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Source: Journal of Wildlife Diseases, 47(3) : 494-500

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-47.3.494

Journal of Wildlife Diseases, 47(3), 2011, pp. 494–500

INBREEDING DEPRESSION INCREASES SUSCEPTIBILITY TO BOVINE TUBERCULOSIS IN LIONS: AN EXPERIMENTAL TEST USING AN INBRED–OUTBRED CONTRAST THROUGH TRANSLOCATION

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ABSTRACT: Disease can dramatically influence the dynamics of endangered wildlife populations, especially when they are small and isolated, with increased risk of inbreeding. In Hluhluwe-iMfolozi Park (HiP), a small, enclosed reserve in South Africa, a large lion (*Panthera leo*) population arose from a small founder group in the 1960s and started showing conspicuous signs of inbreeding. To restore the health status of the HiP lion population, outbred lions were translocated into the existing population. In this study, we determined the susceptibility to bovine tuberculosis (bTB), and the prevalence of antibody to feline viruses of native lions, and compared the findings with those from translocated outbred lions and their offspring. Antibodies to feline herpesvirus, feline calicivirus, feline parvovirus, and feline coronavirus were present in the lion population, but there was no significant difference in antibody prevalence between native and translocated lions and their offspring, and these feline viruses did not appear to have an effect on the clinical health of HiP lions. However, feline immunodeficiency virus (FIV), which was previously absent from HiP, appears to have been introduced into the lion population through translocation. Within 7 yr, the prevalence of antibody to FIV increased up to 42%. Bovine tuberculosis posed a major threat to the inbred native lion population, but not to translocated lions and their offspring. More than 30% of the native lion population died from bTB or malnutrition compared with <2% of the translocated lions and their offspring. We have demonstrated that management of population genetics through supplementation can successfully combat a disease that threatens population persistence. However, great care must be taken not to introduce new diseases into populations through translocation.

Key words: Bovine tuberculosis, disease, feline immunodeficiency virus, inbreeding, *Panthera leo*.

INTRODUCTION

Infectious disease can dramatically influence the dynamics of wildlife species and populations, and has emerged as a central issue in carnivore conservation (Scott, 1988), with viruses causing several major declines in carnivore populations (Young, 1994). Canine distemper virus killed over 35% of lions in the Serengeti, East Africa (Roelke-Parker et al., 1996); rabies caused population declines in Ethiopian wolf (*Canis simensis*; Sillero et al., 1996) and African wild dog (*Lycaon pictus*; Kat et al., 1995); and bovine tuberculosis (bTB) is a pathogen of growing concern in free-ranging wildlife in Southern Africa (Keet et al., 1996). In the Kruger National Park, South Africa, bTB spread rapidly in wildlife populations resulting in morbidity and mortality in buffalo (*Syncerus caffer*; Keet et al., 1996), lion (Keet et al., 1996), and cheetah (*Acinonyx jubatus*; de Lisle et al., 2002) populations.

When wildlife populations are small and isolated, they are more threatened by infectious disease because they face the risk of inbreeding (Reid et al., 2003). Mortality in natural populations is not random, and inbreeding can have a significant impact on the dynamics of wildlife diseases (Acevedo-Whitehouse et al., 2003). For example, inbred rabbits (*Oryctolagus cuniculus*) are more susceptible to tuberculosis than outbred rabbits (Dorman et al., 2004). In Hluhluwe-iMfolozi Park (HiP), a small, enclosed

reserve in South Africa, a lion population established from a small group of founders in the 1960s started showing conspicuous signs of inbreeding by the early 1990s (Maddock, 1996). Lions in HiP showed little genetic variation, and records of abscesses, generally poor condition, and postmortem evidence of reduced immune-competence were all thought to indicate inbreeding (Stein, 1999). To restore the health status of lions in HiP, outbred lions were translocated into the existing population (Trinkel et al., 2008).

In the present study, we evaluated the susceptibility to various diseases of inbred and outbred lions. Specifically, we assessed the impact of bTB on native and translocated animals and their offspring, and determined the prevalence of antibody to important feline pathogens: feline immunodeficiency virus (FIV), canine distemper virus (CDV), feline herpesvirus (FHV), feline parvovirus (FPV), feline coronavirus (FCoV), and feline calicivirus (FCV).

MATERIALS AND METHODS

Study area and study animals

Hluhluwe-iMfolozi Park (900 km^2) is in KwaZulu-Natal, South Africa between 28°00′S and 28°26′S, and 31°43′E and 32°09′E. The entire perimeter of HiP is fenced and borders on rural communities. The native HiP lion population descends from five individuals: a male who entered the park on his own in 1958 and two females with two cubs who were introduced in 1965. The population increased to 140 individuals by 1987 (Maddock, 1996) and started displaying indications of inbreeding, including low reproductive rate and high adult mortality (Maddock, 1996; Stein, 1999). In 1999, the HiP population consisted of about 80 lions (Trinkel et al., 2008). To restore the genetic diversity of the native HiP lion population, 16 outbred lions from the Pilanesberg National Park and the Madikwe Game Reserve, South Africa were translocated into HiP between 1999 and the beginning of 2001. The lions were sourced from Pilanesberg and Madikwe because they originated from Etosha National Park in Namibia, thus maximizing the genetic distance from the HiP population. By the end of 2004, the native HiP population had decreased to 20 lions, while translocated lions and offspring involving at least one translocated animal comprised 42 animals (Trinkel et al., 2008). Descendents with at least one translocated lion in their lineage replaced the entire purebred native lion population by 2006 (Trinkel et al., 2008). Between 1999 and 2004, systematic call-ups (tape-recorded vocalizations to attract lions) were conducted and all individuals were captured and individually marked with a brand. This enabled us to accurately track native and translocated individuals and their offspring.

Bovine tuberculosis

The predominant route by which lions become infected with *Mycobacterium bovis* is through consumption of infected buffalo (Keet et al., 1996). Buffalo are considered to be one of the four preferential prey species of lions (Mills, 1995), and frequent exposure to large amounts of infectious buffalo tissue can lead to a spread of bTB within lion prides in areas where bTB prevalence in buffalo is high (Michel et al., 2006). In 1999 herd bTB prevalence in buffalo was as high as 20% in the northern part of HiP to 60% in selected herds in the south of HiP. A bTB control program started in 1999: over 5,500 buffalo were captured and tested, and bTB-positive buffaloes (>1,000) were culled (Michel et al., 2006). By 2009, there was a reduction in prevalence to <10% in northern HiP and <25% in the southern part of the park (Michel et al., 2006; Jolles, 2007; D. Cooper, unpubl. data).

Between 2000 and 2009, native and translocated lions and their offspring that died or were destroyed due to poor condition were subjected to postmortem examination during which specimens of selected organs and lesions were tested for bTB. The organ specimens for histopathologic examination were preserved in 10% buffered formalin and shipped to the Department of Pathology, Faculty of Veterinary Science, Onderstepoort, South Africa, or to Vetdiagnostix, Veterinary Pathology Services, Cascades, South Africa. Sections for light microscopic examination were cut using routine procedures. The sections were stained with hematoxylin and eosin. Selected tissue sections were stained by the Ziel-Neelsen method for the detection of acid-fast bacteria. Specimens of the spleen, lungs, kidneys, heart, and various affected lymph nodes of the lions were processed and cultured, and any organisms isolated were identified as previously described (Bengis et al., 1996).

TABLE 1. Native lions dying from bovine tuberculosis (bTB) and native lions that were destroyed due to poor condition between 2000 and 2005; translocated lions and offspring dying from bovine tuberculosis and those that were destroyed due to poor condition from 2000 to 2005 and from 2006 to 2009, Hluhluwe-iMfolozi Park, South Africa.

Population	Years	Population size	Lions dying from bTB (n, %)	Lions destroyed (n, %)
Native lions	2000–2005	104	14.4 (15)	17.3 (18)
Translocated lions and offspring[a]	2000–2005	63	0.0 (0)	1.6 (1)
Translocated lions and offspring[a]	2006–2009	>100	<2.0 (2)	<3.0 (3)

[a] Involving one introduced parent.

Serologic assays

In 2001 and 2002, blood samples were collected from native lions (n=31), translocated lions (n=5), and offspring involving one introduced parent (n=3). In 2006, blood samples of 19 lions were taken. Based on the results of Trinkel et al. (2008), we presumed that all 19 lions resulted from out-crossed pairings. Serologic assays were performed to assess the lions' exposure to FIV (Osofsky et al., 1996), CDV (Roelke-Parker et al., 1996), FHV, FPV, FCoV, and FCV (Hofmann-Lehmann et al., 1996). Serum samples were frozen in a −20 C freezer and sent to the Department of Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, South Africa, for analysis. For data analysis, we treated "translocated lion" and "offspring involving one translocated parent" as one group called "translocated lion and offspring."

RESULTS

Between 2000 and 2005, 15 native lions died from bTB (Table 1). An additional 18 native lions were euthanized due to poor condition. Two of these native lions were negative for bTB, 16 others were not tested (too much time had passed since death for postmortem assessment) and the reason for their poor condition was unknown. During the same time period, none of the translocated lions and none of the offspring involving at least one translocated parent died from bTB (Table 1). One translocated female lion was destroyed due to poor condition, and she was negative for bTB. Between 2000 and 2005, there was a significant difference in the frequency of bTB deaths between native and translocated lions and offspring (χ^2=9.98, df=1, P<0.005). Combining bTB deaths and lions destroyed between 2000 and 2005, there was a significant difference in the frequency of deaths between native and translocated lions and offspring (χ^2=21.98, df=1, P<0.001). Between 2006 and 2009, two lions had died from bTB and three died from malnutrition. There was a significant difference in the frequency of bTB deaths between native lions and translocated lions and offspring sampled between 2006 and 2009 (χ^2=10.30, df=1, P<0.005). Combining bTB deaths and lions dying from malnutrition, there was a significant difference in frequency of deaths between native lions and translocated lions and offspring (χ^2=2403, df=1, P<0.001). Based on the results of Trinkel et al. (2008), we assumed that all lions sampled between 2006 and 2009 were out-crossed pairings.

Lions in HiP had antibodies against FCoV, FHV, FCV, FPV and FIV, but not against CDV. In 2001 and 2002, the overall antibody prevalence was highest for FHV (97%), followed by FCV (51%), FPV (41%), FIV (15%), and FCoV (10%). For all viruses tested, there was no significant difference in antibody prevalence between native and translocated lion and offspring (Fisher's exact test: FHV, P=0.79; FCV, P=0.13; FPV, P=0.12; FIV, P=0.58; FCoV, P=0.37). In 2001, FIV was recorded in HiP for the first time.

TABLE 2. Prevalence of antibody to six feline viruses in native lions and translocated lions and offspring in 2001–2002 and 2006, Hluhluwe-iMfolozi Park, South Africa.

Population	Year	Sample size	Antibody prevalance[a] (%)					
			CDV	FCoV	FHV	FCV	FPV	FIV
Native lions	2001–2002	31	0	3	97	58	45	13
Translocated lions and offspring	2001–2002	8	0	13	100	25	13	25
Translocated lions and offspring	2006	19	0	21	100	0	15	42

[a] CDV = canine distemper virus; FcoV = feline coronavirus; FHV = feline herpesvirus; FCV = feline calicivirus; FPV = feline parvovirus; FIV = feline immunodeficiency virus.

Although not statistically significant (Fisher's exact test, $P=0.58$) antibody prevalence was about two times higher in introduced lions and offspring (25%) compared with native lions (13%; Table 2). Two translocated lions (one female, one male) were positive for FIV antibody. Native FIV antibody–positive lions were two females living together in the same pride with the translocated FIV antibody–positive male, and two nomadic males, which were observed in contact with an introduced FIV antibody–positive lioness. In 2006, CDV was still absent from HiP. Compared with the native lion population in 2001 and 2002, by 2006 there was a significant increase up to 42% in prevalence of antibody to FIV in the lion population (Fisher's exact test, $P=0.04$).

DISCUSSION

Lions in HiP were exposed to and became infected with various feline viruses, including FHV, FCV, FCoV, and FPV, with antibody prevalences comparable to those in other ecosystems (Packer et al., 1999; Spencer, 1992). We did not find any significant differences in prevalence of antibody to these viruses between inbred native and outbred translocated lions, suggesting that these diseases were not the reason for the decline of the inbred native HiP lion population. In fact, none of these feline viruses seemed to have great importance for the clinical health of HiP lions and they did not threaten population persistence. Canine distemper is an infectious disease of carnivores with a high fatality rate (Appel and Summers, 1995) and killed 35% of the Serengeti lions in East Africa within 6 mo (Roelke-Parker et al., 1996). There was no evidence of CDV infection in HiP lions. Similarly, Spencer (1998) reported the absence of canine distemper from other reserves in South Africa, where wildlife and domestic animals are usually separated by fencing, making it less likely that canine distemper from domestic dogs surrounding the ecosystem is transmitted (Roelke-Parker et al., 1996; Spencer, 1998).

Bovine tuberculosis was a major problem in native HiP lions but not in translocated lions and offspring. The primary route of bTB infection in lions is through consumption of infected buffalo; thus, the probability of infection with bTB for native and translocated lions, provided that the health status of both groups is similar, should be equal. However, this was not the case. More than 30% of native lions died from bTB or were dramatically emaciated and subsequently destroyed. There was a clear benefit from introducing new genetic material via translocation: bTB in HiP lions was reduced to less than 2%. As a result of the bTB control program, the number of infected buffalos has been greatly reduced, which reduced the risk of spillover into predators. Therefore, one possibility for the absence of bTB deaths in the translocated animals and their offspring might be a long

incubation period of bTB. In cattle the symptoms of bTB usually take months to develop (Iowa State University College of Veterinary Medicine, 2009). Infections can also remain dormant for years and reactivate during periods of stress or disease (Iowa State University College of Veterinary Medicine, 2009). The latency of infection in lions is unknown. However, >50% of native lions that died from bTB during our study were less than 5 yr old. Thus, we can dismiss length of exposure as a reason for fewer bTB deaths in translocated lion and offspring. Native HiP lions showed indications of inbreeding, including reduced reproductive rate and high adult mortality (Maddock et al., 1996; Trinkel et al., 2008), and inbred lions are especially susceptible to infectious disease (Packer et al., 1991; Kissui and Packer, 2004). Inbreeding depression, therefore, could explain the native HiP lions' high bTB infection rate. The reason why translocated lions and their offspring coped better with bTB may be due to a healthier immune system (Springer, 1990).

Before 2000, FIV was absent from HiP (Van Vuuren et al., 2003). In this study, we found an FIV antibody prevalence of 14% in 2001–2002, which increased to 42% in 2006. Feline immunodeficiency virus is considered to be nonpathogenic in lions (Packer et al., 1999) and, so far, FIV does not seem to have great importance to the clinical health of HiP lions. However, the interaction between diseases also has to be considered. Coinfection with FIV in lions in Kruger National Park, South Africa, was suggested to be the reason for a high mortality rate caused by bTB: 90% of Kruger lions monitored in an area with high herd prevalence of bTB in buffalo became infected (Keet et al., 1996); prevalence of FIV infected lions in that area was 83% (Spencer, 1992). Coinfection does not necessarily need to become such an important problem in HiP. In the Serengeti, where bTB is also present in wildlife (Cleaveland et al., 2005), more than 90% of lions are FIV positive (Hofmann-Lehmann et al., 1996) but bTB is not a problem in Serengeti lions.

Translocation of lions without consideration of their disease status can lead to unexpected consequences. It may be that FIV was transferred into HiP when outbred lions were introduced. However, all translocated lions were tested for FIV prior to being translocated, which highlights unanticipated risks of inconclusive disease testing. Our study illustrates the importance of disease surveillance prior to translocations (IUCN, 2010) and emphasizes the need for reliable disease tests and handling to reduce false-negative results. Most importantly, we have demonstrated that management of population genetics through supplementation can successfully combat a disease that threatens a population's persistence.

ACKNOWLEDGMENTS

We thank the HiP management staff, who were an integral part of the project from its inception to completion, and who spent many hours (particularly at night) working on the introduction and monitoring that followed. We thank the University of Kwazulu Natal (UKZN) research assistants for data collection, especially Pete MacFarlane, Luca Turelli, Jan Graf, Dale Airton, Caroline Reid, and Angela Reid. This innovative project would not have been possible without Gus van Dyk, Dave Balfour and Pete Hartley. We thank Dave Balfour, Sue van Rensburg, Owen Howison, Ruth Howison, and Dave Druce from Ezemvelo Kwazulu Natal (KZN) Wildlife Hluhluwe Research Centre for logistical support, and providing access to Ezemvelo Kwazulu Natal (EKZN) Wildlife data. This project was funded by: THRIP grant to N. Ferguson, National Research Foundation and UKZN grants to R. Slotow, National Science Foundation, Walt Disney Foundation and MGM Grand Hotel grants to C. Packer, Wild about Cats, Hluhluwe Tourism Association, Bateleurs and Wildlife Conservation Trust (KZN). We thank the staff and interns who provided assistance and information.

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Submitted for publication 22 September 2010.
Accepted 2 March 2011.