PATHOGEN PREVALENCE IN A HIGHLY VULNERABLE POPULATION OF AFRICAN LIONS IN THE NORTHERN TULI GAME RESERVE, BOTSWANA

by

KIMBERLY R. MCDERMID

(Under the Direction of Michael J. Yabsley)

ABSTRACT

African lion (*Panthera leo*) numbers are decreasing and populations are becoming smaller and fragmented. Diseases are one reason for declines and low density populations are particularly vulnerable to disease epidemics. This study focused on viral and hemoparasite prevalence and characterization in a threatened lion population in Botswana. Blood and serum samples were collected on 50% of the adult/sub-adult lions in the Northern Tuli Game Reserve. Serology results revealed low antibody prevalence for feline panleukopenia (7%), calicivirus (15%) and canine distemper virus (15%). Higher prevalence occurred for feline immunodeficiency virus (76%) and herpesvirus (84%). All lions were seronegative for feline coronavirus and PCR-negative for *Trypanosoma* spp. Reverse line blot testing for *Anaplasma, Theileria* and *Ehrlichia* were negative; however, all lions tested positive for *Babesia*. Cloning and sequencing of amplicons from four lions revealed four *Babesia* spp. including variants of *B. felis, B. lengau, B. canis vogeli* and a *Babesia* sp. which likely represents a novel species most similar to *B. microti*.

INDEX WORDS: African lion, *Babesia* spp., Botswana, DNA sequencing, *Panthera leo*, pathogen, reverse-line blot testing, serology

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DEDICATION

This thesis is dedicated to Farasi, whose free-ranging roots but captive existence changed the trajectory of my life, and S.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

African lion *(Panthera leo)* numbers have declined up to 50% in the last two decades (IUCN, 2006). Lions are currently listed as Vulnerable on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species™ and the United States Fish and Wildlife Service is considering reclassifying the status of African lions as Threatened (U.S. Fish and Wildlife, 2015). In 1960, there were an estimated 450,000 lions but by 1994, only 100,000 remained (Myers, 1975, Nowell and Jackson, 1996). Current estimates range from only 23,000 to 32,000 (Bauer and Van der Merwe, 2004, IUCN 2006, Riggio et al., 2013). Overall, the distribution of lion populations has decreased by 75% of their historical range (Riggio et al., 2013). Increasing human populations create environments that are hostile to large carnivores, which are particularly sensitive to human population growth. Since carnivores may kill livestock and occasionally humans, they are rarely tolerated and persecution of large carnivores is widespread (Woodroffe, 2000). The primary reason for lion population declines are retaliatory and indiscriminate killing by landowners in response to livestock-predator conflict; however, habitat loss due to human encroachment, an increase in infectious disease epidemics, and loss of genetic diversity due to fragmented and isolated

populations are also regarded as important factors for population reductions (Kissui and Packer, 2004, Woodroffe and Frank, 2005, Cleveland et al., 2007, Bauer et al., 2008, IUCN 2012).

Human encroachment into areas previously inhabited only by wildlife often results in domestic animal-livestock-wildlife interactions, which can lead to the introduction of novel pathogens into wild carnivore populations (Woolroffe, 1999). In addition, human encroachment can cause fragmentation of populations which may lead to inbreeding and subsequent loss of genetic diversity; further, less genetically diverse individuals may be more susceptible to infectious diseases (Acevedo-Whitehouse et al., 2003). A combination of these factors could be devastating for threatened populations considering low-density, isolated populations can be eliminated by an infectious disease epidemic (Alexander and McNutt, 2010). Infectious disease outbreaks are of particular concern for free-ranging lion populations given that approximately 40% of the extant populations are low-density; of the 67 areas on the African continent that contain lion populations, 26 are inhabited by less than 50 individuals (Riggio et al., 2013).

The current study investigated the emerging role of infectious diseases in a vulnerable, low-density lion population in eastern Botswana (Figure 1.1). This lion population resides primarily in the Northern Tuli Game reserve (NTGR) and has been subjected to human encroachment, fragmentation, and human-lion conflict (Snyman et al., 2014). Inbreeding has also been observed in this population and currently, there are no data on pathogen exposure in this lion population. We aimed to survey at least 50% of the adult and sub-adult lions in the NTGR for various feline viruses and blood parasites. These data will provide a foundation for future health monitoring of the Tuli lion

population. In addition, information gained from this study will be useful for the conservation and management of this species, as the NTGR lions will eventually be incorporated into a larger metapopulation with the development of the Greater Mapungubwe Transfrontier Conservation Area (GMTFCA).

Literature Review

Infectious Disease Risks to Free-Ranging African Lions

Pathogen identification and infectious disease management are important components to large carnivore conservation considering most carnivore species are already threatened by adverse anthropological factors such as habitat fragmentation/loss, decreased genetic diversity and over-exploitation of carnivores or their prey species (Murray et al., 1999). Importantly, infectious diseases can have devastating consequences on threatened, lower density populations and many wild carnivore species are susceptible to pathogenic viruses or parasites harbored by domestic species (Roelke-Parker et al., 1996, Murray et al, 1999, Woodroffe, 1999, Cleaveland et al., 2007). Examples in which pathogens of domestic animal origin have had devastating effects on free-ranging large carnivore populations in Africa include canine distemper virus and rabies in African wild dogs *(Lycaon pictus)* (Ginsberg et al., 1995, Kat et al., 1995), canine distemper virus in spotted hyenas *(Crocuta crocuta)* and lions (Roelke-Parker et al., 1996), *Mycobacterium bovis* in lions (Keet et al., 1996) and rabies in lions (Berentsen et al., 2013). Increased contact among domestic animals, livestock and wildlife,

combined with the emergence and changing pathogenicity of a number of infectious agents, has had devastating consequences for certain populations (Cleveland et al., 2007, Smith et al., 2009).

A prime example of a vulnerable carnivore population experiencing infectious disease epizootics due to anthropogenic environmental changes is the Ngorongoro Crater lion population in Tanzania. Prey and water sources in the Crater are plentiful; however, the area surrounding the Crater has become closed off due to human settlement. Close inbreeding has been documented among the Crater lions and four documented disease outbreaks have swept through the population (Packer et al., 1991). In 1962, heavy rains contributed to an explosion of stable flies (*Stomoxys calcitrans*) in the Crater. Lions were the preferred host and suffered from severe skin infections and emaciation, leading to an 80% decline in population numbers (Fosbrooke, 1963, Packer et al., 1991). Disease investigations were not permitted in this population from 1991 to 2001 and in 1994 and 1997 the population experienced declines of approximately 30% due to an undiagnosed disease outbreak (Kissui and Packer, 2004). In 2001, 35% of the population died as a result of a CDV and *Babesia* co-infection (Munson et al., 2008).

Consequently, this genetically vulnerable lion population is living below carrying capacity (Kissui and Packer, 2004). Research has concluded that his population is particularly susceptible to infectious disease outbreaks driven by the close proximity to human/domestic animal settlements and a history of inbreeding. The Crater lion population has a history of isolation and fragmentation; however, for the majority of African carnivore populations, these are novel challenges due to more recent habitat losses. This population illustrates that vulnerable, depleted populations are likely to

struggle even with ample space, water and prey if they are isolated and subjected to close human and domestic animal contact. This population can provide insight into the fate of other lion populations on the continent currently experiencing similar conditions (Kissui and Packer, 2004).

Lion Social Structure Influences Infectious Disease Transmission

African lions have a unique and extremely complex social structure. This social structure makes them highly susceptible to direct and indirect intraspecific transmission of pathogens. Lions are the only social felid, living in familial prides of 1-21 adult females, their dependent offspring, and 1-9 adult males (Mosser and Packer, 2009). Females communally raise cubs in a crèche (Packer and Pusey, 1995) and males leave the pride when they become adults at four years of age after which they often form coalitions with other males (Packer and Pusey, 1987). Nomadic male coalitions may travel substantial distances in search of territory and pride residency and will challenge resident males for access to prides (Packer et al., 2001). Pride lions, resident and nomadic males have occasional contact while defending territory, mating and hunting/consumption of kills (Craft et al., 2011). A study conducted in the Serengeti ecosystem found that direct pride-to-pride contact was more likely to facilitate pathogen transmission than nomadic males (Craft 2008). However, the authors cautioned that these data may not apply to lion populations in other ecosystems with different lion densities per unit area, population numbers or habitat restrictions (Craft 2008, Craft et al., 2011). Finally, disease outbreaks in lion populations can result in an increased rate of infanticide. Infanticide occurs when there is a pride takeover and aging or less fit resident males are ousted from the pride by

another male, or a coalition of males. Thus, when resident males are reduced by disease, social factors intensify the effects of epidemics by increasing infanticide rates (Kissui and Packer, 2004).

Pathogens of African Lions

Overall, data related to pathogen prevalence or infectious disease dynamics in African lion populations is sparse and limited to a small number of populations and/or sample sizes (Table 1.1). Yet, these surveys have contributed valuable information regarding infectious pathogens circulating in lion populations and confirm co-infections can exacerbate clinical disease (Munson et al., 2008). In addition, there are several pathogens that do not necessarily cause overt clinical disease in lions; however, sub-lethal effects on host fitness has yet to be determined.

Viruses

Viral pathogens that are not associated with clinical disease or mortality in otherwise healthy lions include feline herpesvirus (FHV), feline calicivirus (FCV), feline coronavirus (FEC), and feline panleukopenia virus (FPV). Feline leukemia virus (FeLV), has yet to be reported in free-ranging lion populations. Viral pathogens associated with notable reductions in fitness levels or mortality includes canine distemper virus (CDV) and feline immunodeficiency virus (FIV-*Ple*).

Canine distemper virus (CDV) is one of the most significant infectious diseases of carnivore species world-wide. Although this virus is most often associated with domestic dogs, one study determined that domestic dogs (*Canis lupus familiaris*) were not the only

vector for CDV infection in lions and that persistence of this virus likely involves a larger network of multiple host species (Viana et al., 2015). In lions, this virus has been associated with neurological disease, seizures, encephalitis and pneumonia (Roelke-Parker et al., 1996). However, several studies have found antibodies to CDV in lions that were healthy at the time of sampling. In Botswana, twenty-one samples collected from 2003 to 2005 found a low prevalence of CDV antibodies (4.8%) in lions from the Khutse and Central Kalahari Game Reserves (Ramsauer et al., 2007). However, lions in Uganda had a high prevalence rate (79%) for CDV antibodies in samples collected in 1998-1999. One of the most extensive studies conducted to date on CDV in lions involves prides within the Serengeti ecosystem. CDV seroprevalence has varied between years in adults and immature lions (< 4 years). Lions were also found to have experienced both lethal and "silent" CDV epidemics from 1984 to 2007 as the population in general had low prevalence rates for several years and then spikes of transmission as noted by seroconversion. Some of the CDV peaks were associated with clinical disease whereas some were not (Munson et al., 2008). It is worth noting that during a 1993-1994 outbreak, lions in this ecosystem had a CDV prevalence rate of over 85% (Packer et. al., 1999).

Feline immunodeficiency virus (FIV) is a lentivirus that infects domestic cats (*Felis catus*) and numerous wild felids species world-wide. Species-specific strains of FIV have been isolated from domestic cats, African lions, leopards *(Panthera pardus)* and pumas *(Puma concolor)* (Brown et al., 1994, Carpenter et al., 1996, Carpenter et al., 1998). Although it has generally been accepted that FIV-*Ple* does not cause obvious clinical disease in lions, recent research suggests certain subtypes of FIV-*Ple* could be

contributing to a loss in immune competence in lions (Roelke et al., 2009, Troyer et al., 2011, O'Brien et. al., 2012). One study conducted from 1999 to 2006 compared lions in Botswana and Tanzania that were FIV-*Ple* positive (n=47) to FIV-*Ple* negative lions (n=17). FIV-*Ple* positive lions presented with opportunistic infections, weight loss, lymphadenopathy and altered serum chemistry profiles and histopathologies consistent with immune deficiencies caused by lentivirus infection in domestic cats (FIV-Fca), Asian macaques (SIV) and humans (HIV) (Roelke et al., 2009). Additionally, FIV-*Ple* associated depletion of CD4 T-lymphocytes has been documented in biopsies of lymphoid tissues from infected lions (Roelke et. al., 2009).

Furthermore, there are six genetically distinct FIV-*Ple* subtypes and some are possibly more pathogenic than others. Two lion subtypes (FIV-*Ple* A and E) are found in Botswana and three types (FIV-*Ple* A, B and C) circulate in the Serengeti ecosystem (Troyer et al., 2004). Recent research indicates that lions infected with CDV during a 2001 outbreak in the Ngorongoro Crater, Tanzania were twice as likely to survive if infected with FIV-*Ple* sub-type B, in contrast to lions infected with FIV-*Ple* A or C (Troyer et al., 2011). Although this study was conducted on a low-density population, the results warrant further investigation. The high degree FIV-*Ple* diversity suggests that this retrovirus has been endemic in lion populations for thousands of years (Brown et al., 1994, Carpenter and O'Brien, 1995). Considering the high prevalence of FIV-*Ple* in most surviving lion populations, it appears that infected lions are capable of thriving under normal circumstances; however, reductions in fitness levels caused by FIV-*Ple* may be more evident in times of nutritional or environmental stress or during co-infection. To date, only two surveyed populations, one in Etosha National Park, Namibia

and another in Hluhluwe-Umfolozi Reserve, South Africa, have tested negative for FIV-*Ple* (Spencer et al., 1992, Spencer, 1993). The fact that some lion populations are FIV-*Ple* negative should be taken into consideration if translocation or reintroduction of individuals is under consideration.

Feline herpesvirus (FHV) occurs with high prevalence in both domestic and wild felid species. FHV can cause upper respiratory tract and ocular disease in felines. Infected felids become latent carriers and episodes of viral shedding and reactivation can occur, especially in stressed individuals (Gaskell et al., 2007). FHV is endemic in the majority of lion populations on the continent, with some populations approaching 100% prevalence. Other detrimental effects of this virus could include abortion and failure to thrive in cubs; however it would be difficult to discern if cub loss is due to FHV or other factors, such as predation (Spencer 1991). Likewise, determining the possible sub-lethal effects of FHV on lion fitness is problematic because of the exceptionally high prevalence in most populations thus, control groups are not available for comparison (Packer et al., 1999).

Feline calicivirus (FCV) can cause upper respiratory tract infections and oral and/or subcutaneous ulcerations. Highly virulent forms of FCV have evolved that result in systemic infection that can be fatal in domestic felines (Radford et al., 2007). The first documented case of FCV in lions occurred in the Serengeti population; samples obtained from 1984-1991 revealed a 70% prevalence rate (Hofmann-Lehmann et al., 1999). Lions in Queen Elizabeth National Park, Uganda also have a high prevalence of FCV (79%)

(Driciru et al., 2006). A drastically lower prevalence rate was found in Central Kalahari lions in Botswana (9.5%) and lions surveyed in Etosha National Park and Kruger National Park both reported (0%) prevalence (Spencer 1993, Spencer 1991).

Feline coronavirus (FEC) is found in domestic and wild felids, including cheetahs (*Acinonyx jubatus*) and lions (Heeney et al., 1990, Hofmann-Lehmann et al., 1996). FEC most commonly presents as a mild enteric infection; however, the virus can also present as virulent feline infectious peritonitis. In adult domestic felids, the infection is often sub-clinical; however, young animals are more susceptible to clinical signs associated with this virus (Dewerchin et al., 2005). Only lions in Tanzania report (> 50%) prevalence, other sampled populations report extremely low or (0%) prevalence.

Feline panleukopenia virus (FPV) is a parvovirus that can cause severe disease in domestic and wild felids. FPV is a systemic infection that can cause panleukopenia, acute enteric disease and lethargy (Steinel et al., 2001). The first reported case in free-ranging lions occurred in Kruger National Park, South Africa, with the highest prevalence reported to date (84%) of any lion population sampled. Researchers postulate that this virus could have entered the ecosystem via domestic felids, however, no sampling of lions was done prior to the arrival of domestic cats; therefore, it is not possible to know if domestic animals are responsible for transmitting this virus to free-ranging felids (Spencer 1991). High to moderate prevalence rates for FPV in lions also occur in Tanzania (75%) and Uganda (36%). Recently, a related parvovirus (canine parvovirus) has been found in wild and domestic felids in North American pumas and bobcats (Allison et al., 2013).

Finally, rabies virus can infect all mammals, including felidae. A recent study surveyed free-ranging lions for rabies virus and found that 40% of the sample populations in South Luangwa National Park and Liuwa Plain National Park, Zambia tested positive for rabies virus neutralizing antibodies. None of the lions exhibited behavioral or clinical signs of rabies and the researchers theorize that the lions became exposed by eating rabies-infected prey (Berentsen et al., 2013).

Bacteria

Mycobacterium bovis, the causative agent of bovine tuberculosis, is found world-wide and is associated with severe disease in domestic cattle. Clinical signs of *M. bovis* in predators include alopecia, depression and emaciation (Keet et al., 1996). Pathologic findings in ungulates include granulomatous lesions in lymph nodes; however, necropsy findings of tuberculous-infected lions and cheetahs (*Acinonyx jubatus*) primarily include pulmonary lesions such as, extensive granulomatous lung lesions, pneumonia and pneumothorax (Keet et al., 1996). In Kruger National Park, South Africa, this pathogen has resulted in clinical disease and mortality in African buffalo (*Syncerus caffer*), lions and cheetahs (Keet et al., 1996, Keet et al., 2000). In Serengeti National Park, Tanzania, samples collected from Serengeti and Ngorongoro Crater populations between 1984-2000 revealed that (4%) of the lions were infected (Cleaveland et al, 2005). In certain parts of Africa, *M. bovis* has spilled over from domestic cattle to African buffalo, which are now considered maintenance hosts. By 1992, prevalence rates of *M. bovis* were as high as 70% in some Kruger buffalo herds and predators are at serious risk for contracting *M.*

bovis by ingesting infected prey (Keet et al., 1996). Given that the NTGR is geographically situated directly north of Kruger National Park, testing NTGR lions for *M. bovis* would be beneficial; however, current diagnostic assays available for use in Botswana are limited. New anti-mortem methods for detecting antibodies to *M. bovis* in lions are currently being evaluated (Miller et al., 2012).

Several tick-borne bacteria can infect felids including hemotropic *Mycoplasma, Bartonella, Anaplasma*, and *Ehrlichia* spp. Hemotropic mycoplasma species are found in felids world-wide and the most common clinical sign is hemolytic anemia. Although hemotropic *Mycoplasma* has been studied in domestic felids, research into the prevalence, transmission and health impacts of *Mycoplasma* in wild felids is lacking. One study determined that nine out of fifteen wild felid species from Europe, South America and Africa were positive for *Mycoplasma* infections. This study also found that Serengeti lions were co-infected with several hemoplasmas (Willi et al., 2007). In the Ngorongoro Crater, Tanzania, ticks were collected from ten immobilized lions in 2001 that contained DNA from *M. haemofelis* and *Candidatus M. haemominutum* (Fyumagwa et al., 2008).

Bartonella species are also capable of infecting domestic and wild felids. Clinical signs in domestic cats include bacteremia and lymphadenopathy. Blood samples from fifty-eight lions and seventeen cheetahs from several African countries were analyzed for *Bartonella henselae* and results revealed low prevalence rates of (5%) in lions and (6%) in cheetahs (Molina et al., 2004). In Zambia, all lions sampled were negative for

Bartonella Spp. (Williams et al., 2014). Testing the NTGR lions for *Mycoplasma* and *Bartonella* Spp. is complicated because no labs in Botswana or South Africa provide diagnostic molecular assays for these two pathogens.

Anaplasma and *Ehrlichia* bacterial organisms infect erythrocytes and leukocytes, respectively, but are only rarely reported from felids. A multi-pathogen survey of 484 feral cats in the United States found that *Anaplasma* and *Ehrlichia* Spp. were absent (Luria et al., 2004). Further, only two studies, one conducted in Zambia, the other in Botswana, have evaluated exposure of lions to *Anaplasma* and/or *Ehrlichia* and no evidence was found (Ramsauer et al., 2007, Williams et al., 2014).

Protozoans

Wild and domestic felids are host to several *Babesia* spp. Clinical signs include anemia, lethargy and anorexia. In domestic cats, several *Babesia* spp. have been reported including *Babesia felis* and *B. lengau* from southern African countries (Penzhorn et al., 2004; Bosman et al., 2013), *B. cati* from India (Mudaliar et al., 1950), *B. canis canis* from Spain and Portugal (Criado-Fornelio et al., 2003), *B. canis presentii* from Israel (Baneth et al., 2004), *Babesia canis vogeli* from Thailand (Simking et al., 2010), a *Babesia microti*-like species (previously referred to as *Theileria annae*) from Portugal (Criado-Fornelio et al., 2003) and *B. hongkongensis* from a feral cat in Hong Kong (Wong et al., 2012). In addition, uncharacterized species have been reported from domestic cats from France, Germany, Thailand, and Zimbabwe (Stewart et al., 1980; Jittapalapong and Jansawan, 1993; Bourdeau, 1996; Moik and Gothe, 1997). Immunocompromised and older domestic cats may be more susceptible to infection, especially if they are co-

infected with pathogens such as FIV (Barr et al., 1989). Clinical babesiosis in domestic cats has been reported in South Africa and is primarily associated with *B. felis* (Jacobson et al., 2000), but severe disease in domestic South African cats has recently been associated with *B. lengau* (Bosman et al., 2013). Rarely, clinical signs have been documented in domestic cats infected with *B. canis* subsp. *presentii* and *B. herpailuri* (Stewart et al., 1980; Baneth et al., 2004). No clinical disease was noted in natural and experimental infection of domestic cats with *B. cati* and *B. leo*, respectively (Futter et al., 1980; Lopez-Rebollar et al., 1999; Ayoob et al., 2010).

In contrast to domestic cats, wild felids rarely develop clinical disease when infected with *Babesia* sp.; however, mortality has been reported in immunosuppressed lions experiencing a concurrent CDV and *Babesia* outbreak (Munson et al, 2008). *Babesia* species reported from wild felids include *B. lengau* from South African cheetahs and lions in Zambia (Bosman et al., 2010; Williams et al., 2014), *B. felis* from African wild cats (*Felis silvestris*), caracals (*F. caracal*), cheetahs, lions, and servals (*Leptailurus serval*) from Africa (Penzhorn et al., 2004; Bosman et al., 2007; Williams et al., 2014), *B. leo* from lions and leopards (*Panthera pardus)* from South Africa and Zambia (Penzhorn et al., 2001; Bosman et al., 2007; Williams et al., 2014), *Babesia pantherae* from African leopards (Dennig and Brocklesby, 1972), and *B. herpailuri* from the jaguarundi (*Herpailurus yaguarondi*) from Central America (Dennig, 1967). Undescribed species have been reported from the West African civet cat (*Viverra civetta*) (Wenyon and Hamerton, 1930), the Indian leopard (*Panthera pardus fusca*) (Shortt, 1940), pampas cats (*Leopardus pajeros*), genets (*Genetta tigrina*) in Brazilian zoos (André et al., 2011),

bobcats (*Lynx rufus*) (Shock et al., 2013), and the Florida puma (*Puma concolor*) (Yabsley et al., 2006). Importantly, there is currently no known vector for any felid *Babesia* sp.

In lions, the prevalence of *Babesia* is high. At least four species have been reported (*B. felis*, *B. leo*, *B. lengau,* and a *B. gibsoni*-like sp.) in surveyed lion populations in Tanzania, South Africa, and Zambia (Penzhorn et al., 2001, Munson et al., 2008, Williams et al., 2014). As previously mentioned, otherwise healthy lions infected with *Babesia* spp. are presumed to be asymptomatic; however, clinical signs can occur related to environmental and nutritional stress, immunosuppression and co-infection with viral pathogens. Several *Babesia* spp. were associated with severe disease in lions during a simultaneous CDV outbreak (Munson et al., 2008). The synergistic effects of these two pathogens resulted in an estimated 35% reduction (~1,000 lions) in lion numbers during two outbreaks in the Serengeti National Park and the Ngorongoro Conservation Area. (Roelke-Parker et al., 1996, Munson et al., 2008).

Finally, *Trypanosoma* spp. are protozoal parasites that can infect numerous mammalian species, including felids. Trypanosome infections result in thousands of human deaths per year world-wide and infected livestock creates a serious economic issue throughout Africa (Maudlin, 2006). *T. brucei* and *T. congolense* have been reported in lions in the Luangwa Valley, Zambia while three species, *T. brucei brucei*, *T. congolense* and *T. brucei rhodesiense*, have been reported in lions in the Serengeti ecosystem (Welburn et al., 2008, Anderson et al., 2011). *T. brucei brucei* and *T. congolense* appear to be non-pathogenic; however, the subspecies *T. brucei rhodesiense*, the causative agent of human sleeping sickness, is a pathogenic species. Given that

numerous wildlife species are susceptible to this parasite, further investigation into wildlife reservoir hosts is warranted (Anderson et al., 2011). Interestingly, the Serengeti lion study determined that lions infected with non-pathogenic *T. brucei brucei* and *T. congolense* had developed age-related resistance to the pathogenic *T. brucei rhodesiense* species. By age six, lions that had been infected with *T. brucei rhodesiense*, apparently cleared the infection due to cross-immunity created by the two non-pathogenic *Typanosome* species. It is worth noting that this was the first study to demonstrate acquired immunity in a host species against *Trypanosome* parasites (Welburn et al., 2008).

Ectoparasites

Literature is scarce regarding actual ectoparasites recovered from lions and most is on tick species. Most tick species found on lions are generalists and most of the ticks collected from lions are adults. Six species of ticks have been documented on nine lions in three southern African countries (Horak et al., 2010). Numerous tick species were found on twenty-two lions in Kruger National Park and Umfolozi Game Reserve, South Africa (Horak et al., 1987, Horak et al., 2000) (Table 1.2). Additionally, several tick species have been collected from lions; however, no information is available regarding the locations where these ticks were obtained (Walker et al., 2000) (Table 1.3). As stated previously, several tick species are suspected vectors of *Babesia* spp. that infect free-ranging felids, but the vectors have yet to be identified (Penzhorn et al., 1999). However, researchers theorize that the 1994 and 2001 high mortality epidemics in lions within the Serengeti ecosystem were partly attributed to severe babesiosis caused by a sharp upsurge

in the number of tick vectors (Munson et al., 2008). Numerous ixodid ticks such as, *Rhipicephalus appendiculatus, Rhipicephalus simus* and *Amblyomma hebraeum* can infest both carnivores and ungulate species and are considered potential vectors for *Babesia* spp. (Horak et al., 2000, Penzhorn et al., 2001). Further, Serengeti lions that consumed tick-infested buffalo had significantly higher hemoparasite levels compared to lions that were not exposed to the buffalo (Munson et al., 2008).

Ectoparasites other than ticks have been reported from lions, including a *Demodex* mite species from a single lion in Tanzania (Bjork et al., 2000) and Sarcoptic mange mites (*Sarcoptes scabiei*) are known to have caused infections in lions in Kruger National Park (Young 1975, Pence, D. B., & Ueckermann, E., 2002). A study conducted in the Masai Mara National Reserve, Kenya concluded that *S. scabiei* infestation spreads from prey species to cheetahs and lions (Gakuya et al., 2011). Lions were the preferred host for hordes of Stable flies (*Stomoxys calcitrans*) in the Ngorongoro Crater that resulted in an 80% mortality rate for the Crater lions (Fosbrooke, 1963, Packer et al., 1991) and the dog fly (*Hippobosca longipennis),* has also been found on lions as well as other African carnivores and domestic canids and felids (Iowa State University, 2009). Finally, four different flea species including, *Ctenocephalides* spp., *Echidnophaga* spp., *Procaviopsylla* spp., and one species classified as "Other" were collected from 23 lions in Kruger National Park and Umfolozi Game Reserve, South Africa (Horak et al., 2004).

Review of Pathogens detected in Lions from Botswana

The estimated lion population in Botswana in 2002 was approximately 3,000 individuals (Bauer et al., 2005) and while lion numbers were considered stable as

recently as 2006, significant decreases have recently been noted. This decrease is occurring despite the fact that Botswana has placed a moratorium on lion trophy hunting and has also preserved large allocated areas for wildlife that provide open natural habitat for lions. Lions play an important role in the ecological dynamics of ecosystems in Botswana and are vital to the economy as a tourist attraction.

Three infectious disease surveys have been completed specifically for lions in Botswana. First, in 1996, 31 lions throughout Botswana were tested for antibodies to feline leukemia virus (FeLV) and feline lentiviruses using a puma lentivirus isolate and domestic feline immunodeficiency virus (FIV) isolate. Eight of the 31 lions (25%) were positive for antibodies to feline lentivirus and no lions were positive for FeLV (Osofsky et al., 1996). The next study was conducted from 1999-2006 on 64 lions from Botswana and Tanzazia found a (74%) seropositive rate for FIV-*Ple*. Two locations in Botswana had extremely high prevalence rates (Okavango Delta (96%) and Chobe National Park (100%)) and one site had lower prevalence (Makgadikgadi and Nxai Pans (18%)) (Roelke et al., 2009). Last, the only published multi-pathogen survey for viral and parasitic pathogens of lions from Botswana was conducted from 200-2005 on 21 lions from the Khutse and Central Kalahari Game Reserve populations. Results of this study indicated high prevalence of antibodies to (FIV-*Ple*) (71%) and feline herpesvirus (100%) and a low prevalence of CDV (5%) and feline calicivirus (10%). All lions sampled were seronegative for FeLV, FEC, FPV, *Ehrlichia canis* and *Anaplasma phagocytophilum* (Ramsauer et al., 2007).

Importance of Infectious Disease Surveys for the Tuli Lion Population

Tuli lion population numbers have been closely monitored since 2000. It is thought that the NTGR originally supported ~ 75 lions (Snyman, pers comm. 2015) but between 2000 and 2008 the lion population averaged only 19 individuals. Lion numbers further decreased to a low of 11 individuals in 2009. Many lions lost between 2005 and 2011 were due to human impacts (e.g., 19 lions were known to have been snared, poisoned or shot) (Snyman et al., 2014). At the beginning of the current study (Summer 2014) there were 39 known individuals in the NTGR lion population and 31 of these lions were within Botswana's borders at that time. Population demographics at the time of sampling are as follows: ten adults, twelve sub-adults and nine cubs. Age classification for lions are; cubs, 0-2 years, sub-adults, 2-4 years and an adults are 4+ years (Schaller 1972).

The NTGR is a privately-held, mostly unfenced reserve (Figure 1.2). However, human encroachment and an increase in livestock numbers are impacting this lion population by either restricting lion movement patterns (Snyman et al., 2014) and possibly increasing exposure risk to pathogens. The NTGR covers a limited land area (720 km^2) and lions regularly cross international borders to travel to adjacent areas. These areas include agricultural zones, tribal grazing lands and hunting concessions. Occasionally, lions emigrate from South Africa or Zimbabwe and become residents of the Northern Tuli region. These immigrations, along with the establishment of the future metapopulation, should increase lion population numbers as well as genetic diversity. Metapopulation theory predicts that increasing migration between sub-populations via construction of corridors or active translocation is beneficial overall for conservation of

species; however, decreasing disease transmission between sub-populations is imperative (Gog et al., 2002). Therefore, a multi-pathogen screening conducted on the NTGR lions will provide data that can be used to implement metapopulation management strategies to decrease the impact of pathogens on this lion population and possibly other carnivore species in the area.

Comprehensive pathogen screenings are critical given the likelihood that increased domestic animal/livestock/wildlife interactions will occur resulting in spillover events (canine distemper virus, rabies virus, *Mycobacterium bovis*, etc.) or climate change that may result in lethal pathogen interactions (*Babesia* and CDV) (Roelke-Parker et al., 1996, Munson et al., 1996, Munson et al., 2008). Infectious disease epidemics are one reason for the rapid decline in lion numbers on the continent and high mortality epizootics can have devastating consequences on small, genetically vulnerable populations such as the NTGR lion population. Furthermore, infectious diseases may persistently hold populations below carrying capacity (Mitchell and Power, 2003).

Wildlife disease surveillance is an important aspect of wildlife management especially with the establishment of the Mapunugbwe Transfrontier Conservation Area (Figure 1.3). This development could be vital for this population as larger metapopulations are better able to recover from the effects of sporadic infectious disease outbreaks (Alexander et al., 2010). However, the development of Transfrontier Conservation Areas in Southern Africa may increase infectious disease risks as wildlife populations can move freely across international borders and interact with other wild carnivores, domestic animals and livestock.

To address the need for pathogen exposure data for the NTGR lion population, we conducted a multi-pathogen survey on 59% of the adult and sub-adult lions for viruses and hemoparasites known to infect lions. This study aimed to identify, conduct physical exams and collect biological samples on NTGR adult and sub-adult lions. Data provided by this study will establish a baseline for future health monitoring of this population and assist with developing conservation initiatives and management strategies to minimize the effects of infectious diseases on the NTGR lions, as they become part of a larger metapopulation.

Table1.1. Reports and prevalence rates of select viruses from African lion populations in six countries from 1984-2013.

Area	Country	Years	No. positive for antibodies/No. tested (%)					
			CDV*	FEC	FCV	FHV	FPV	FIV-*Ple*
West Africa Etosha National Park	Namibia	1989-1991		2/66 (3)	0/66 (0)	44/66 (67)	0/66 (0)	0/66 (0)
Southern Africa S. Luangwa & Liuwa Plain NP's	Zambia	prior to 2013	0/21 (0)				1/21 (5)	
Various locations	Botswana	early 1990's						8/31 (26)
Okavango Delta & Chobe NP	Botswana	1999-2006						47/64 (73)
Khutse & Central Kalahari Game Reserves	Botswana	2003-2005	1/21 (5)		2/21 (10)	21/21 (100)		15/21 (71)
Kruger National Park	SA	1987-1990		0/32 (0)	0/32 (0)	29/32 (91)	27/32 (84)	50/55 (91)
East Africa Queen Elizabeth NP	Uganda	1998-1999	11/14 (79)		11/14 (79)	9/14 (64)	5/14 (36)	10/14 (71)
Serengeti NP	Tanzania	1984-1991	(endemic)	149/254 (58)	170/254 (67)	252/253 (99)	192/253 (75)	221/243 (91)
Ngorongoro Crater	Tanzania	1984-1991	(endemic)	28/51 (55)	1/51 (2)	51/51 (100)	14/51 (27)	41/44 (93)
Lake Manyara	Tanzania	1984-1991		1/5 (20)	0/5 (0)	5/5/ (100)	3/5 (60)	4/5 (80)

*Canine distemper virus (CDV), Feline coronavirus (FEC), Feline calicivirus (FCV), Feline herpesvirus (FHV), Feline panleukopenia virus (FPV), Feline immunodeficiency virus (FIV-*Ple*)

Table 1.2. Tick species collected from lions in five African countries.

Country	**Tick Species**	**Citation**
Botswana	*Amblyomma hebraeum* *Hyalomma truncatum* *Rhipicephalus simus*	Horak et al., 2010
Namibia	*Hyalomma truncatum* *Rhipicentor bicornis* *Rhipicephalus turanicus*	Horak et al., 2010
Tanzania	*Rhipicephalus carnivoralis* *Rhipicephalus sanguineus*	Walker et al., 2000 Fyumagwa et al., 2008
South Africa	*Amblyomma hebraeum* *Haemaphysalis leachi* *Rhipicephalus simus* *Hyalomma truncatum* *Amblyomma hebraeum* *Amblyomma marmoreum* *Amblyomma tholloni* *Boophilus decoloratus* *Haemaphysalis leachi* *Haemaphysalis zumpti* *Hyalomma truncatum* *Rhipicephalus appendiculatus* *Rhipicephalus evertsi evertsi* *Rhipicephalus simus* *Rhipicephalus turanicus* *Rhipicephalus zambeziensis* *Amblyomma hebraeum* *Haemaphysalis elliptica* *Hyalomma truncatum* *Rhipicephalus simus*	Horak et al., 1987, Apanaskevich, D. A., & Horak, I. G., 2008 Horak et al., 2000 Horak et al., 2010
Zambia	*Rhipicephalus carnivoralis*	MacLeod, J., & Mwanaumo, B., 1978

Table 1.3. Tick species collected from lions in which geographic location is unknown.

Country	**Tick Species**	**Citation**
Country-unknown	*Hyalomm albiparmatum*	Apanaskevich, D. A., & Horak, I. G., 2008
	Rhipicephalus appendiculatus *Rhipicephalus aquatilis* *Rhipicephalus compositus* *Rhipicephalus evertsi evertsi* *Rhipicephalus evertsi mimeticus* *Rhipicephalus exophthalmos* *Rhipicephalus humeralis* *Rhipicephalus hurti* *Rhipicephalus jeanneli* *Rhipicephalus kockhi* *Rhipicephalus longus* *Rhipicephalus lunulatus* *Rhipicephalus maculatus* *Rhipicephalus masseyri* *Rhipicephalus muhsamae* *Rhipicephalus praetextatus* *Rhipicephalus pravus* *Rhipicephalus sp. near pravus* *Rhipicephalus pulchellus* *Rhipicephalus sp. near punctatus* *Rhipicephalus senegalensis* *Rhipicephalus simus* *Rhipicephalus tricuspis* *Rhipicephalus turanicus* *Rhipicephalus zambeziensis* *Rhipicephalus zumpti*	Walker et al., 2000

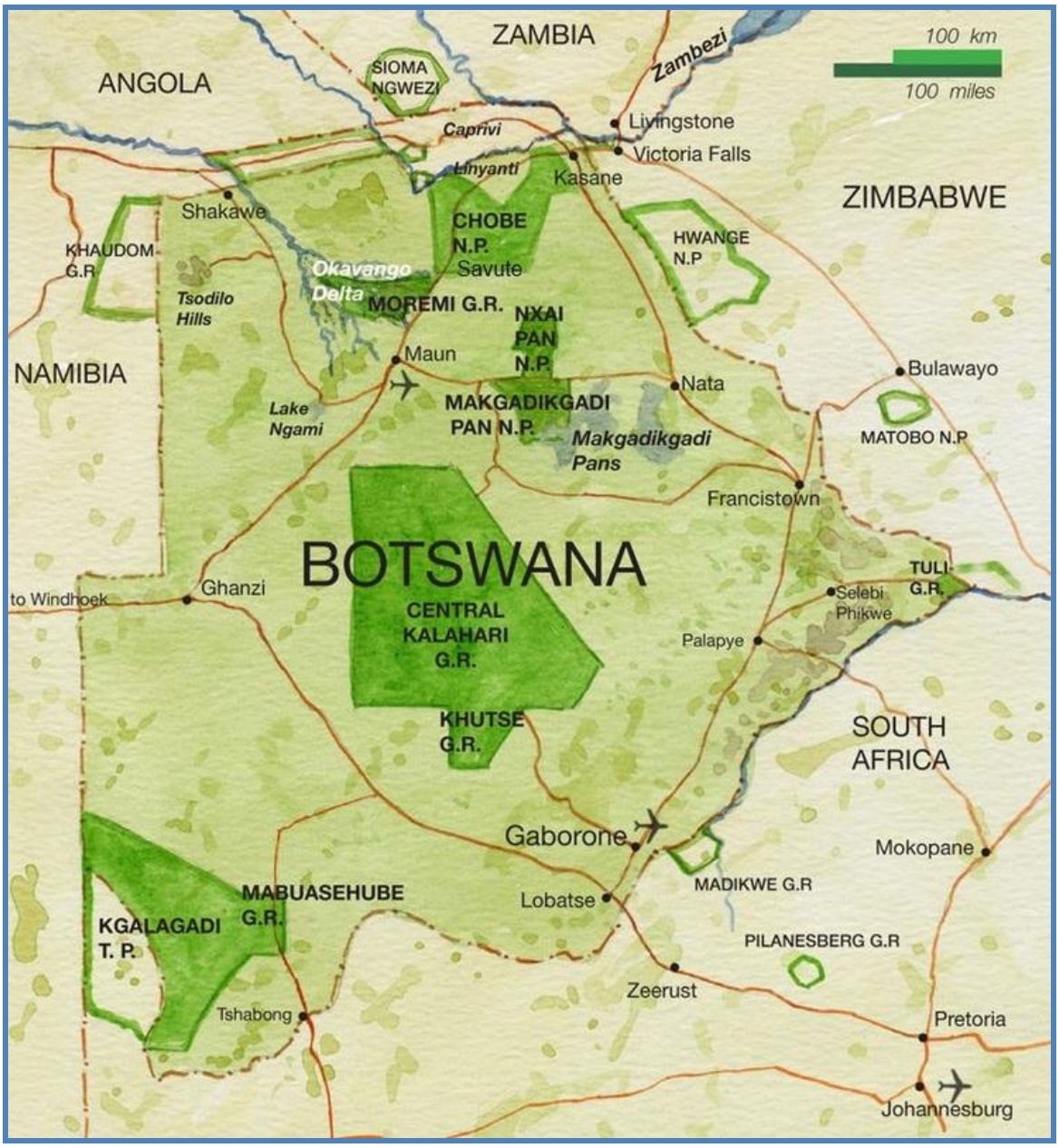

Figure 1.1. Botswana with the Northern Tuli Game Reserve (noted as TULI G.R.) highlighted in the eastern corner.

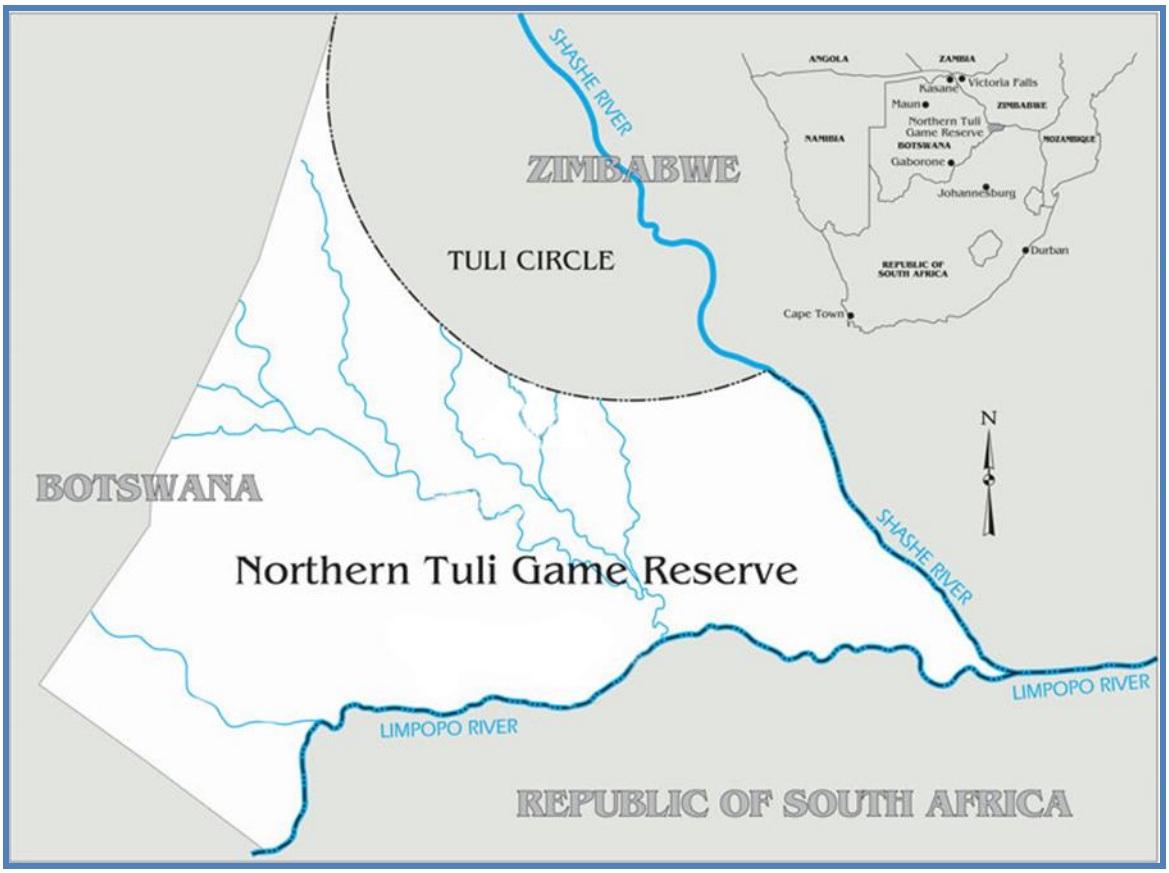

Figure1.2. The Northern Tuli Game Reserve (NTGR).

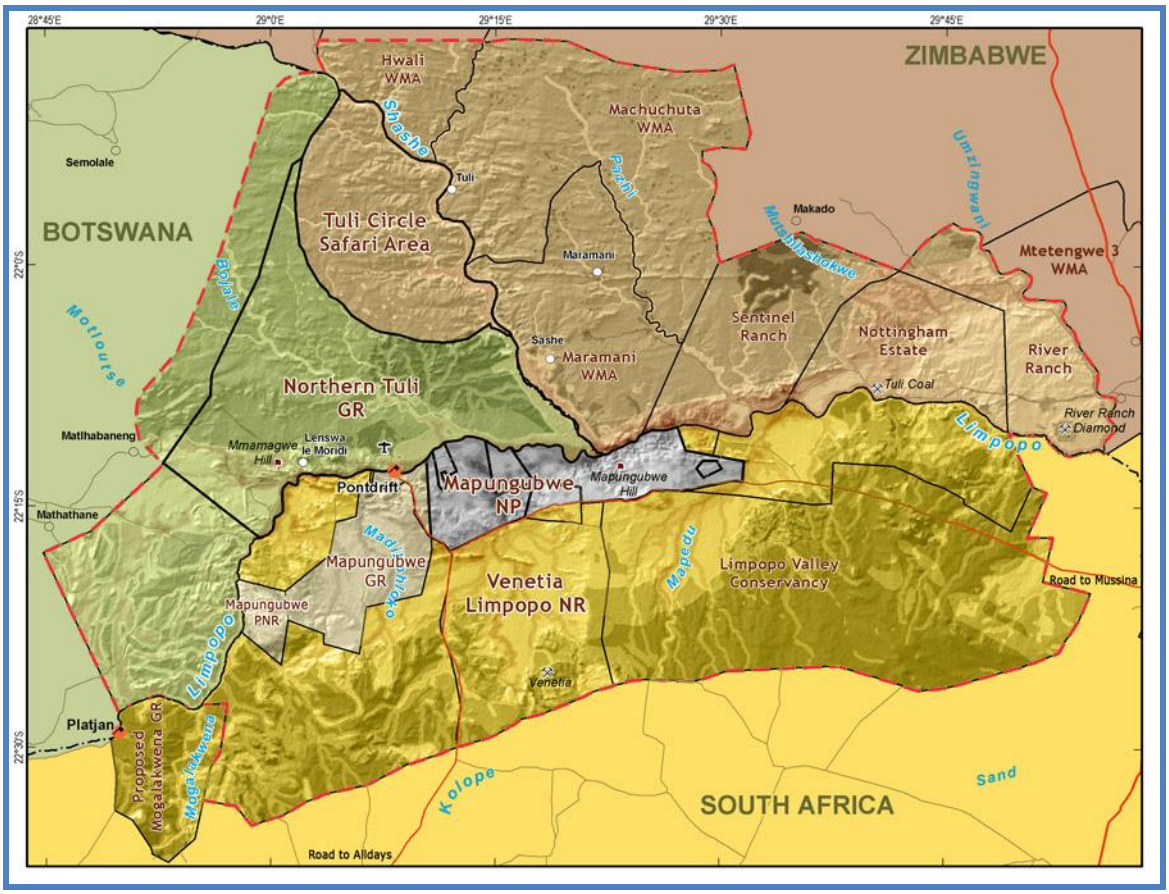

Figure 1.3. The Greater Mapungubwe Transfrontier Conservation Area showing how the NTGR will be incorporated into the larger conservation area.

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CHAPTER 2

SURVEILLANCE FOR VIRAL AND PARASITIC PATHOGENS IN A VULNERABLE AFRICAN LION (*PANTHERA LEO*) POPULATION IN THE NORTHERN TULI GAME RESERVE, BOTSWANA[1]

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Abstract

African lion (*Panthera leo*) numbers are decreasing rapidly and populations are becoming smaller and more fragmented. Diseases are one reason for population declines and low density populations are particularly vulnerable to disease epidemics. This study was conducted to obtain data on the prevalence and diversity of viral and parasitic pathogens for a threatened lion population in southeastern Botswana. Blood and serum samples were collected on 59% (n=13) of the adult/sub-adult lions in the Northern Tuli Game Reserve. Serology results revealed low prevalence of antibodies to feline panleukopenia virus (7%), canine distemper virus (15%) and feline calicivirus (15%) and higher prevalence to feline immunodeficiency virus (76%) and feline herpesvirus (84%). All lions were seronegative for feline coronavirus. All lions were PCR negative for *Trypanosoma* spp. Reverse line blot testing for *Anaplasma*, *Theileria and Ehrlichia* spp. were also negative; however, all lions tested positive for *Babesia*. Sequencing of amplicons from four lions revealed four *Babesia* spp. including genetic variants of *B. felis*, a variant of *B. lengau*, *B. canis vogeli* and a *Babesia* sp. which is only 96% similar to other *Babesia* spp. and likely represents a novel species most similar to *B. microti*. These data provide a baseline for future health assessments of this population and can be utilized to identify management and conservation strategies to decrease the impact of pathogens as there is an initiative to incorporate this population into a larger metapopulation of lions from South Africa and Zimbabwe.

Introduction

Surveillance for and management of infectious diseases are important components to large carnivore conservation considering most carnivore species are already threatened due to habitat fragmentation and loss, diminished genetic diversity, over exploitation of carnivores themselves or their prey species and persecution by humans (Murray et al., 1999, Treves and Karanth, 2003, Woodroffe and Frank, 2005, Frank et al., 2006). Infectious diseases can have devastating consequences on threatened, lower density populations and many wild carnivore species are susceptible to lethal or debilitating pathogens harbored by domestic species (Roelke-Parker et al., 1996, Murray et al, 1999, Woodroffe, 1999, Cleaveland et al., 2007,) and co-infections can exacerbate clinical disease (Munson et al., 2008). The combination of infectious diseases and sub-optimal environmental conditions such as climate change, loss of habitat, overexploitation and environmental pollution, increases the risk of local extinctions (Smith et al., 2009). In addition, increased contact with domestic animals and livestock can result in emergence of significant pathogens for wildlife (Cleveland et al., 2007, Smith et al., 2009). For example, canine distemper virus and rabies in African wild dogs *(Lycaon pictus)* (Ginsberg et al., 1995, Kat et al., 1995), canine distemper virus in spotted hyenas *(Crocuta crocuta)* and lions (Roelke-Parker et al., 1996), *Mycobacterium bovis* in lions (Keet et al., 1996) and rabies in lions (Berentsen et al., 2013).

Infectious disease outbreaks are of particular concern for free-ranging lion populations given the current population estimate is 32,000 and the majority of populations are small and isolated (Bauer et al., 2012, Riggio et. al., 2013, IUCN 2014). Of the approximately 67 known areas in Africa where resident lion populations exist, 26

(39%) contain fewer than 50 individuals (Riggio et al., 2013). Given the rapid decline in lion numbers, protecting extant populations is critical. It is important from a conservation standpoint to evaluate how infectious diseases contribute to declining lion numbers and increased local extinction risk.

Our primary objective was to conduct a serologic and molecular surveillance survey for potential pathogens in a lion population from the Northern Tuli Game Reserve (NTGR) in eastern Botswana. At the beginning of the current study (Summer 2014) there were 39 known individuals in the NTGR lion population and 31 of these lions were within the borders of Botswana at that time. Although this population is currently experiencing a limited degree of immigration and emigration, the development of a 4870 km^2 Greater Mapungubwe Transfrontier Conservation Area (Figure 1.2) with lands from Botswana, South Africa and Zimbabwe will result in a larger metapopulation of lions.

Materials and Methods

Study Area

The privately owned NTGR covers 720 km^2 and consists of farms and commercially run wildlife lodges. The NTGR occupies a unique geographical area, as it sits within 30 km^2 wide block of land between Zimbabwe and South Africa (Figure 2.1). The NTGR's eastern border with Zimbabwe is demarcated by the Shashe River and the southern boundary is defined by the Limpopo River. The only boundary fences are along the western boundary and a section of the South African border; however, this fence is not well-maintained nor do these fences restrict the movement of lions and other large

carnivores (Snyman et. al., 2014). This region supports a diverse range of wildlife and has the ability to support a greater number of large predators due to ample space, water and prey species (Snyman et. al., 2014).

Sample Collection

Lions were located within the NTGR by reports from wildlife officers, global positioning system (GPS) satellite locations or radio telemetry from collared animals, baiting and calling stations, and tracking spoor. Samples were collected from 13 lions including 4 adult and 8 sub-adult lions (6 males, 6 females) in June and July 2014 and an adult male in 2012. This represents 59% of the 22 adult and sub-adult known lions (31 lions total including cubs) present in NTGR at the time of sampling in 2014. Lions were immobilized with a combination of 0.03-0.05 mg/kg medetomidine 20ml/ml (Kyron Laboratories) and 0.5-1.0 mg/kg Zoletil 100 (tiletamine-zolazepam) (Virbac, South Africa) based on estimated weight by dart injection by a Botswana registered wildlife veterinarian. Medetomidine was reversed with 0.2 mg/kg atimapezole 5mg/ml (Pfizer, South Africa) administered intramuscularly. Lions were identified by age and distinguishing characteristics and given complete physical exams. Blood samples were collected into EDTA and serum separator tubes via femoral or cephalic venipuncture. Clotted blood was centrifuged at 1,250 g for 15 minutes and serum separately frozen at -20 C until diagnostic testing. Representative ectoparasites were collected and preserved in 100% ethanol and submitted to Onderstepoort Veterinary Institute, South Africa for identification.

Serologic and Microbiological Diagnostic Testing

Blood and serum samples were submitted to the University of Pretoria, Department of Veterinary Tropical Diseases for serologic and molecular testing for selected pathogens. Serum samples were tested for antibodies to known viral pathogens of lions including canine distemper virus (CDV), feline coronavirus (FEC), feline calicivirus (FCV), feline herpesvirus (FHV), feline panleukopenia virus (FPV) and feline immunodeficiency virus (FIV-*Ple*) using indirect fluorescent antibody tests (CDV, FEC, FCV, FHV and FPV) and an enzyme-linked immunosorbent assay for FIV (using a strain of FIV from a wild felid).

Blood samples were tested for DNA of several tick borne pathogens by reverse line blot PCR assay. DNA was extracted from whole blood using the QIAamp DNA mini kit (Qiagen, Whitehead Scientific, South Africa) and tested for *Anaplasma/Ehrlichia* and *Babesia/Theileria* by amplification of the 16S and 18S rRNA genes, respectively, and hybridization with genus-wide probes. For individual species identification, amplicons were hybridized with the following species-specific probes as described: *E. ruminantium, E. chaffeensis, E. canis, A. centrale, A. marginale, A. phagocytophilum, A. bovis, A.* sp. omajienne, *B. bovis, B. bigemina, B. divergens, B. bovis, B. occultans, B. canis rossi, B. canis canis, B. canis vogeli, B. gibsoni, B. bicornis, B. caballi, B. sable, B. microti, B. felis, B. leo, B. lengau, T. bicornis, T. sable, T.* sp. Kudu, *T. annulata, T. equi, T. buffeli, T. sp. buffalo, T. mutans, T. parva, T. taurotragi, T. velifera, T. lestoquardi, T. ovis, T. annae* (actually a *B. microti*-like sp.), and *T. separata* (Bosman et al., 2010; Ros-Garcia et al., 2011). Nested PCR testing for *Trypanosoma* spp. was conducted as described (Delespaux et al., 2003). For two samples positive with only the *Babesia*

genus-wide probe, DNA was purified and directly bidirectionally sequenced (Boseman et al., 2010) (Inqaba Biotec, Pretoria, South Africa). For two additional lions which were positive with more than one species specific probe, purified DNA was cloned and seven clones were sequenced for each lion (Inqaba Biotec). Sequences were analyzed and assembled in Geneious R7 (Biomatters Ltd., Auckland, New Zealand). Sequences obtained from this study and related sequences were aligned using the multisequence alignment tool in MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 program (Kumar et al. 1994). Phylogenetic analysis using the neighbor-joining algorithm using the Kimura 2-parameter model and maximum parsimony using a heuristic search was conducted in MEGA. Sequences will be submitted to GenBank.

Results

Serologic Testing

Serologic testing indicated that lions from the NTGR population had exposure to all tested viral pathogens with the exception of FEC (Table 2.1). Low prevalence rates were noted for FPV (7%), FCV (7%) and CDV (15%). High prevalence rates were detected for FIV-*Ple* (76%) and FHV (84%). No notable differences in prevalences were detected based on age or sex, however this might be attributed to the small sample size.

Molecular Testing and Molecular Characterization

All 13 lions were positive for *Babesia* genus-wide probes. Only two lions were positive for any tested species probes; one lion, (lion 2) was positive for *B. felis*, *B.*

lengau, *B. microti*, and *T. bicornis* probes and the other lion, (lion 3) was positive for *B. felis, B. lengau* and *B. microti* probes. These two lions are brothers who formerly predated on cattle near one of the villages in the region.

Sequences from the seven clones from lion 2 showed three *Babesia* species were present including five related to *B. felis*, one related to *B. lengau*, and one novel *Babesia* sp. Lion 3 was infected with at least three *Babesia* species; two sequences were related to *B. lengau*, one to *B. felis*, and two unique sequences (99.1% similar to each other) were related (95.6-96%) to the novel *Babesia* sp. detected in lion 2. Phylogenetically, the two sequences from lion 3 (clones 2 and 7) formed a separate clade from other *Babesia* spp. and was distinct from the sequence from lion 2, clone 9 (Figure 2.1). The four full-length *B. felis*-like sequences formed a separate clade which was related to a *Babesia* sp. from a caracal (*Caracal caracal*). The three *B. lengau*-like sequences were included in a clade with other *B. lengau* sequences from a cheetah (*Acinonyx jubatus*) and domestic cat.

Sequences from two lions only positive for the genus-wide probe were 99.9% similar (1510/1511 bp) to each other and were related to various *Babesia canis* sequences (most similar [98.1%] to *Babesia* sp. Xinjiang (AB083376). Phylogenetically, these sequences formed a distinct group from all of the four recognized subspecies of *B. canis* (Figure 2.1). All lions were RLBH negative for genus-wide probes for *Anaplasma, Ehrlichia,* and *Theileria* and were PCR negative for *Trypanosoma*.

Four species in the Ixodidae family of ticks were found on six lions (from three different prides) in the NTGR and identified as, *Rhipicentor nuttalli, Rhipicephalus simus, Rhipicephalus sulcatus and Rhipicephalus appendiculatus* (Table 2.2).

Discussion

This NTGR lion population is at potential risk, because it is a low-density population, similar to ~ 40% of other lion populations which contain < 50 individuals (Riggio et al., 2013). These populations are under threat from numerous deterministic and stochastic threats, including infectious diseases, so knowledge on exposure rates is critical. Prior to this study, only a single multi-pathogen survey had been conducted on lions in Botswana (Central Kalahari and Khutse Game Reserves [CKGR and KGR] which are over 700 km^2 from NTGR) and this survey was restricted to twenty-one lions and was based on serologic testing for selected viruses and tick borne pathogens (Ramsauer et al., 2007).

Similar results were obtained for serologic testing for viruses in lions from the CKGR/KGR and NTGR. Low prevalence rates of FEC, FPV, and CDV indicate that both of these populations are at risk for clinical disease should the pathogen(s) be introduced, densities increase or if lions become stressed or co-infected with other pathogens that increase disease synergistically (Munson et al., 2008). Among these three viruses, CDV has been most often associated with clinical disease in free-ranging lions. Lions co-infected with CDV and *Babesia* spp. suffered high morbidity and mortality rates in 1994 in the Serengeti ecosystem and in 2001 in the Ngorongoro Crater populations in Tanzania (Munson et al., 2008). Only two lions in NTGR were seropositive for CDV and both belong to the same pride; a male (lion 2) that had previously predated on cattle near a large village, and a related female (lion 12).

High antibody prevalence rates for both FIV-*Ple* and feline herpesvirus was anticipated, as these pathogens are endemic to most lion populations sampled in southern and eastern Africa (Spencer et al., 1991, 1992 and 1993, Hofmann-Lehmann et al., 1996, Packer et al., 1999, Driciru et al., 2006, Ramsaur, et al., 2007, Roelke et al., 2009). Feline herpesvirus is a common virus found in domestic and free-ranging felid populations (Thompson et al., 1971). All serosurveys published on free-ranging lions report exposure to feline herpesvirus. Lions sampled in Namibia and Uganda have reported prevalence rates of 67% and 62% (Spencer 1992, Driciru et al, 2006); however, this study found lions in the NTGR had an 84% prevalence rate; this is in accordance with populations sampled in South Africa and Botswana with rates approaching 90% or above (Spencer 1991 and 1993, Roelke et al., 2009) and populations sampled in Tanzania are close to or at 100% prevalence for FHV (Hofmann-Lehmann et al., 1996, Packer et al., 1999).

All published FIV-*Ple* surveys of free-ranging lion populations report high prevalence of FIV-*Ple* with the exception of lions in Etosha National Park, Namibia, which is the only population surveyed with published evidence of no FIV-*Ple* exposure (Spencer 1992). The NTGR population displayed a high prevalence rate of 76% for FIV-*Ple*. A recent FIV-*Ple* study conducted on lions in the Okavango Delta, Chobe National Park and Makgadikgadi and Nxai Pans, Botswana found FIV-*Ple* prevalence rates of 96%, 100% and 18% respectively (Roelke et al., 2009). It is generally been accepted that strains of FIV-*Ple* do not cause obvious clinical disease in lions; however, recent research suggests FIV-*Ple* could be contributing to a loss in immune competence (Roelke et al., 2009, O'Brien et. al., 2012) and some subtypes of FIV-*Ple* are possibly more pathogenic

than others (Troyer et al., 2011). Further, recent research on the variance in pathogenicity of each of the six genetically distinct subtypes of FIV-*Ple* found that the CDV infected lions in the 1994 outbreak were twice as likely to survive the outbreak if they were infected with FIV-*Ple* subtype B, compared to lions infected with subtypes A or C. Higher mortality among lions coinfected with CDV and FIV-*Ple* subtypes A or C resulted in modified FIV-*Ple* strain prevalence as lions infected with subtype C declined, but lions with subtype B experienced lower mortality during the outbreak (Troyer et al., 2011, O'Brien et al., 2012).

Lions are common hosts for *Babesia* parasites and at least four described species have been reported (*B. felis, B. leo, B. lengau,* and *B. gibsoni*) with the former three species being most common (Penzhorn et al., 2001; Munson et al., 2008; Williams et al., 2014). In addition, an uncharacterized *Babesia* species has been reported from lions in Tanzania, but no sequences were available for comparison. Of the three most common species reported from lions, we detected *B. felis* and *B. lengau*. The lack of *B. leo* infections was somewhat surprising because this parasite has been reported in numerous populations of lions in South Africa, Zambia, Swaziland, and Tanzania (Bosman et al., 2007; Munson et al., 2008; Williams et al., 2014); however, during a 20 year study in Tanzania, *B. leo* was only detected in two lions from the 1980s.

Coinfection with multiple *Babesia* spp. has been reported in lions (Bosman et al., 2007; Munson et al., 2008) and based on sequencing of several clones from two lions, we detected at least three *Babesia* species infecting each lion. A longitudinal study in Tanzania noted that coinfections were common and infections were randomly detected

over time. Importantly, however, *Babesia* coinfections were correlated with high mortality rates in two lion populations during concurrent CDV outbreaks in 1994 and 2001 (Munson et al., 2008).

Molecular characterization of samples from four lions revealed four species most similar to *B felis, B. lengau, B. canis vogeli* and an undescribed species related to *B. microti*. Sequencing of cloned PCR products from two lions indicated that a high diversity of unique *Babesia* sequences were present, and nine of these unique sequences were similar to two described species (*B. felis* and *B. lengau*). The remaining three sequences represent either one or possibly two novel *Babesia* species. In general, they were related to a large group of small babesid parasites of carnivores and rodents. Additional studies are needed to better understand the natural history of this novel *Babesia*. Finally, we detected two lions infected with a variant of *B. canis*. Although one subspecies of *B. canis* has been reported from felids (*B. c. presentii*), the sequences from the Botswana lions were more similar to a *B. canis* sequence from a red-cheeked ground squirrel (*Spermophilus erythrogenys*) from China (Zamoto et al., 2004).

The four species in the Ixodidae family of ticks collected from the NTGR lions were identified as, *Rhipicentor nuttalli, Rhipicephalus simus, Rhipicephalus sulcatus and Rhipicephalus appendiculatus*. *Rhipicentor nuttalli* is one of two species in the genus and occurs only in Africa. The adult ticks preferred hosts are free-ranging canids, felids and South African hedgehogs (*Atelerix frontalis*) (Fourie et al., 2002). Although ticks in the *Rhipicephalus* genus occur worldwide, approximately 60 of the 80+ species are found in Africa. *Rhipicephalus appendiculatus* is economically an important parasite as it is a known vector for several infectious pathogens in livestock and humans (Walker et al.,

2000). In another study, ticks collected from three lions in Botswana were identified as *Hyalomma truncatum*. *Amblyomma hebraeum* and *Rhipicephalus simus* were also recovered from two lions in the Limpopo Province, South Africa (Horak et al., 2010). The ticks *Amblyomma hebraeum, Haemaphysalis leachi,* and *Rhipicephalus simus* were found in large numbers on a lioness in Kruger National Park (Horak et al., 1987). Several of these tick species are suspected vectors for *Babesia* species that infect free-ranging felids; however, the vector(s) have yet to be identified (Penzhorn et al., 1999).

All of the lions sampled in this study appeared to be in good physical condition and although most of the viral and parasitic pathogens detected in our study are found in healthy lions, disease may be triggered by various factors such as stress due to loss of habitat, human persecution and over exploitation. In addition, individuals immunocompromised from co-infections, increases in population densities, or changes in interactions with sympatric wild or domestic carnivores can elicit clinical disease signs. The NTGR population has many of these possible concerns including being low-density which can result in loss of genetic diversity and reduced disease resistance (Acevedo-Whitehouse, et al., 2003, Kissui and Packer, 2004). The current study provides baseline pathogen data which will be critical once the NTGR is incorporated into the Greater Mapungubwe Transfrontier Conservation Area. This development should overall, be advantageous for this population, as metapopulations are better equipped to recover from the effects of stochastic events, including disease outbreaks (Alexander et al., 2008) and genetic diversity of the population should be improved. Additional efforts should be made to minimize interactions of free-ranging lions with domestic dogs and livestock to minimize pathogen spill-over events (e.g., buffer zones between wildlife and domestic

animal areas (Smith et al., 2009), vaccination campaigns for domestic canines and livestock (Cleveland et al., 2007) and well-informed decisions regarding the translocation of individuals. Lions will continue to experience greater habitat restriction and fragmentation due to human and domestic animal encroachment and transmission of infectious agents from domestic animals to free-ranging carnivores is likely to become more frequent and a conservation matter of increasing importance (Murray et al., 1999).

Table 2.1 Demographics and results of serologic testing for selected viruses in 13 lions from the Northern Tuli Game Reserve.

Lion No.	Age (yrs)	Sex	Sample Date	Antibody Titers CDV*	Antibody Titers FEC	Antibody Titers FCV	Antibody Titers FHV	Antibody Titers FPV	Antibody to FIV-*Ple*
1	9	M	2012	-	-	-	**1:320**	-	-
2	3	M	May 2014	**1:20**	-	-	**1:160**	-	+
3	3	M	May 2014	-	-	-	**1:320**	-	+
4	2	M	June 2014	-	-	-	**1:320**	-	+
5	2	F	June 2014	-	-	**1:20**	**1:160**	-	+
6	6	M	June 2014	-	-	**1:80**	**1:40**	**1:80**	+
7	6	M	June 2014	-	-	-	**1:80**	-	+
8	3	F	July 2014	-	-	-	**1:80**	-	+
9	3	F	July 2014	-	-	-	**1:80**	-	+
10	3	F	July 2014	-	-	-	**1:640**	-	+
11	3	M	July 2014	-	-	-	**1:640**	-	-
12	15	F	July 2014	**1:10**	-	-	-	-	+
13	8	F	July 2014	-	-	-	-	-	-
Overall prevalence (%)				**15**	**0**	**15**	**85**	**8**	**77**

Canine distemper virus (CDV), Feline coronavirus (FEC), Feline calicivirus (FCV), Feline herpesvirus (FHV), Feline panleukopenia virus (FPV), Feline immunodeficiency virus (FIV-*ple*)

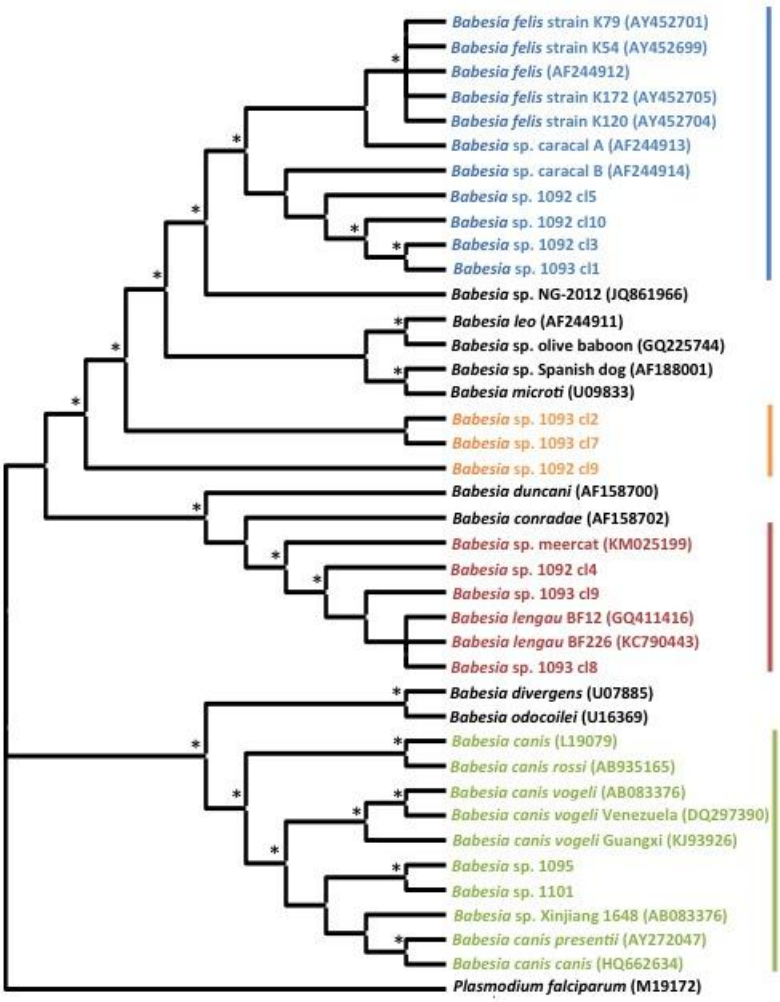

Figure 3.1. Phylogenetic tree showing relationships of the various *Babesia* detected in lions from Northern Tuli Game Reserve (NTGR). *Babesia felis* and related sequences are shown in blue, *Babesia lengau* and relatives are shown in red and *Babesia canis* subspecies are shown in green. The novel *Babesia* detected in two lions in NTGR are shown in orange.

Table 2.2. Ticks detected on six lions from Northern Tuli Game Reserve.

Lion	Date Collected	Tick Identification
Male Lion 1	2012	*Rhipicentor nuttalli* 2♂
Male Lion 3	24 May 2014	*Rhipicentor nuttalli* 1♂ *Rhipicephalus appendiculatus* 2♀
Female Lion 5	29 June 2014	*Rhipicentor nuttalli* 1♀
Male Lion 6	30 June 2014	*Rhipicephalus simus* 18♂ 2♀ *Rhipicephalus sulcatus* 1♀
Male Lion 7	30 June 2014	*Rhipicephalus simus* 7♂ 2♀ *Rhipicephalus sulcatus* 1♀
Female Lion 8	02 July 2014	*Rhipicentor nuttalli* 1♂ 1♀

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CHAPTER 3

CONCLUSIONS

This study was conducted to obtain data on the prevalence and diversity of viral and parasitic pathogens for the Northern Tuli Game Reserve (NTGR) lion population in eastern Botswana. We identified, conducted physical exams and collected blood, serum and ectoparasites from 59% of the adult and sub-adult NTGR lions. Exposure to specific viruses was determined by serology and exposure to select hemoparasites was verified by standard PCR and reverse line blot hybridization assay. Sequencing of amplicons from four lions revealed four species of *Babesia*. Four species in the Ixodidae family of ticks were collected and identified from six lions in the NTGR.

Conservation Implications

Although the most severe threats to lions are from human persecution and habitat loss, infectious diseases are capable of not only reducing host fitness; but, decimating lower-density, isolated populations. Infectious disease epidemics are one reason for the rapid decline in lion numbers on the continent and high mortality epizootics can have devastating consequences on small, genetically vulnerable populations such as the NTGR lion population. The NTGR lion population is representative of numerous extant lion populations in Africa today, low-density, fragmented, persecuted by humans and living outside the borders of a national park.

Wildlife disease surveillance is an important aspect of wildlife management and conservation, especially with the continued development of the Mapunugbwe Transfrontier Conservation Area. The baseline data collected on the NTGR lions in this study provides critical information moving forward as these lions are incorporated into a larger metapopulation with lions from South Africa and Zimbabwe. The importance of serological surveys to accrue baseline data on pathogen prevalence paired with field-based research is an essential component to protecting and conserving carnivore populations.

This disease survey is only the second known multi-pathogen assessment conducted on lions in Botswana; therefore, these findings allow for the larger examination of disease threats to free-ranging lions in Botswana and contribute data to ongoing research regarding the ecology and epidemiology of carnivore pathogens in African ecosystems.