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The epidemiology of lion lentivirus infection among a population of free-ranging lions (*Panthera leo*) in the Kruger National Park, South Africa

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ABSTRACT

Feline immunodeficiency virus is a lentivirus of domestic cats that causes significant lifelong infection. Infection with this or similar lentiviruses has been detected in several nondomestic feline species, including African lions (*Panthera leo).* Although lion lentivirus (FIVple) infection is endemic in certain lion populations in eastern and southern Africa, little is known about its pathogenic effects or its epidemiological impact in free-ranging lions. This report describes the epidemiological investigation of lentivirus positivity of free-ranging lions in the Kruger National Park, South Africa. A nested polymerase chain reaction assay for virus detection was performed on all whole blood samples collected. In addition, serum samples were tested for cross-reactive antibodies to domestic feline lentivirus antigens and to puma lentivirus synthetic envelope peptide antigen. The results were analysed in conjunction with epidemiological data to provide a descriptive epidemiological study on lion lentivirus infection in a free-ranging population of lions. The overall prevalence of lentivirus infection was 69 %, with a prevalence of 41 % in the north of the park, and 80 % in the south. Adult males had the highest prevalence when combining the factors of sex and age: 94 %. The lowest prevalences were found among juveniles, with male juveniles at 29 %. Adults were 5.58 times more likely to test positive for FIVple than juveniles, with adult males being 35 times more likely to be test positive for FIVple compared with juvenile males. This research represents the 1st epidemiological study of the lion lentivirus among free-ranging lions in the Kruger National Park.

Keywords: feline immunodeficiency virus, Kruger National Park, lentivirus, prevalence, lions, *Panthera leo,* South Africa.

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INTRODUCTION

Feline immunodeficiency virus of domestic cats (FIVfca) is a member of the genus *Lentivirus* in the *Retroviridae* family and causes a significant lifelong infection in domestic felines *(Felis catus).* The virus was first discovered in domestic cats with a history of chonic recurrent and opportunistic infections that resembled the human immunodeficiency virus (HIV) infection[17]. Lentiviral infections have also been detected in several nondomestic feline species, including African lions (*Panthera leo)* populations in eastern and southern Africa[4,5,13,15,19]. Feline lentivirus infection leads to immunological dysfunction and immunosuppressive disease in domestic cats. However, little is known about the pathogenic effects of infection or its epidemiological impact on free-ranging and captive populations of lions. The lentivirus infecting lions in southern Africa has never been isolated and few published studies exist on the epidemiology of the virus among free-ranging lion populations. As a result, little is known about the characteristics of the lentivirus in lions at the host and population levels.

An estimated 2000 African lions (*Panthera leo)* reside in the Kruger National Park (KNP) in the South Africa and are regarded as one of the largest subpopulations of lions in southern Africa (D Keet, pers. obs., 2006). The park is South Africa's largest wildlife refuge and a critical biodiversity resource, bordering Zimbabwe to the north and Mozambique to the east. From north to south, KNP stretches roughly 320 km, and 65 km from east to west. The lions of KNP have previously demonstrated serological evidence of infection with lion lentivirus, with virus-specific antibodies detected in the population as early as 1977[19]. The purpose of this investigation was to provide an epidemiological description of the lion lentivirus among the lions in KNP.

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MATERIALS AND METHODS

Sample collection and storage

The samples available for evaluation were from a 2004 census of 77 lions in KNP, from which 73 whole blood and 63 serum samples were taken. All samples were stored at –80 °C until the time of processing and testing, in 2006.

Epidemiological data

Epidemiological data were collected from 77 KNP lions at the time of blood collection. Data gathered included pride name, as designated by KNP research staff; approximate location in KNP, as designated by recognisable landmarks; animal's identification number (microchip ID number implanted upon 1st immobilisation in each KNP lion; numbers designated by KNP state veterinarian); sex; age as estimated by tooth morphology (D Keet, pers. comm., 2006); body weight in kilograms; and body condition score (1–5) as follows: 5, Excellent: hindquarters well rounded and no ribs showing; general appearance in relation to posture and coat sheen excellent; 4, good: hindquarters rounded, ribs showing slightly; 3, fair: hindquarters angular in appearance and ribs well defined; 2, poor: pelvic bones and pelvic-femoral joint prominent and ribs protruding, tail root sunken in, dorsal spine of vertebrae becomes apparent; 1, very poor: skeletal details clearly visible and general appearance, posture, and coat condition deteriorated, dorsal and lateral processes of vertebrae clearly visible.

DNA extraction

Epidemiological data were correlated with the results from an indirect enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) assay

for each animal for which all data were available. DNA was extracted from the samples using a QIAGEN DNeasy Blood and Tissue Extraction kit (Invitrogen, Carlsbad, CA, USA), resulting in 100 $\mu\ell$ of purified DNA. Briefly, 100 $\mu\ell$ of whole blood sample was mixed with 20 $\mu\ell$ of proteinase K(>600 mAU/mℓ) and 100 $\mu\ell$ of 1X phosphate buffered saline (PBS; 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) and 200 $\mu\ell$ of Buffer AL (lysis buffer) and incubated at 56 °C for 10 min. Added to the mixture was 200 $\mu\ell$ of 100 % ethanol, after which the mixture was placed into a spin column and centrifuged, followed by 2 washes with Buffer AW1 and Buffer AW2, respectively, centrifugation, and a final elution in 100 $\mu\ell$ of Buffer AE (10 mM Tris, pH 9.0; 0.5 mM EDTA).

PCR

A nested conventional PCR was performed on the purified DNA, using a protocol developed for FIVple and optimised for use in testing southern African lions[22,23]. Primers were derived from GenBank published sequence information for FIVfca (M25381 & U11820), FIVpco (*Puma concolor* lentivirus; U03982), and FIVoma (*Otocolobus manul* lentivirus; U56928)[22]. Degenerate primers were developed to detect viral DNA from the reverse transcription region of the Pol gene (RT-Pol) of the lentivirus genome, resulting in a nested 576 base pair product (including nested primers). The 5′ and 3′ outer primer sequences were TGGCCWYTA WCWAATGAAAARATWGAAGC (referred to as P1F) and GTAATTTRTCTT CHGGNGTYTCAAATCCCC (referred to as P2R), respectively. The nested primer sequences were TGAAAARATWGAAG CHTTAACAGAMATAG (referred to as P2F) and GTAATTTRTCTTCHGGNG TYTCAAATCCCC (referred to as P1R), respectively. All primers were synthesised by Sigma Genosys (Sigma, The Woodlands, TX, USA), and were reconstituted to a 20 pM working solution.

The successful nested primer set was used to generate DNA using Platinum PCR SuperMix (containing 22 U/mℓ complexed recombinant *Taq* DNA polymerase with Platinum *Taq* Antibody, 22 mM Tris-HCl at pH 8.4, 55 mM KCl, 1.65 mM MgCl2, 220 μmol dGTP, dATP, dTTP, and dCTP, and stabilisers [Invitrogen, Carlsbad, CA, USA]). The 54 $\mu\ell$ total volume, 1st round PCR reaction mixture consisted of 45 $\mu\ell$ PCR SuperMix, 2 $\mu\ell$ (20 pM) of primer P1F, 2 $\mu\ell$ (20 pM) of primer P2R, and 5 $\mu\ell$ of sample template. The cycling conditions for the 1st round PCR reaction consisted of 3 min at 94 °C, 45 cycles of 30 s at 94 °C, 30 s at 52 °C, and 45 s at 72 °C, with a final extension for 10 min at 72 °C. Three $\mu\ell$ of the resultant PCR product from round 1 were added to a nested PCR reaction mixture consisting of 45 $\mu\ell$ of PCR SuperMix, 1 $\mu\ell$ of primer P2F and 1 $\mu\ell$ of primer P1R, for a total volume per sample of 50 $\mu\ell$. The cycling conditions for the nested reactions were the same as for the 1st round PCR reaction. All reactions were carried out in an Eppendorf Mastercycler personal PCR thermocycler (Eppendorf, Westbury, NY, USA).

Agarose gel electrophoresis

Agarose gel electrophoresis was performed on all PCR samples to visualise amplification products. Ten microlitres of each amplified DNA product was combined with 2 $\mu\ell$ of 6X loading buffer and was loaded into a 1 % agarose gel stained with ethidium bromide in 1X TBE buffer (Tris 0.9 M, Borate 0.02 M, and EDTA 0.02 M, pH 8.3). A 1 Kb DNA ladder (exACTGene 100 bp DNA Ladder, Fisher Scientific, Pittsburgh, PA, USA) was used for size estimation of all samples. The agarose gel with loaded samples was then placed in a 1X TBE buffer bath and was electrophoresed for 25 min at 125 V, after which the gel was transilluminated to reveal DNA products.

FIVpco ELISA

Serum samples were tested for cross-reactive antibodies with an indirect ELISA using a synthetic FIVpco peptide (CPFKDICQL, AA 610618, GenBank U03983) located on the envelope glycoprotein, corresponding to the peptide P237 of FIVfca (CNQNQFFCK)[12]. Prior research with this ELISA demonstrated cross-reactivity to lion IgG antibodies[12,24].

Briefly, the peptide was diluted in PBS at 1:1000 (10 μg/mℓ was diluted in 10 $\mu\ell$ Ag, 10 mℓ PBS), added to a 96-well microplate (Immulon, Thermo Lab Systems, Waltham, MA, USA) at 100 $\mu\ell$/well, and incubated at between 2–8 °C overnight. Plates were then washed 4 times with PBS-Tween$_{20}$ (0.5 mℓ polyoxyethylene sorbitan monolaurate/litre PBS = 0.05 % Tween$_{20}$), and blocked by adding 200 $\mu\ell$ of PBS-Tween$_{20}$ and waiting for 1 h. Serum was diluted to 1:25 in PBS-Tween$_{20}$ (40 $\mu\ell$ serum, 960 $\mu\ell$ PBS-T) and 100 $\mu\ell$ diluted serum was added to the appropriate wells. Plates were sealed and incubated for 1 h at 37 °C, followed by 4 washes with PBS-T as described above. Next, 100 $\mu\ell$ of anti-cat IgG HRPO conjugate was added to all test wells, followed by sealing, incubating, and washing 4 times as described above. After washing, 100 $\mu\ell$ of 2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt substrate was added to each well, and plates were placed in the dark for ±25 min until good colour development was achieved, followed by reading at 405 nm (Bio-Tek EL808 Microplate Reader, Bio-Tek, VT, USA). Cut-off values were equal to double the negative value and positive values were considered to be greater than or equal to the cut-off value[12].

FIVfca ELISA

Thirty-three serum samples were tested for cross reactive antibody against FIVfca *gag* p24 protein antigens with an ELISA known as the SNAP Combo FeLV Ag/FIV Antibody Test (IDEXX Laboratories, Westbrook, ME, USA), according to the manufacturer's instructions[14]. Samples were selected using a random number generator. Domestic cat FIV *gag* products are most likely to be highly immunogenic yet conserved among different feline lentivirus isolates, including FIVple isolates[6,8]. Briefly, serum samples (if positive, containing antibodies to FIV) were mixed with inactivated FIV antigen-enzyme conjugate (provided) and placed into the sample well of the SNAP device, which contained FIVfca antigens in the matrix. Test results were interpreted after 10 min. A positive test result was indicated by colour development in the FIV antibody sample spot, according to the manufacturer's instructions, along with the appropriate colour development in the positive control spot.

Calculation of test prevalence

For an overall measure of prevalence, a test bank approach was used, referred to as the combined test prevalence (CTP)[21]. If a sample tested positive to all tests or 2 out of 3 tests, it was classified as a positive result. In addition, if a sample tested positive to PCR and was sequence-confirmed as FIVple, it was classified as a positive. If a sample tested negative to all tests, it was classified as a negative. All other intermediate results were discarded from the combined test prevalence calculations.

RESULTS

Of the 77 lions for which epidemiological data were collected during the 2004 census, a total of 73 whole blood samples and 63 serum samples were obtained for lentivirus testing. Of the 63 serum samples, all were tested with the FIVpco ELISA; however, only 52 samples were banked in frozen storage and thus available for further testing (FIVfca ELISA). Of the 73 samples tested, PCR yielded 44 positive and 29 negative samples. Of the PCR positive samples, 9 were from the 23 lions sampled from the north of KNP and 35 positives were from the 50 lions

sampled from the south. Thirty-eight of 63 FIVpco ELISA samples were positive, with 11 positives from lions in the north and 27 positives from lions in the south. Nineteen of 23 FIVfca ELISA samples were positive from lions in the south of KNP. No FIVfca ELISAs were performed on lion samples from the north, as no serum samples were available for testing from KNP northern lions (Table 1).

Table 1: **Results of samples tested with all three tests for feline lentivirus in lions in the Kruger National Park, South Africa.**

Sample ID	FIVfca ELISA (pos/neg)	FIVpco ELISA (pos/neg)	PCR (pos/neg)
21848	Neg	Neg	Neg
A251B***	Pos	Pos	Neg
D062B**	Pos	Neg	Neg
E3D00	Pos	Pos	Pos
550205	Pos	Pos	Pos
534E04***	Pos	Pos	Neg
B176607***	Pos	Pos	Neg
67B35	Pos	Pos	Pos
40085E	Pos	Pos	Pos
33833	Pos	Pos	Pos
F1F6314	Pos	Pos	Pos
D655B	Pos	Pos	Pos
321408	Pos	Pos	Pos
D184C4C	Pos	Pos	Pos
B2C2D	Neg	Neg	Neg
5A 5278	Neg	Neg	Neg
95270	Pos	Pos	Pos
249 A3A	Pos	Pos	Pos
44 A559AF76	Neg	Neg	Neg
C4560*	Pos	Neg	Pos
80263*	Pos	Neg	Pos
E4223****	Pos	Neg	No whole blood available
Bill-LP	Neg	Neg	Neg
Ben-LP	Neg	Neg	Neg

*Discrepancy between FIVfca and FIVpco ELISA; PCR agreement with FIVfca ELISA.
**Discrepancy between FIVfca and FIVpco ELISA; PCR agreement with FIVpco ELISA.
***Discrepancy between PCR and ELISAs; agreement between FIVfca and FIVpco ELISAs.
****Discrepancy between FIVfca and FIVpco ELISA; no whole blood available for PCR.

Of the 77 lions sampled in KNP, 58 were classified as adults 3 years of age or older, and 19 lions were juveniles less than 3 years of age, for an age category ratio of 3:1, respectively[20]. Lions in the north had an age ratio of 1.6:1 adults to juveniles, and lions in the south had an age ratio of 4.7:1 adults to juveniles. There were 27 males sampled and 50 females sampled, giving a sex ratio of 1.9:1 females to males. The sex ratio for northern lions was 4.2:1 females to males, and for the south was 1.3:1 females to males. There were no bachelor male coalitions sampled from the northern prides; however, 2 of the 9 prides sampled in the south were male coalitions, which may help to explain the more equivalent sex ratios in the south, as compared with the northern lions.

The average body condition score (BCS) was high overall, at 4.65 out of 5 (SD 0.565, range 3.05.0). The BCS for lions in the north was slightly higher, at 4.8 (SD 0.450, range 3.55.0), than those in the south, at 4.6 (SD 0.605, range 3.05.0). Only 3 of 77 lions received the lowest score recorded of 3 out of 5, and it can be noted that all 3 of these lions were part of a bachelor male coalition in the south. Of the 77 lions sampled, 26 lions were from the north and 51 lions were from southern KNP.

The results of the combined test prevalence calculations are shown in Figs 1–4. In addition, the CTP was calculated for the various combinations of host factors of age, sex and geographical location of the lions. The results are provided in Table 2.

The overall CTP for KNP was 69 %, with a lower prevalence of 41 % in the north and a higher prevalence of 80 % in the south. Adult males had the highest prevalence, at 94 %, when combining the factors of sex and age. When geographical location was included, adult males in the north were highest at a prevalence of 100 %. The lowest prevalences were found among juveniles, with male juveniles at a prevalence of 29 %. When geographical location was included, female juveniles in the north and male juveniles in the south were lowest, at 20 % and 25 %, respectively. Figs 34 show the prevalence of FIVple by pride in northern and southern KNP, respectively.

Odds ratios (OR) were calculated to explore the association of prevalence to host factors of age, sex, and geographical location of the lions (Table 3). Adults were 5.6 times more likely to be test positive for FIVple than juveniles, with adult males being 35.0 times more likely to be test positive for FIVple over juvenile males. This likelihood increased further to an OR of 48.0 when comparing adult and juvenile males in the south. Although there was virtually no difference in likelihood of infection for males versus females (OR: 1.48), adult males were 5.9 times more likely to test positive for FIVple than adult females. There was no difference (OR: 0) in the likelihood of infection between adult and juvenile males in the north; adult males and females in the north; and between adult males in the north versus the south.

DISCUSSION

The African lion is listed as a vulnerable species by the International Union for Conservation of Nature, which notes declining numbers in the last 20 years from an estimated 75 000 lions to around 30 000. Although the species exists throughout sub-Saharan Africa today, it is

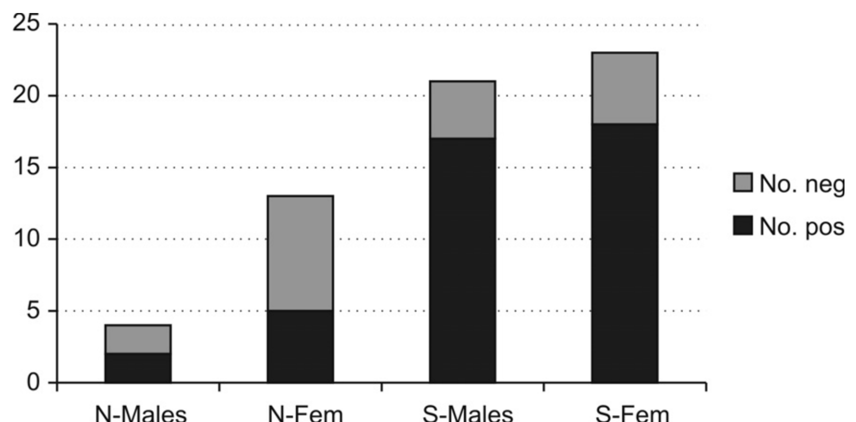

Fig. 1: **Combined test prevalence** (CTP) **by location and sex of free-ranging lions in the Kruger National Park in 2004. N-Males: males in northern subset; CTP = 50 %. N-Fem: females in northern subset; CTP = 38 %. S-Males: males in southern subset; CTP = 81 %. S-Fem: females in southern subset; CTP = 78 %.**

not a contiguous population, but rather exists as a series of smaller, fragmented subpopulations. These subpopulations of lions are genetically isolated from the rest of their species. Genetic exchange is only possible with human intervention such as translocation or the creation of habitat corridors.

The conservation management of free-ranging lions must consider the infectious agents to which they are susceptible. Of these, the lentiviruses are a major focus of investigation. The genetic make-up of the host, as well as the genomic variation and characteristics of the feline lentiviruses are key factors in the dynamic interplay between the host, the agent, and their environment. These three factors – host, agent, and environment – create a triad in which a change in any one factor will have an effect on the others in turn. Thus all three factors should be considered when discussing any health or conservation management strategy for the African lion.

The lion population in South Africa is estimated to be approximately 3000, with the largest free-ranging population of 2000 lions in KNP. The KNP is a fenced habitat with human encroachment to the park's borders, thus limiting the potential territory size for the population of lions that exists within the boundaries of KNP.

However, a new initiative to create a larger transfrontier conservation area is underway, joining KNP with national parks in the neighbouring countries of Mozambique and Zimbabwe. The newly established transfrontier park will be known as the Greater Limpopo Transfrontier Conservation Area (GLTCA), and will allow for the movement of wildlife into new and larger territory. This will create opportunities for lion populations that are currently constrained by genetic isolation to come into direct contact with one another, facilitating genetic exchange between both the lions and the lentiviruses they harbour. The corridor connecting the KNP as a transfrontier park lies in the north of KNP; thus the impact may initially be observed among the lions of northern KNP. As new territory opens for lions in the north, lions in the south of KNP may in turn migrate northward, thus impacting the overall social dynamics, and subsequently the viral epidemiology of FIVple subtypes presently circulating in the entire GLTCA.

This investigation examined lentivirus infection in southern African lions and represents the 1st descriptive epidemiological study of FIVple among the lions of KNP. Sampling of animals was not random but was conducted on an opportunistic basis as part of routine surveillance. It may therefore not be a true representation of

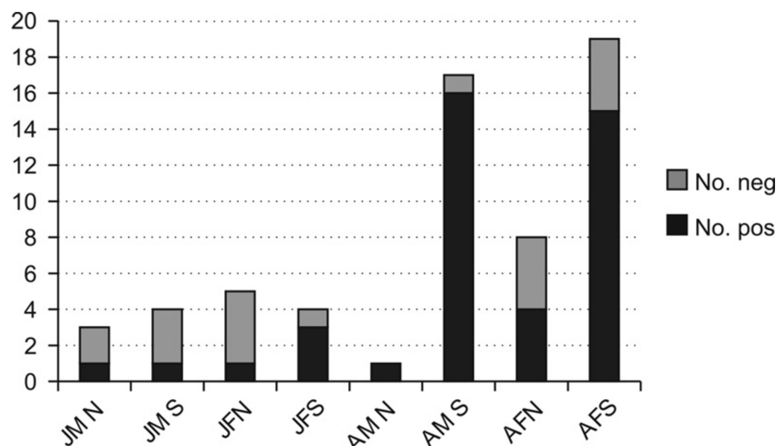

Fig. 2: **Combined test prevalence** (CTP) **by age and sex of free-ranging lions in the Kruger National Park in 2004. JMN: juvenile males in the northern subset; CTP = 33 %. JMS: juvenile males in southern subset; CTP = 25. JFN: juvenile females in northern subset; CTP = 20 %. JFS: juvenile females in southern subset; CTP = 75 %. AMN: adult males in northern subset; CTP = 100 %. AMS: adult males in southern subset; CTP = 94 %. AFN: adult females in northern subset; CTP = 50 %. AFS: adult females in southern subset; CTP = 79 %.**

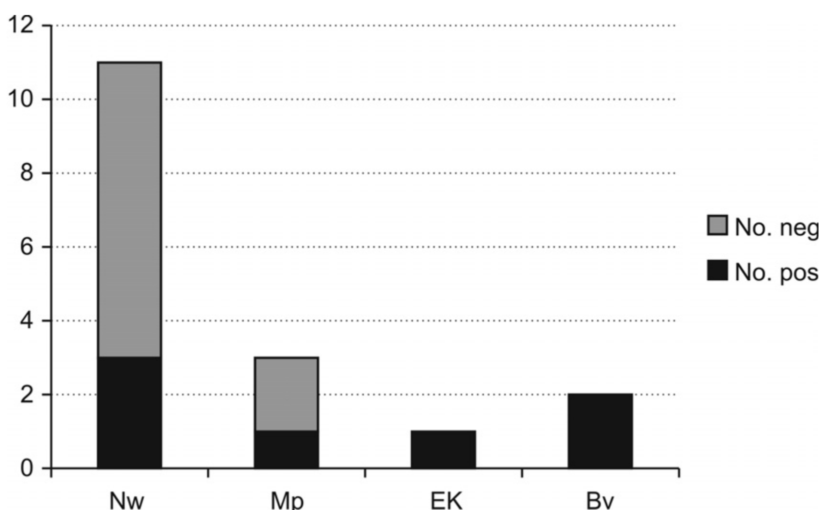

Fig. 3: **Combined test prevalence** (CTP) **by pride of free-ranging lions in the north of the Kruger National Park in 2004. Nw: Nwashitumbe pride; CTP = 25 %. Mp: Mpenza pride; CTP = 33 %. EK: Elands Kiel pride; CTP = 100 %. Bv: Biesiesvlei pride; CTP = 100 %.**

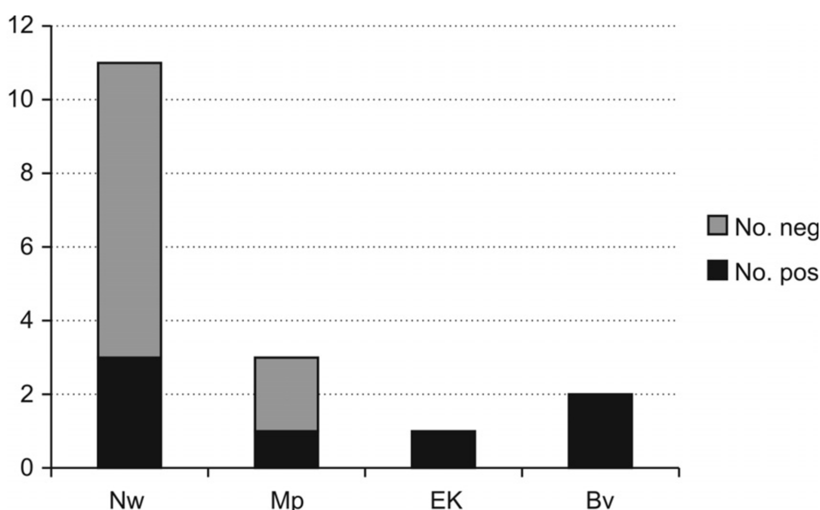

Fig 4: **Combined test prevalence** (CTP) **by pride of free-ranging lions in south of the Kruger National Park in 2004. Jeep: Jeep pride; CTP = 0 %. Nz: Nlanganzwane pride; CTP = 50 %. Dz: Dzuweni pride; CTP = 67 %. Mp: Mpanamana pride; CTP = 70 %. PL: Power lines pride; CTP = 80 %. LS: Lower Sabie pride; CTP = 100 %. BP: Bob Picket pride; CTP = 100 %. Gez: Gezentombi pride; CTP = 100 %. Ng: Ngomondwane pride; CTP = 100 %.**

Table 2: **Combined test prevalence of FIVple among the lions in the Kruger National Park, by host factors of age and sex, and by location within KNP.**

Category/subset	No. positive	No. negative	Prevalence (%)
Overall	42	19	69
North	7	10	41
South	35	9	80
Females	22	13	63
Males	15	6	71
Adults (≥ 3 years)	31	10	79
Juveniles (<3 years)	5	9	36
North-females	5	8	38
North-males	2	2	50
North-adults	5	4	56
North-juveniles	2	4	33
South-females	18	5	78
South-males	17	4	81
South-adults	31	5	86
South-juveniles	4	4	50
Female-adults	19	8	70
Female-juveniles	4	5	44
Male-adults	14	1	94
Male-juveniles	2	5	29
North-female-adults	4	4	50
North-female juveniles	1	4	20
South-female-adults	15	4	79
South-female-juveniles	3	1	75
North-male-adults	1	0	100
North-male-juveniles	1	2	33
South-male-adults	16	1	94
South-male-juveniles	1	3	25

the population of lions in general, or of FIVple specifically, from their respective geographical regions. When dealing with a free-ranging wildlife species whose population status is vulnerable, and anaesthetic immobilisation is required in order to obtain samples, it is necessary and acceptable to sample opportunistically[10,25].

The estimated population of lions in KNP was 2000, according to the last recorded census in 2002 (M van Vuuren, University of Pretoria, pers. comm., 2007). Thus, the population of lions sampled for this study represents roughly 4 % of the total population of KNP lions. In general, the density of lions is higher in the south of the park. This may help to explain why there were more males and juveniles sampled in the south, as access to females is greater and allows males increased opportunities to mate with females in oestrus. This may also explain why no bachelor male coalitions were sampled in the north, as bachelor males may be driven to roam further south for access to females, as well as for access to prey species. Approximately twice as many animals were sampled from the south. This is partly due to the increased lion density in the south described above, as well as due to the location of the veterinary staff headquarters in the south of KNP, facilitating easier access to the lions of the south than in the more remote areas in northern KNP.

The body condition score for lions in KNP was high overall, at 4.6 out of 5. The slightly higher score for northern lions may be due to the lower density of lions, with decreased competition for resources among prides and roaming males. The 3 lions with the lowest BCS were part of the same adult male coalition in the south. These 3 lions were also FIVple positive. It is possible that the stress of life as a bachelor male lion may affect an individual's overall health, which is reflected in a lower BCS. A lower BCS may also be due in part to muscle wasting and emaciation from lion lentivirus infection. In a limited observational study of lions in captivity at the North Carolina Zoo, muscle wasting was the primary clinical sign noted among FIVple positive lions[1].

Roughly 2/3 of the lions sampled from KNP were positive for FIVple by serology and/or virus detection. The higher prevalence for lions sampled in the south may be due to the increased lion density there, allowing for increased opportunities for virus transmission. Adult males had an extremely high prevalence of FIVple infection. This is expected, because the reported seroprevalence for both domestic and exotic feline lentivirus infections increases with sexual maturity, suggesting that horizontal transmission, as with bite wounds, is the primary mode of transmission[23]. Overall, juveniles had the lowest prevalence of infection. These results suggest that FIVple infection among the lions studied is most likely primarily due to bite transmission. This may be a more efficient means of transmission than *via* maternal transmission or other direct contact such as grooming between pride members (saliva), which are established as means of transmission in domestic cats[2,3,7,11,16,18].

Many of the calculated ORs were not found to be statistically significant; however, this finding could be due to the small sample sizes in several of the strata. Among the lions of KNP, adults, and specifically adult males, were more likely to be infected than juveniles (and specifically juvenile males). This likelihood increased further when comparing adult and juvenile males in the south. This observation supports the theory that male lions are at greater risk for infection due to the increased propensity for aggressive and roaming behaviour. The higher density of lions in southern KNP may also contribute to an increase in males fighting and a resultant higher individual stress, as well as increased opportunities for direct contact with other infected lions, as demonstrated in East African lion populations[9].

Owing to the dynamic, ever-changing nature of the agent–host–environment relationship and the obstacles to gathering comprehensive data on free-ranging wildlife, it is difficult to predict the spread of infection with FIVple or if and when it may occur in a previously unexposed free-ranging population of lions. This alone may be enough to demonstrate that monitoring FIVple infection among populations of lions is crucial in order to better elucidate and document the patterns of virus-host co-evolution and adaptation over time.

Researchers are only now beginning to understand the complex interplay that exists between the lion, its immune competence, and the co-evolution with FIVple that may or may not contribute to the manifestation of serious disease in some individuals. Unfortunately, when dealing with free-ranging populations of wildlife, there is limited opportunity to conduct long-term, exhaustive, invasive research into the effects of an infectious agent on a population; thus it is important to glean as much information as possible from the limited opportunities available for gathering data. Finally, lion conservation management would benefit from a more comprehensive knowledge of FIVple evolution, adaptation, and cross-species transmission for use when planning the development of a newly protected area, when planning strategies for the translocation of animals, as well as to plan for potential management and intervention

Table 3: **Odds ratios for FIVple positivity among free-ranging lions in the Kruger National Park, according to host factors and geographical location. Odds ratios** (OR), ***P*-value, and 95% confidence intervals** (CI) **were calculated using Epi Info Statcalc** (version 3.4.1).

Category	OR	OR 95% CI	*P*-value
S *vs* N*	5.56	1.42–22.61‡	0.0040†
M *vs* F	1.48	0.40–5.60‡	0.52†
A *vs* J*	5.58	1.27–25.78	0.010
N-M *vs* N-F	1.60	0.09–28.34	0.99
S-M *vs* S-F	1.18	0.21–7.00	0.99
S-F *vs* N-F*	5.76	1.05–32.55‡	0.030
S-A *vs* N-A	4.96	0.70–34.21	0.063
M-A *vs* F-A	5.89	0.64–279.87	0.12
F-A *vs* F-J	2.97	0.48–18.82	0.23
M-S *vs* M-N	4.25	0.22–71.26	0.23
M-A *vs* M-J*	35.00	1.87–1766.69	0.004
J-S *vs* J-N	2.00	0.15–33.32	0.63
J-F *vs* J-M	2.00	0.17–30.82	0.63
J-M-N *vs* J-M-S	1.50	0.01–78.25	0.99
J-F-S *vs* J-M-S	9.00	0.21–626.24	0.49
A-M-S *vs* J-M-S*	48.00	1.43–2663.96	0.011
J-F-S *vs* J-F-N	12.00	0.31–782.99	0.21
A-F-S *vs* J-F-S	1.25	0.02–21.43	0.99
J-M-N *vs* J-F-N	2.00	0.02–58.70	0.99
A-M-N *vs* J-M-N	0.00	0.00–39.00	0.99
A-F-N *vs* J-F-N	4.00	0.21–245.34	0.56
A-F-S *vs* A-F-N	3.75	0.45–30.87	0.18
A-M-S *vs* A-F-S	4.27	0.35–222.99	0.34
A-M-N *vs* A-F-N	0.00	0.00–48.75	0.99
A-M-N *vs* A-M-S	0.00	0.00–663.00	0.99

Key: N: north; S: south; M: male; F: female; A: adult; J: juvenile.
*Statistically significant.
†Used Maentzel-Hansel test (EpiInfo Statcalc; http://www.cdc.gov/epiinfo/about.htm); all other *P*-values are from the fisher exact 2-tailed test.
‡Indicates Cornfield's 95 % CI (EpiInfo Statcalc); for all others, exact CI used.

strategies in the case of an outbreak of disease or other health crisis in a population.

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