the Bulweni land system are made up of Karoo and Soutpansberg sedimentary rocks, both sandstone and shale. Sandstone regions are occupied by broad leaved bushveld whereas the shale soils are dominated by broad-leaved woodlands, or mopanis. Like Vutome, these sodic soils are nutrient poor and only receive around 450-500 mm of rainfall per year (Venter *et al.* 2003). The relative locations of each locality within the Kruger National Park are classified into the corresponding land systems in Figure 1.

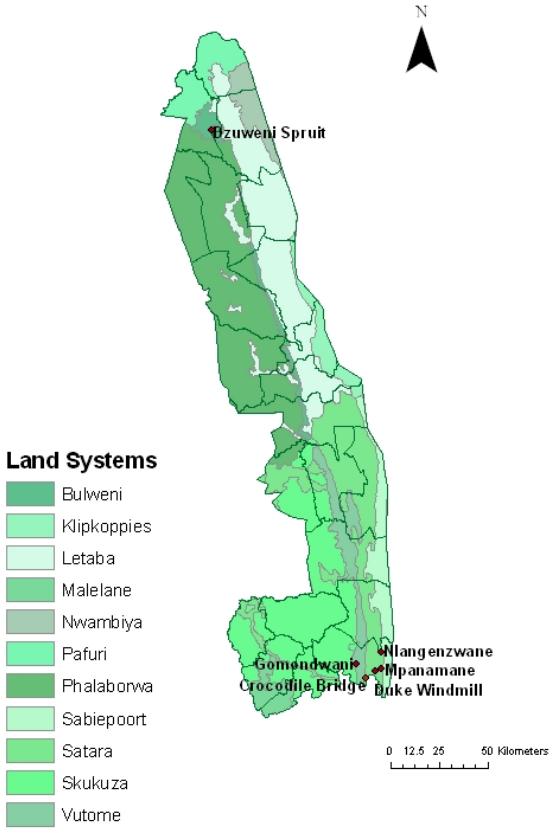

Figure 1. Localities and land systems of the Kruger National Park

Due to the high degree of similarity between the Vutome and Bulweni land systems, for the purpose of this study, these systems were classified together as nutrient poor soils. In contrast, the Satara land system was classified as nutrient rich.

Study Species

The African lion, is a carnivorous member of the Felidae family found in sub-Saharan Africa. Lions are the most social of the cats and on average live in prides ranging from 4-6 adults. In Kruger National Park, the average lion pride consists of about 13 lions (1.7 adult males, 4.5 adult females, 3.8 sub adults, and 2.8 juveniles). Large prides may never actually assemble in one place and are often found in smaller groups of 3-5 lions, especially when hunting (Schaller 1972). Lions tend to live at higher densities than most other felids, but with a wide variation from 1.5 adults per 100 km² in southern African semi-desert to 55/100 km² in some parts of the Serengeti (Sunquist and Sunquist 2002). Home range size depends on prey density and can vary from 13 km² to 248 km². Medium to large ungulates, like antelopes, zebras, and wildebeests make up the bulk of their prey, however, scavenging is also considered to be an important food source for most prides. Savannas and plains are the most common habitats for the African lion due to the high density of prey in these areas (Estes 1999). African lions are categorized as vulnerable on the red list of endangered species (Bauer *et al.* 2008). Major threats to the species include indiscriminate killing, trophy hunting, and disease (Bauer 2008, Packer *et al.* 2006, Ray *et al.* 2005).

DNA Extraction

DNA was extracted from lion blood using a Qiagen DNeasy® Blood and Tissue Kit (Qiagen Sciences, Maryland, USA). Two hundred microliters of non-nucleated blood were added to a 1.5 mL microcentrifuge tube containing 20 µL of proteinase K, and the volume was adjusted to 220 µL with 1X Phosphate Buffered Saline. To this 200 µL of Buffer AL was added, and the contents were mixed thoroughly by inversion followed by incubation for 10 minutes at 56°C. After incubation, 200 µL of 96% ethanol was added and the samples were mixed again by inversion. Samples were then pipetted into the DNeasy Mini spin column placed in a 2 mL collection tube and centrifuged at 8,000 rpm for 1 minute. The DNeasy Mini spin column was then placed in a new 2 mL collection tube followed by the addition of 500 µL of Buffer AW2, and centrifuged for 3 minutes at 12,000 rpm. The DNeasy Mini spin column was removed and placed on the bench to dry overnight while the flow-through and collection tube were discarded. Buffer AE was preheated to 55°C and 400 µL was transferred to the DNeasy Mini spin column and placed in a new 2 mL collection tube. DNA was finally eluted into the collection tube by 1 minute of centrifugation at 8,000 rpm. The collection tube containing DNA was placed in the freezer until further processing and the DNeasy Mini spin column was discarded.

Spectrophotometry

DNA concentration was determined using a Biorad SmartSpec Plus spectrophotometer (Biorad, Hercules CA, USA). DNA samples were diluted 20-

fold(5 µL DNA in 95 µL ddH_20). After the machine was turned on and the program for double stranded DNA was selected, the cuvette was washed 2 times with 100 µL of ddH_20. The machine was then blanked by loading the cuvette with 100 µL of ddH_20 and the absorbance was measured at 260 nm. After blanking, 100 µL of each sample was individually pipetted into the cuvette and the absorbance was recorded. In between the processing of each sample, the cuvette was washed with 100 µL of ddH_20 to prevent cross-contamination.

Determining DNA Concentration and Dilution

The concentration of the total extracted DNA was determined based on spectrophotometry results using equation 1. This DNA was subsequently diluted to a concentration of 1 ng/µl. The dilution factor used in this experiment was 20.

Equation 1. DNA concentration (ng/µl) = (OD at 260nm) x (50ng/µl) x (dilution factor)

Polymerase Chain Reaction

Semi-quantitative Polymerase Chain Reaction (PCR) was used to quantify the total hemoparasite DNA present in each sample. The PCR amplifications were carried out using Red*Taq* polymerase (Sigma, St Louis, MO, USA). PCR reactions were performed in a total volume of 17 µL containing: 11.05 µL of double distilled H_20, 1.50 µL of PCR buffer (10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5 mM $MgCl_2$), 0.75 µL of 2.50 mM dNTPs, 0.75 µL of 2 mM of each primer, 0.20 µL of 2U of DNA polymerases, and 2.00 ng of template DNA (2

μL). Due to the high morphological and genotypic similarity between hemoparasites, distinct species have been difficult to identify on a molecular level (Penzhorn *et al.* 2001). For this reason, gene-specific PCR primers (BabN1: 5' GGG GCA TTC GTA TTT AAC TGT CAG 3' and BabN2: 5' AGA CTT TGA TTT CTC TCA AGG TGC TG 3') were designed to amplify a 223 bp fragment of the 18 s rRNA gene in known *Babesia sp., Theileria sp., Cytauxzoon sp.,* and *Hepatozoan sp.* PCR amplifications were carried out in PTC-225 thermocycler (MJ Research, Waltham, MA, USA). The PCR program included five minutes of initial denaturation at 94°C, followed by 31 cycles composed of 30 seconds of denaturing at 94°C, 30 seconds of annealing at 61°C, and 30 seconds of extension at 72°C. The PCR program was terminated by doing a final extension for 5 minutes at 72°C and then held at 10°C until further processing. All samples were run in duplicate and averaged.

DNA fractionation by Gel Electrophoresis

One percent agarose gels were made by dissolving agarose in SB buffer (20X stock - 8 g NaOH, 45 g boric acid in 1 L water) in a conical flask and applying heat in a microwave for 1 minute. The agarose was then allowed to cool for several minutes on the bench. Upon cooling, 4 μL of ethidium bromide (0.5 mg/mL) were added per 100 mL of buffer. The solution was then poured into a gel cast tray fitted with combs to create wells, and allowed to solidify for 10-20 minutes.

Three-hundred and fifty milliliters of SB buffer were added to a gel box in order to completely cover the surface of the gel. The PCR products were loaded into the gel along with 3 µL of a low mass ladder (Invitrogen, Carlsbad, CA, USA) and run at 135 volts for 15 minutes. The gel was then removed, visualized with UV light and photographed using a Gel Documentation System (Alpha Innotech, San Leandro CA, USA).

DNA Quantization

Hemoparasite DNA was quantitated with the software package, AplaImager IS-2200 (Alpha Innotech, San Leandro CA, USA).The Spot Denso tool was used to create a calibration curve, which functions to allow the quantization of the bands on a gel based on a set of standards. The standards were obtained from the low mass ladder (Invitrogen, Carlsbad, CA, USA) and consisted of 5 bands corresponding to 50 ng, 30 ng, 20 ng, 10 ng, 5 ng, and 2.5 ng respectively. After the values of the standard bands were entered, values of the unknown bands were automatically calculated and outputted to an excel file. This quantization process, including calibration of the standard curve, was repeated for each individual 17-well electrophoresis gel.

Data Analysis

The average hemoparasite burdens measured from the PCR amplifications were logarithmically transformed in order to normalize the data and then analyzed using SAS version 9 (SAS Institute Inc., Cary, NC, USA). The alpha value was

set to 0.1 due to the small number of lions sampled at each locality, which therefore lacked the power to detect significant differences and also control for variation among prides. A linear ANOVA model was used to detect trends of infection according to age, sex, and land system. Another linear ANOVA was used to detect significant trends based on age, sex, and locality. A Fisher's LSD (least significant difference) was used as a post hoc analysis to test for significant differences between means associated with particular sex, age, soil type, or locality classes.

Results

Total DNA Quantization

In order to determine the amount of DNA that was extracted from whole blood samples, DNA extracts were analyzed by spectrophotometry. Optical Densities, absorbance at 260 nm, and corresponding DNA concentrations of all 39 samples are reported in Table 1.

Table 1. Summary of lion DNA samples including corresponding spectrophotometry and hemoparasite amplification results.
Locality represents the pride's location within the Kruger National Park and soil type (rich or poor) is used as an indicator of land system.

Lane	Lion ID Number	Optical Density	Total DNA Concentration (µg/ml)	Hemoparasite DNA Amplification (ng)	Locality (pride)	Land System (soil type)	Sex	Age (yrs)
1	49354D1067	0.056	56	2.45	Mpanamane	rich	Male	
2	434C656B6B	0.076	76	1.72	Mpanamane	rich	Female	
3	494A36140A	0.06	60	1.90	Mpanamane	rich	Male	1
4	492E73601B	0.043	43	2.26	Mpanamane	rich	Female	1
5	4959717807	0.062	62	1.88	Mpanamane	rich	Male	1
6	492A10157F	0.042	42	4.10	Mpanamane	rich	Male	1
7	49301C174B	0.092	92	2.19	Mpanamane	rich	Male	1
8	434E630268	0.045	45	4.96	Mpanamane	rich	Female	10
9	44155D142B	0.088	88	3.06	Gomondwani	poor	Female	4
10	442E1B4D0F	0.059	59	4.08	Gomondwani	poor	Female	8
11	49482E0022	0.043	43	1.97	Gomondwani	poor	Male	1.67
12	441547114E	0.044	44	7.31	Gomondwani	poor	Female	10
13	4930794722	0.052	52	3.13	Gomondwani	poor	Male	7
14	494748583B	0.055	55	3.56	Gomondwani	poor	Female	9
15	451F726223	0.053	53	2.25	Crocodile Bridge	rich	Male	3
16	454A696521	0.06	60	2.46	Crocodile Bridge	rich	Female	3
17	Ladder			Ladder				
18	456A62761F	0.045	45	4.38	Crocodile Bridge	rich	Female	1
19	4542767511	0.03	30	19.34	Crocodile Bridge	rich	Female	4
20	4536256D07	0.06	60	2.49	Crocodile Bridge	rich	Female	6
21	456E016973	0.055	55	2.95	Crocodile Bridge	rich	Female	1.5
22	456B0D3468	0.099	99	8.19	Crocodile Bridge	rich	Female	1.5
23	494A2A7564	0.049	49	14.47	Crocodile Bridge	rich	Male	1.5
24	494A1B6278	0.051	51	3.65	Dzuweni Spruit	poor	Male	8
25	492D150E07	0.028	28	3.62	Dzuweni Spruit	poor	Male	2
26	4932190F1C	0.052	52	16.76	Dzuweni Spruit	poor	Male	3
27	49302A521B	0.103	103	2.36	Dzuweni Spruit	poor	Female	4
28	4948211C28	0.164	164	2.83	Dzuweni Spruit	poor	Female	5
29	49494C5264	0.062	62	13.44	Dzuweni Spruit	poor	Female	1.5
30	434D092D75	0.125	125	8.93	Duke Windmill	rich	Male	3
31	49492B451E	0.057	57	4.31	Duke Windmill	rich	Female	8
32	456B2A755A	0.05	50	1.64	Duke Windmill	rich	Female	1.5
33	49297D0A60	0.149	149	2.35	Mpanamane	rich	Female	4
34	Ladder			Ladder				
35	492A562508	0.062	62	2.40	Mpanamane	rich	Male	4
36	4115092830	0.072	72	4.03	Mpanamane	rich	Female	8
37	441850741E	0.049	49	4.73	Nlangenzwane Dam	rich	Female	7
38	494A185A30	0.067	67	6.05	Nlangenzwane Dam	rich	Female	9
39	494A247E14	0.301	301	2.14	Nlangenzwane Dam	rich	Female	1.5
40	442D287135	0.051	51	1.27	Nlangenzwane Dam	rich	Male	1.5
41	4958633932	0.121	121	1.81	Nlangenzwane Dam	rich	Female	3
42	Ladder			Ladder				

Hemoparasite DNA Quantization

Following amplification, PCR products were visualized by gel electrophoresis under UV light. Hemoparasite DNA was quantified by comparing band intensity to a standard mass ladder. Amplification of the 223-bp fragment of hemoparasite DNA can be seen in Figure 2. The numerical values representing relative hemoparasite burdens are presented in Table 1.

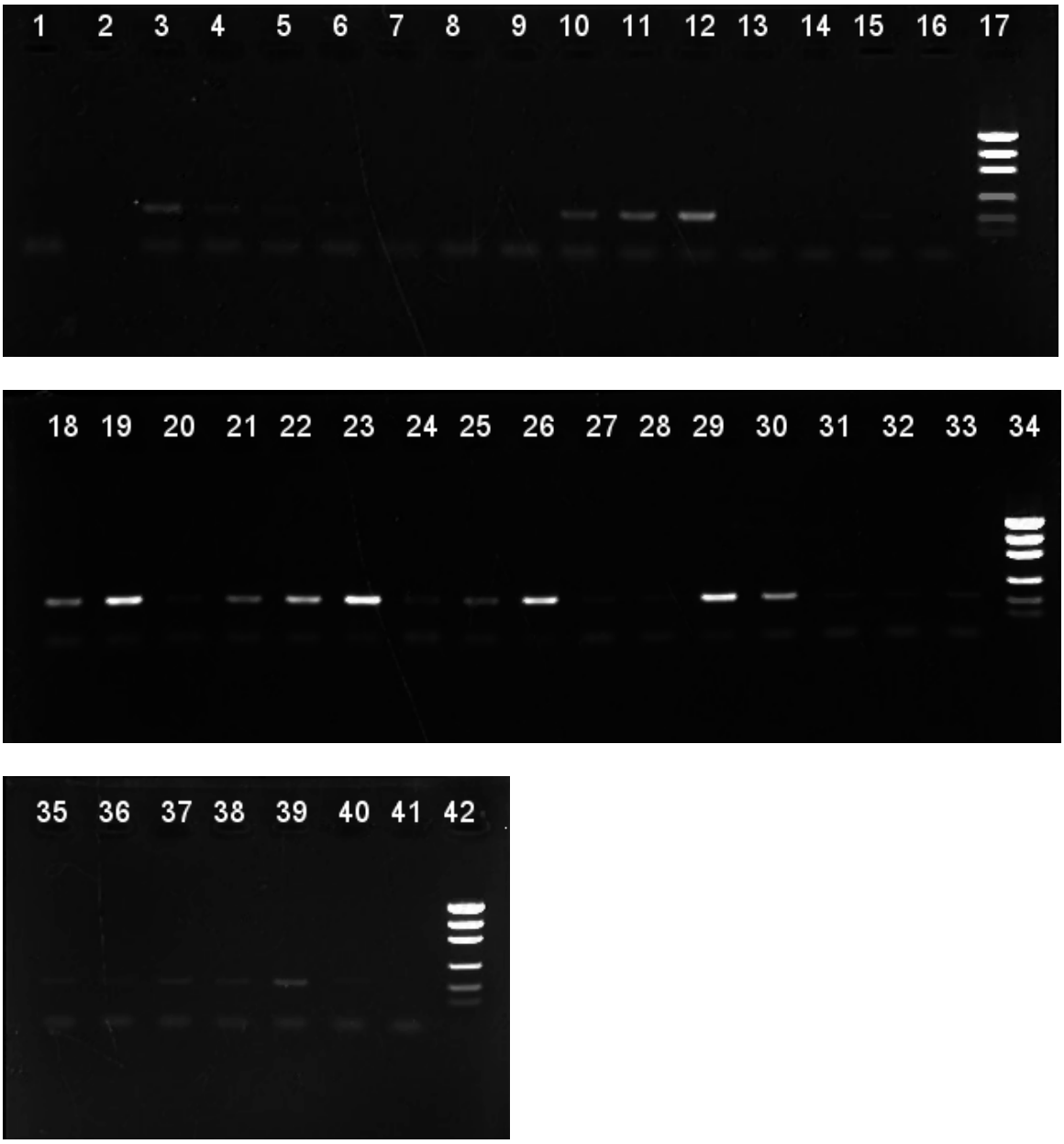

Figure 2. Gel electrophoresis results representing PCR amplification of total hemoparasite DNA in selected lion samples.
Lion identification numbers and quantified hemoparasite burdens are presented according to gel electrophoresis lane in Table 1. The Low Mass Ladder (LML) in lanes 17, 34, and 42 is defined by: band 1, 50 ng, band 2, 30 ng, band 3, 20 ng, band 4, 10 ng, band 5, 5 ng, band 6, 2.5 ng.

Trends of infection according to age, sex, and land system

There was evidence of a significant trend of infection according to lion gender in which males reported higher hemoparasite burdens than females ($F_{(1)}$= 2.65, p = 0.1). Figure 3 demonstrates that males had higher parasite loads in both land systems, independent of soil quality. No trends of infection were found to be significant according to age ($F_{(1)}$= 0.14, p = 0.7) or land system ($F_{(1)}$= 0.10, p = 0.8). When investigating interactions between subjects, land system and gender ($F_{(1)}$= 0.65, p = 0.4) or land system and age ($F_{(1)}$= 0.41, p = 0.5), again no significant trends of infection were observed. The main effect of the model also proved to be insignificant ($F_{(5)}$= 0.73, p = 0.6), therefore, gender was the only subject which affected disease prevalence.

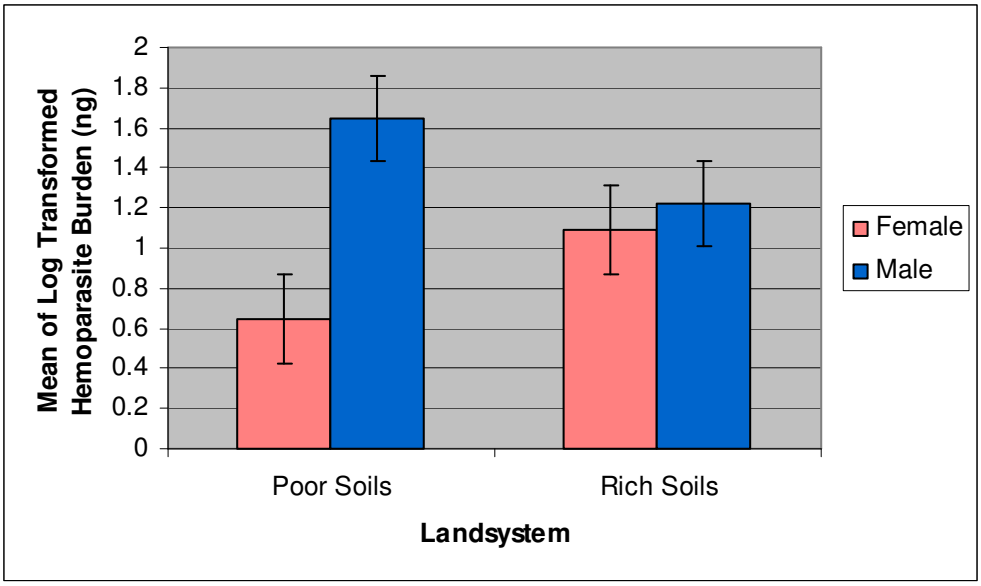

Figure 3: The relationship between male and female hemoparasite burdens based on soil type as an indicator of land system.

Trends of infection according to age, sex, and locality

Male and female lions reported significantly different hemoparasite burdens ($F_{(1)}$= 3.53, p = 0.07), indicating a trend of infection according to gender. Figure 4 demonstrates that males had higher disease levels at all six localities. Like the previous model, no significant trend of infection was found according to age ($F_{(1)}$= 0.99, p = 0.3) or locality ($F_{(5)}$= 1.61, p = 0.2). The main effect also proved to be insignificant ($F_{(7)}$= 1.42, p = 0.2), thus indicating that gender was the only factor that significantly had an effect on disease prevalence.

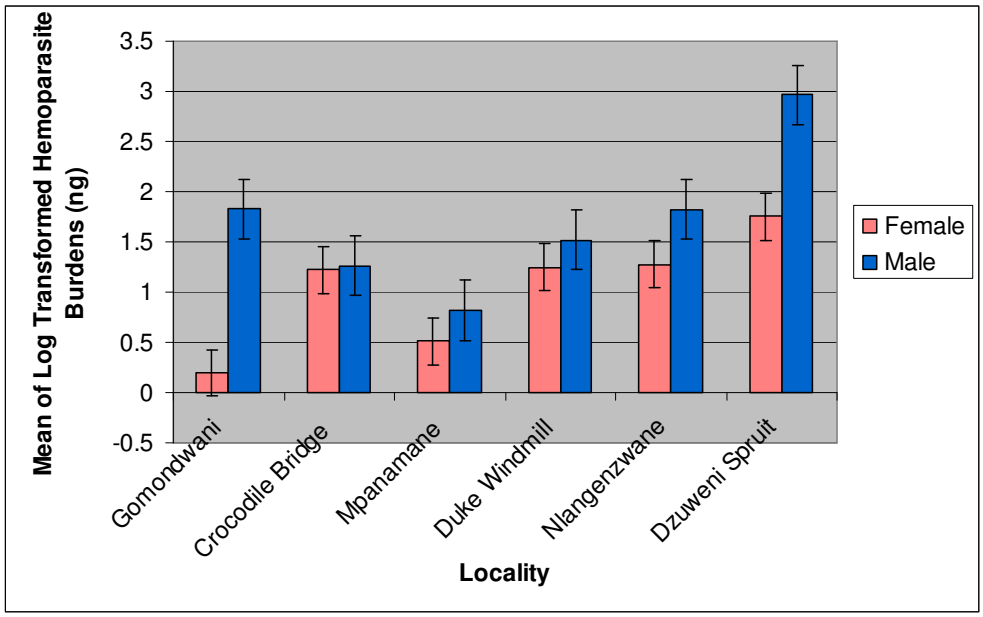

Figure 4. The relationship between male and female hemoparasite burdens based on locality.

Discussion

Contrary to previous studies which found parasitic disease prevalence to vary according to land system (Averbeck *et al*. 1990), the results of this study suggest that trends of infection might actually be dependent on gender. There was no evidence that age, land system, or locality had a significant effect on hemoparasite loads. More specifically, we found that male lions had greater parasite burdens than females (Figure 3 & Figure 4). This finding might indicate that males are more susceptible to disease, independent of age, land system, or locality.

One possible explanation for greater disease prevalence in male lions is due to the gender roles in hunting and eating. In a pride, females do the majority of the hunting, however males will eat first. It is believed that males eat before females so that they can remain strong in order to protect the pride (Schaller 1972). Ungulate prey such as buffalo, kudu, and impala, are the primary hosts to ticks responsible for hemoparasitic infection in lions (Barnett and Brocklesby 1968). Since some of the parasites examined in this study are transmitted by direct ingestion of the disease vector, male lions will be the first to become infected. Once females eat, most, if not all of the infected ticks will have been removed from the prey during male feeding.

Social interactions between and among males could also explain the higher parasite burdens observed in this study. Male lions greet each other in the wild by rubbing faces together. This act involves the exchange of scent markers located in

the corner of the mouth (Schaller 1972). The contact involved in this greeting could also facilitate the transfer of infected ticks between males. Additionally, male lions characteristically fight with each other which involves biting of the neck and nape area (Estes 1999). These acts of aggression facilitates the direct transfer of ticks from one male to another and disease transmission by ingestion of the vector. The social behaviors exhibited by male lions would facilitate the propagation of disease within the male community only, resulting in greater disease prevalence.

There is no direct immunological evidence that male lions are more susceptible to disease than female lions, therefore the pride social behaviors are most likely responsible for the trends observed in this study.

The hemoparasite burdens measured in this study were semi quantitative and therefore further studies will be needed to confirm the results. Lion DNA samples were normalized based on the total DNA measurements by spectrophotometry. Red blood cell counts would have produced a more accurate result. Additionally, PCR is a semi quantitative test and therefore analysis by real-time PCR would be needed. This study investigates lion hemoparasite burdens across the Kruger National Park in order to gain further insight into the current state of health for these mammals. In order to more successfully assess current parasite loads in lions, additional studies would be necessary. Understanding the factors that

contribute to infection is necessary for the enlightened management of hemoparasite propagation in the Kruger National Park, South Africa.

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Summary

Most reports of African Lion (*Panthera leo)* parisitemia come from zoos and managed game reserves without consideration of the lion's origins, diets, and natural habitats. Few studies have looked at parasite loads, specifically hemoparasites, of free ranging lions, (Bjork *et al.* 2000). Hemoparasites are typically hemoflagellates or filarid worms found in animal blood. The following hemoparasites, *Babesia*, *Theileria*, *Cytauxzoon*, and *Hepatozoons*, are the most common parasites found in free ranging lions in sub-Saharan Africa (Munson *et al.* 2008). Alone these parasites do not seem to cause serious illness, but when combined with other infections, or found in immuno-compromised individuals, they can become sick (Sherding 1994).

Babesia, *Theileria*, *Cytauxzoon*, and *Hepatozoon* have all been found in the blood of free ranging lions in South Africa. The Kruger National Park, South Africa, is home to approximately 1,600 free ranging lions (Funston and Ferreira 2006). The purpose of this study is to look at hemoparasite burdens of free ranging lions in various parts of the Kruger National Park. The four parasties that will be investigated included in this study are, *Babesia sp.*, *Theileria sp.*, *Cytauxzoon sp.*, and *Hepatozoon sp*. The hemoparasite DNA values obtained will be used to find trends in parasitic infection according to age, sex, locality, and land system or habitat.

It is important to understand hemoparasite burdens in wildlife, including free ranging lions, for the general health of these animals and to determine the threat their infections pose for domestic species. Enlightened wildlife managers coupled with thorough wildlife disease investigations would be beneficial to resolve epizootiologic problems early and to prevent the needless sacrifice of wildlife populations in emergency situations.

For this study blood samples were collected from 39 free ranging lions in 2006 across six regions in the Kruger National Park, South Africa. While I was in South Africa, I extracted DNA from the blood samples using a specialized kit. The samples were stored in a freezer until they were shipped to the United States for processing. First I calculated the concentration of the DNA I extracted. I did this using spectrophotometry which transmits UV light through the sample to detect how much DNA is present. I used this information to dilute each sample with water so that they would all contain the same amount of DNA. Then I used Polymerase chain reaction (PCR) to quantify how much parasite DNA was present in each sample. PCR is a technique that can detect very specific DNA fragments and amplify them exponentionally so that they can be visually detected. The results of the amplification were photographed with on an agarose gel with UV light. Specialized software was used to quantify each band by comparing the fluorescence to a known standard. I statistically analyzed the PCR results to look for trends of infection.

Contrary to previous studies which found trends of infection according to land system or habitat (Averbeck *et al*. 1990), I found that trends of infection might actually be dependent on gender. There was no evidence that age, land system, or locality had a significant effect on hemoparasite loads. More specifically, we found that male lions had greater parasite burdens than females. This finding might indicate that males are more likely to contract a disease, independent of age, land system, or locality.

One possible explanation for greater disease prevalence in male lions is due to the gender roles in hunting and eating. In a pride, females do the majority of the hunting, however males will eat first. It is believed that males eat before females so that they can remain strong in order to protect the pride (Schaller 1972). Ungulate prey such as buffalo, kudu, and impala, are the primary hosts to ticks responsible for hemoparasitic infection in lions (Barnett and Brocklesby 1968). Since some of the parasites examined in this study are transmitted by direct ingestion of the disease vector, male lions will be the first to become infected. Once females eat, most, if not all of the infected ticks will have been removed from the prey during male feeding.

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