Assessing the impact of feline immunodeficiency virus and bovine tuberculosis co-infection in African lions

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Abstract

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis* (*M. bovis*), is a disease that was introduced relatively recently into the Kruger National Park (KNP) lion population. Feline immunodeficiency virus (FIV_{ple}) is thought to have been endemic in lions for a much longer time. In humans, co-infection between *M. tuberculosis* and human immunodeficiency virus increases disease burden. If BTB were to reach high levels of prevalence in lions and if similar worsening effects would exist between FIV_{ple} and BTB as for their human equivalents, this could pose a lion conservation problem. We collected data on lions in KNP from 1993-2008 for spatio-temporal analysis of both FIV_{ple} and BTB, and to assess whether a similar relation between the two diseases exists in lions. We found that BTB prevalence in the south was higher than in the north (72% vs 19% over the total study period) and increased over time in the northern part of the KNP (0% to 41%). No significant spatio-temporal differences

were seen for FIV_{ple} in the study period, in agreement with the presumed endemic state of the infection. Both infections affected haematology and blood chemistry values, FIV_{ple} in a more pronounced way than BTB. The effect of co-infection on these values, however, was always less than additive. Though a large proportion (31%) of the lions was co-infected with FIV_{ple} and *M. bovis*, there was no evidence for a synergistic relation as in their human counterparts. Whether this results from different immunopathogeneses remains to be determined.

Key index words: feline immunodeficiency virus, bovine tuberculosis, *Mycobacterium bovis*, lion, co-infection, prevalence

Introduction

Both feline immunodeficiency virus (FIV_{ple}) and *Mycobacterium bovis (M. bovis),* causing bovine tuberculosis (BTB), are found in the lion (*Panthera leo*) population in the Kruger National Park (KNP), South Africa. Feline immunodeficiency virus is an endemic pathogen in many lion populations in eastern and southern Africa [1-5], and its presence may even date back as far as the species divergence of the genus *Panthera* [6,7]. Differences have been found recently in the CD4+/CD8+ T-cell subset [4] and the prevalence of AIDS-defining conditions [8] in FIV_{ple}-infected lions compared to non-infected lions, contradicting studies that did not find pathologic effects associated with FIV_{ple} infection [7,9-11]. This may also depend on differences in pathogenicity between FIV_{ple} subtypes [12]. Common haematological and blood chemistry changes that are found in FIV infected domestic cats are lymphopenia, leucopenia, neutropenia, anaemia, hyperproteinaemia and hyperglobulinaemia [13-17]. In lions, FIV_{ple} was associated with dehydration and abnormal red blood cell

parameters, e.g. anaemia, depressed serum albumin and elevated liver enzymes, total protein, globulin and gamma globulin [8].

Mycobacterium bovis was introduced in the southeast corner of the KNP in the 1960's [18], spreading from infected cattle to buffaloes. The first case of lion BTB was found in 1995, likely resulting from consumption of infected buffalo carcasses [19]. A prevalence of almost 80% in the lion population in the south of the KNP was reported in 2000 [20]. Limited information is available about the effect of (B)TB on haematologic and blood chemistry values, but in humans with minimal active tuberculosis a significant rise in the gamma globulin fraction with a corresponding decrease in albumin was found. In far advanced cases, all globulins were increased, but the mean total protein did not differ from the normal value [21]. In a report of a BTB infected lion, leukocytosis, monocytosis, anaemia, neutrophilia, hypoalbunemia and hyperglobulinemia were found [22].

In humans, one of the most well-known pathogen-pathogen interactions is that between human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis (M. tuberculosis)*. HIV is the strongest known risk factor for TB, affecting on the immunity by T-cell depletion [23]. On the other hand, TB can accelerate the progression of HIV [24]. These synergistic interactions magnify the burden of disease of both infections [23,25,26]. In animals, pathogen-pathogen interactions are also described, for example between *Babesia* and canine distemper virus in lions [27] or between various infectious agents in voles [28]. Interactions may be synergistic or antagonistic to one or both of the infectious agents [29], which may be explained by a

variety of mechanisms influencing host susceptibility, pathogenicity or infectiousness in both positive and negative ways [28-31].

Lions are listed as a vulnerable species by the International Union for Conservation of Nature [32] and the relatively recent introduction of *M. bovis* in a population where FIV_{ple} is highly prevalent, could be an even more serious threat to lion conservation if the two infections would enhance each other's effects as their counterparts do in humans. Although it has been reported that 53% of the lions in the southern half of KNP is co-infected with FIV_{ple} and *M. bovis*[33], little is known about the effects of their interaction. Previous literature on FIV_{ple} and *M. bovis* infection in lions in the KNP is scarce and has often only been anecdotal, using small groups of animals. We collected data from 1993-2008, which resulted in a unique data set of 669 lions, that was used

(I) to assess what variables (area, period, age, sex, body condition) are related with FIV_{ple} and BTB infection in lions, studied by a multivariable logistic regression model, and

(II) to assess the pathogen-pathogen interaction of FIV_{ple} and BTB in lions.

For the latter, we used a sub-group of 205 lions that had been subjected to diagnostic tests for both infections. Body condition and haematological and blood chemistry values, that were deemed relevant based on literature [17,22,34], were used as dependent variables in general linear models to assess this potential interaction.

Material and methods

The Kruger National Park

The KNP is a partly fenced, wooded savannah covering about 20 000 km^2. The total KNP lion population is estimated to be about 1600-1700 [35]. For the purpose of this analysis, the KNP was divided into three regions based on the prevalence of *M. bovis* in buffaloes, namely High, Medium and Low Prevalence Zones (HPZ, MPZ, LPZ; roughly corresponding to the southern, central and northern part of the park), separated by the Sabie River (south-central) and the Olifants river (central-north). Prevalences of *M. bovis* in buffaloes in 1998 were respectively 38.2%, 16.0% and 1.5% [36]. Data obtained from lions from adjacent game reserves with open access to the KNP was included in the analyses, according their locations.

Bovine tuberculosis in the KNP is not controlled. This makes the ecosystem unique, as many other ecosystems with *M. bovis* presence have a test-and-removal [37,38] or culling strategy [38].

The animals

Most lions were captured with call-up stations in designated areas in the southern, central and northern part of the park, which were known to have lions, based on ranger information. These stations were randomly distributed as much as logistic considerations allowed (figure 1a). About 25% of the study lions was brought to the Veterinary Station as emaciated or problem lions.

Before handling, all lions were immobilized with a combination of tiletamine and zolazepam (Zoletil® 100, Virbac). Venous blood samples were obtained from the

Figure 1a. Capture locations of lions, covering 93% of the lions from the data set, of which exact locations were known. Multiple lions may have been captured at one location. Locations outside KNP indicate escaped lions. Figure 1b. Pie charts presenting the numbers of lions in the four co-infection groups (n=205) in the three different areas of the KNP. Size indicates the number of lions captured. $FIV_{ple}^{+}BTB^{+}$= black, $FIV_{ple}^{-}BTB^{+}$= dark grey, $FIV_{ple}^{+}BTB^{-}$= light grey and $FIV_{ple}^{-}BTB^{-}$= off-white.

medial saphenous vein as soon as possible after anaesthesia in heparin, EDTA and serum Vacutainer® tubes, which were kept at ambient temperature and were processed within preferably 8, but maximum 24 hours. Serum was collected and stored at -20°C. All lions in this study were aged by examining dental attrition according to Smuts et al. [39]. Body condition score (BCS) was assessed according to criteria that were determined beforehand and ranged from 5 (Excellent) to 1 (Very poor) (table 1). Lions were micro-chipped and were given a unique brand so the animals could be recognized at future captures. Due to the higher lion density in the south (compared to the north) as well as the location of the veterinary staff headquarters in the south of KNP, almost twice as many study lions originated from the south, compared to the central and northern areas.

Table 1 : Definitions of the different BCSs.

Body condition score	Definition
5. Excellent	Hindquarters well rounded and no ribs showing; general appearance in relation to posture and coat sheen excellent.
4. Good	Hindquarters rounded, but 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 is sunken in. The dorsal spinae of the vertebrae becomes apparent.
1. Very poor	Skeletal details clearly visible and general appearance, posture and coat condition deteriorated. The dorsal and lateral processes of the vertebrae clearly visible.

Sample collection

BTB status: The BTB status of individual lions was determined by performing the Single Intradermal Cervical Test (SICT/skin test) as described by Keet et al. [33]. A lion was considered BTB positive when three days after intradermal administration of bovine tuberculin, the skin swelling was 2 mm or larger, irrespective of the response

to the avian tuberculin. The SICT has a sensitivity and specificity of respectively 86.5% and 81%. The SICT appears not to be influenced by FIV_{ple}, in contrast to the tuberculin skin test in humans, which is affected by HIV infection [33,40].

FIV_{ple}-status: Serum samples were tested for FIV_{ple}-specific antibodies at the Department of the Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, using a protocol described by Van Vuuren et al. [41]. The sensitivity of the ELISA, when using the Western blot as the gold standard, is 78.6% and the specificity 100% [41].

Haematology: Haematology analysis was performed in the KNP with a Coulter AcT diff analyzer (Beckman Coulter).

Blood chemistry: Blood chemistry analysis was conducted with a NExCT/VetEX (Bayer Health) at The Clinical Pathology Laboratory, Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science, University of Pretoria.

The data set

From 1993-2008, a large data set has been established, consisting of 669 lions from the KNP and adjacent game reserves. Descriptions of the data set can be found in the electronic supplementary material tables and figures S1 to S5. Small, specific subsets of this extensive data set have been used in various studies in the past [1,5,20,33,42]. Not all information was available for each animal and table 2 gives an overview of the cross-sectional data that has been used for the analyses in the current study.

Table 2 : An overview of the lion dataset (n = 669) and the number of lions that were available for the different analyses. (A difference has been made for lions either captured at a call-up station (cal), lions that were brought to the veterinary station (vet) or lions with an unknown capture method (unk). Ht, haematocrit; WBC, white blood cell count; TSP, total serum protein; Alb, albumin; Glob, globulin; A/G ratio, albumin/globulin ratio; gamma glob, gamma globulin.)

	Number of lions	
	Total (cal.; vet.; unk)	with SICT and FIV_{ple} result (cal.; vet.)
SICT result	240 (191; 49; 0)	205 (165; 40)
FIV_{ple} result	561 (415; 137; 9)	205 (165; 40)
Haematology (Ht, WBC)	Ht:435 (320; 105; 10) WBC: 375 (260; 105; 10)	Ht: 164 (124; 40) WBC: 163 (123; 40)
Blood chemistry (TSP, Alb, Glob, A/G ratio, gamma glob)	All: 500 (358; 133; 9)	All: 172 (132; 40)

Data analysis

As age and BCS are subjective characteristics and age is increasingly difficult to determine in the older ages classes, these nominal variables were recoded to binary variables to facilitate the modelling, resulting in the following variables: age (binary, ≤ 36 months or >36 months, reference level: ≤ 36 months), BCS (binary, BCS 1,2&3 and BCS 4&5, reference level: BCS 4&5), sex (binary, reference level: female), area (nominal, three levels (HPZ, MPZ, LPZ), reference level: MPZ) and period (nominal, three levels (1:1993-1998; 2:1999-2002; 3:2003-2008), reference level: 1).

To assess which variables potentially influenced FIV_{ple} and BTB infection (both binary, reference level: negative), the following multivariable logistic regression models were used:

$$FIV = \mu + age + BCS + sex + area + period + e \quad [1a]$$

$$BTB = \mu + age + BCS + sex + area + period + e \quad [1b]$$

where μ represents the intercept, e residual error and where the other variables are coded as mentioned above. The e^{β} was used to calculate the odds ratio [43].

A total of 205 lions that had been subjected to both FIV_{ple} and BTB-specific diagnostic tests were used to study the potential synergistic effects of the infectious agents on the body condition and seven blood parameters. To study the effects of FIV_{ple} and *M. bovis* and their interaction on BCS, the following multivariable logistic regression model was used:

$$BCS = \mu + FIV + BTB + FIV \times BTB + e \quad [2]$$

where μ represents the intercept, e residual error and BCS, FIV and BTB are binary variables coded as defined above.

Blood parameters were selected that were deemed relevant in the literature for either or both of the infections: haematocrit (Ht), white blood cell count (WBC), total serum protein (TSP), albumin (ALB), globulin, gamma globulin and albumin:globulin (A:G) ratio [17,22,34,44]. To assess the effects of FIV_{ple} and *M. bovis* and their interaction,

as well as other potentially influencing variables on these parameters, the following full general linear regression model was used:

$$\text{blood parameter} = \mu + FIV + BTB + FIVxBTB + age + BCS + sex + area + e \quad [3]$$

with variables as defined above.

The Akaike Information Criterion (AIC) was used to rank the models [45], following backward stepwise selection. Models with smaller values of the raw AIC values were preferred for each step, unless the difference between the two smallest models was less than 2. In those situations, the principle of Occam's razor was used to select the "simplest" model with the least parameters, i.e. with the highest information gain [46,47], following standard statistical methods [43]. All models were checked for normality and homoscedasticity. For data analysis R version 2.15.0 was used (including packages Hmisc and modeest)[48].

Results

The models assessing the dependency of FIV_{ple} and BTB on the variables area, period, age, sex and BCS, showed that FIV_{ple} positivity was significantly related with sex (males were more likely to be FIV_{ple} positive), higher age and lower BCS. *M. bovis* infection was related with the area (lions were more likely to be infected in the south of the park) and the period (lions were more likely to be infected in the last time period) (table 3). Additional information can be found in the electronic supplementary material (tables and figures S7 to S10).

Table 3 : Results of the two final logistic regression models with dependent variables FIV_{ple} and BTB. (Odds ratios are given with their 95% confidence interval between brackets. n.s., these variables were not statistically significant in the model (based on the AIC) and were not included in the final model.)

	FIV_{ple}	BTB
Sex (M)	1.5 (1.0 to 2.2)	NS
Age (>36 months)	3.5 (2.4 to 5.1)	NS
BCS (1,2,3)	1.7 (1.1 to 2.6)	NS
Area		
LPZ/north	NS	0.1 (0.0 to 0.3)
MPZ/central	NS	1
HPZ/south	NS	2.2 (1.2 to 4.3)
Period		
Period 1 (1993-1998)	NS	1
Period 2 (1999-2002)	NS	0.8 (0.4 to 1.6)
Period 3 (2003-2008)	NS	3.4 (1.2 to 11.1)

The 205 lions with test results for both FIV_{ple} and BTB were divided into four groups: $FIV_{ple}^{+}BTB^{+}$, $FIV_{ple}^{-}BTB^{+}$, $FIV_{ple}^{+}BTB^{-}$ and $FIV_{ple}^{-}BTB^{-}$. No significant differences were found between the observed group sizes and the expected group sizes based on the FIV_{ple} and BTB prevalences (electronic supplementary material table S7a); neither for the total KNP, nor for the three areas separately (figure 1b, for details see electronic supplementary material table S6). This may indicate there is no significant relation between the pathogens, but may also result from a balance between an increased incidence and an increased mortality caused by co-infection of FIV_{ple} and BTB, and should thus be interpreted with caution. When assessing the effects of FIV_{ple} and BTB on the BCS, the co-infected lions had a slightly higher percentage of lions with a low BCS (figure 2) compared to the other groups, but this was not statistically significant (the logistic regression model confirmed this finding)[model 2].

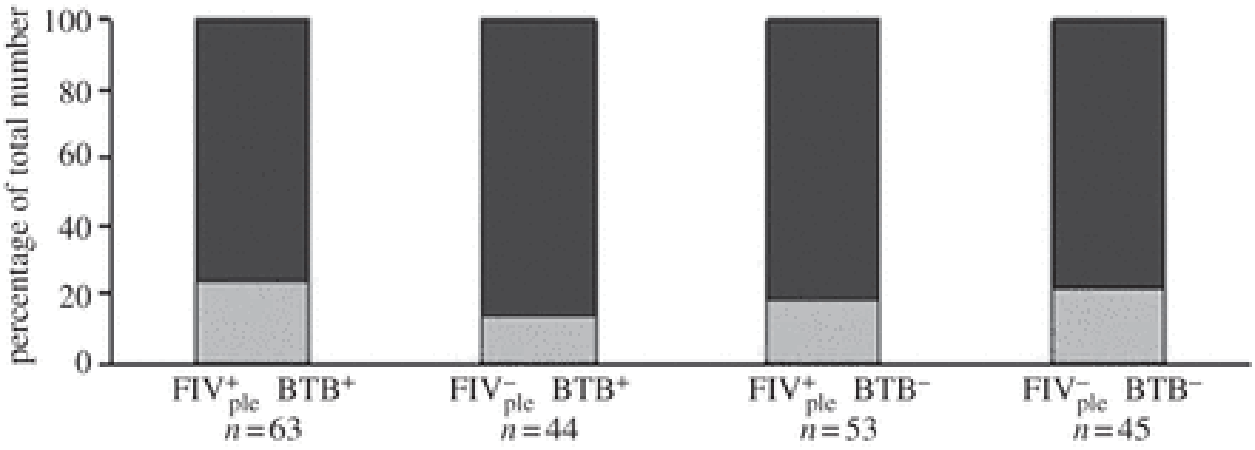

Figure 2. Lions (n=205) grouped according to their FIV_{ple} and BTB status and their BCS. Light grey = lions with a low BCS (BCS 1,2,3). Dark grey = lions with a high BCS (BCS 4,5).

Linear regression models to determine associations with FIV_{ple} and/or *M. bovis* infection showed that in co-infected animals there is a statistically significant antagonistic interaction between FIV_{ple} and *M. bovis* for three of the seven blood parameters, resulting in less deviation than expected from the sum of the individual effects (table 4). Effects of FIV_{ple} were more pronounced, except for the hyperproteinaemia. Neither FIV_{ple} nor BTB nor their interaction had a significant influence on the white blood cell count or the gamma globulins. Comparisons with reference values from zoo lions can be found in supplementary table S11.

Discussion

Infectious diseases are an important issue in conservation, having the power to dramatically influence the dynamics of wildlife species and populations [27,49,50], especially in dwindling populations [51,52]. Because it is difficult to determine their effect, it is common to deal with each infectious disease as a separate entity caused by a single pathogen [31]. However, in nature multiple pathogens are often encountered

Table 4 : Final multivariable models for seven haematologic and blood chemistry values. (Beta coefficients are given with their 95% confidence interval between brackets. To determine the effect of interaction, all values of a blood parameter should be added, for example, for globulin: 9.3 + 7.5 – 6.6 = 10.2. n.s., these variables were not statistically significant (based on the AIC) and were not included in the final model.)

	Dependent variables						
Explanatory variables	**Ht (%)**	**WBC (*10^9/L)**	**TSP (g/L)**	**Alb (g/L)**	**Glob (g/L)**	**Gamma glob (g/L)**	**A:G ratio**
N	164	163	172	172	172	172	172
Observed mean values in population	34.3	18.6	84.0	28.3	55.7	24.9	0.5
FIV	-2.0 (-3.5 to -0.6)	NS	6.9 (3.1 to 10.6)	-2.4 (-3.5 to -1.2)	9.3 (5.7 to 12.9)	NS	-0.1 (-0.2 to -0.1)
BTB	NS	NS	7.8 (3.3 to 12.3)	NS	7.5 (3.3 to 11.8)	NS	-0.1 (-0.2 to 0.0)
FIVxBTB	NS	NS	-6.7 (-12.2 to -1.1)	NS	-6.6 (-11.9 to -1.3)	NS	0.1 (0.0 to 0.2)
Age	1.7 (0.2 to 3.2)	2.6 (0.7 to 4.5)	NS	NS	NS	3.5 (1.9 to 5.0)	NS
BCS	-6.9 (-8.5 to -5.3)	4.8 (2.8 to 6.9)	-4.8 (-8.1 to -1.5)	-6.7 (-8.1 to -5.4)	NS	1,9 (0.2 to 3.7)	-0.1 (-0.2 to -0.1)
Sex	1.8 (0.4 to 3.2)	NS	NS	NS	NS	NS	NS
TB area							
HPZ/south	-3.3 (-4.9 to -1.8)	NS	-3.8 (-7.1 to -0.5)	-0.4 (-1.8 to 0.9)	-3.3 (-6.4 to -0.2)	NS	NS
MPZ/central (reference)							
LPZ/north	2.0 (0.0 to 3.9)	NS	4.1 (0.3 to 7.9)	2.3 (0.7 to 3.8)	1.4 (-2.2 to 5.0)	NS	NS
Intercept	36.1	15.6	80.0	30.8	49.8	22.2	0.7

simultaneously by individual hosts, which can lead to additive, antagonistic, or synergistic effects on hosts and pathogens [53]. It was expected that co-infection with FIV_{ple} and *M. bovis* in lions would have a synergistic effect, similar to the human counterparts [25]. However, though there was an indication that co-infected lions more often had a low body condition score (BCS), this was not statistically significant

(figure 2). FIV_{ple} alone was significantly correlated with a lower BCS. However, though *M. bovis*-infected lions were noted to often have a "scruffy and unthrifty look" (DK, personal observations), BTB was not significantly correlated with BCS. This was surprising as pathologic lesions have been described in *M. bovis* infected lions with a poor condition [19,20]. In a study of BTB in buffaloes, it was found that 70% of the infected animals examined post mortem had only a mild infection and were unlikely to have shown symptoms while alive [54]. Our dataset contained several lions with necropsy results, but unfortunately their number was too small to allow statistically significant conclusions. Therefore, whether there are patterns in the clinical signs in lions, like in buffaloes, remains to be seen.

Effects of both FIV_{ple} and *M. bovis*-infection on the various blood parameters may indicate chronic disease, such as anaemia [44,55], but could also be exaggerated by the effect of age, for example the hyperglobulinaemia, as older lions are more likely to be FIV_{ple} or BTB positive and in general show an increase in globulins [55]. The values might be slightly biased by the delay between sampling and analysis and possible temperature differences during this delay, though the majority of the blood samples was collected at night in the winter season. For all blood parameter values the interaction of the infections was shown to be less than additive. Explanations for this may be that the immune response already reaches maximum capacity for one infection, or that the body is able to keep the various parameters between homeostatic limits.

Although not specific for either infection, the direction of changes in blood parameter values was comparable to that observed in previous studies on FIV in cats [13-17] and FIV_{ple} in lions [8], and to results from the less abundant literature on (B)TB [21,22].

Anaemia and hypoalbuminaemia are associated with progression to AIDS and death in human pre-AIDS patients [56,57]. In contrast to the decrease of CD4+ and CD8+ T-cell counts observed in HIV-infected humans, previously also reported for FIV_{ple} in lions [4], no decrease of white blood cells was seen in FIV_{ple}-positive lions in the present study. White blood cells were not further typed, which would be needed to determine possible changes in the numbers of the different cell types.

M. bovis prevalence was significantly different between the three areas in the KNP, coinciding with observations on *M. bovis* prevalence in the buffaloes [58], one of the four preferential prey species of lions in the KNP [59]. Surprisingly, this did not result in an age distribution skewed to the younger ages in the high prevalence zone compared to the low prevalence zone (electronic supplementary material table and figure S5). Also, the prevalence of *M. bovis* infected animals in the northern part of KNP increased significantly with time from 0% to 41% (Fisher's exact test, two tailed, p=0.014; electronic supplementary material table S7c). These findings suggest that *M. bovis* infection in lions is caused by an external source of infection, i.e. that lions are spillover hosts, in agreement with [60,61]. This contradicts suggestions from the past about their role as maintenance host [19,62]. FIV_{ple} on the other hand shows no significant relation with the external factors measured, supporting the intraspecies transmission route.

It was expected that, like HIV and *M. tuberculosis* in humans, a synergy between FIV_{ple} and *M. bovis* was to be found in lions, but this large data set shows no proof for detrimental synergy in the tested parameters. There may be various reasons for not finding a similar relation of FIV_{ple} and *M. bovis* like in humans.

- The immunopathological characteristics of BTB can vary in different species [63] and macroscopic lesions in *M. bovis* positive lion have been found to be very different from those in ungulates and non-human primates [20]. This may mirror a difference in susceptibility to infection, but the knowledge on immunopathogenesis is still very limited in lions. Differences in immune response in feline and simian species have also been noted for immunodeficiency viruses, related with specific virus-host co-adaptation and viral load [9,12,64]. One reason for a difference in immunopathogenesis may be that lions have co-evolved with the endemic disease FIV_{ple}, whereas *M. bovis* is a recently introduced pathogen, in contrast to the situation in humans, where *M. tuberculosis* has been in the population for many centuries, and HIV/AIDS was introduced relatively recently [64].

- It remains possible that even our extensive dataset was not suitable to detect a pathogen-pathogen interaction. In literature on the HIV-TB interaction, emphasis has been laid on the changes in CD4+/CD8+ T-cell counts, pathology and the collection of longitudinal data [25,65,66]. The present data set was not collected for the purpose of assessing FIV_{ple}- *M. bovis* interaction and therefore lacks results on these important parameters. This precludes determining the directionality of any interactions, while order of infection can be crucial in the outcome of the pathogen-pathogen interaction [67]. Also, stage of infection and time of infection for both BTB and FIV_{ple} were not known, but may affect for example blood parameter values like WBC, and this could thus be a potential source of error. Selection pressures that were not determined in this study, for example prey availability, could also be confounding the pathogen-pathogen interaction [35].

- Although the use of call-up stations is accepted for the non-lethal capture of wildlife [68], our sampling method is likely not to have been truly random. For example,

relatively few young animals have been captured (electronic supplementary material S3). These are likely to be more cautious approaching a call-up station. This may also count for animals in a bad condition, since fewer animals than expected were captured at call-up stations in poor conditions. Lions that were brought to the veterinary station were collected with different efforts over the three areas. They had a lower mean BCS and higher mean age compared to lions captured at call-up stations, but the prevalence of FIV_{ple} and *M. bovis* infection for the lions (resp. 63% and 47%) brought to the veterinary station were not statistically different (χ^2-tested, P-value resp. 0.40 and 0.82) from prevalence found for the lions sampled at call-up stations (resp. 61% and 55%), and either including or excluding the emaciated lions had little influence on the various statistics (results not shown); therefore they were included to increase the power of the analyses. Though we tried to control for bias as much as possible, field data sets like these may include unmeasured biases, and results should be interpreted with caution.

Finally, we remark that the pathogen-pathogen interaction may become more important when the lions are under additional stress, for example due to high parasite load or bad nutritional status when there is a low prey density [30,50,67,69]. This complexity of disease in general and especially the interaction of pathogens, necessitates an extensive, long-term research program requiring large sample sizes from the host population [31]. In future studies, besides CD4+/CD8+ T-cell counts, macro parasite infestation [70,71], and social interaction networks to assess the infectiousness of individual animals [72] could be valuable inclusions.

With the tested parameters, no evidence was found that FIV_{ple} or *M. bovis*, or a co-infection of these, is currently causing a serious conservation threat to KNP lions. However, a significant spatio-temporal increase of BTB was found, which may impact on lion health, as previous studies have related BTB to diverse pathological lesions [73]. In buffaloes, the population growth rate was negatively affected by BTB without altering the population age distribution significantly, thus reducing the resilience of the population to disturbances [54], which may also apply to lions. With the recent creation of the Greater Limpopo Transfrontier Conservation Area and the knowledge that the co-infection of FIV_{ple} and *M. bovis* is most likely also present in lions in other African parks [7,74], it should be closely monitored how the KNP and other co-infected lion populations respond to a severe environmental perturbation compared to populations that are infected with only one or neither of these two agents.

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This project was approved by the South African National Parks conservation and ethics committees as well as the Animal Use & Care Committee of the University of Pretoria.

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Electronic Supplementary Material

- Description of the whole data set
- Description of the data set for lions with results for either FIV_{ple} and BTB or both

Description of the whole data set

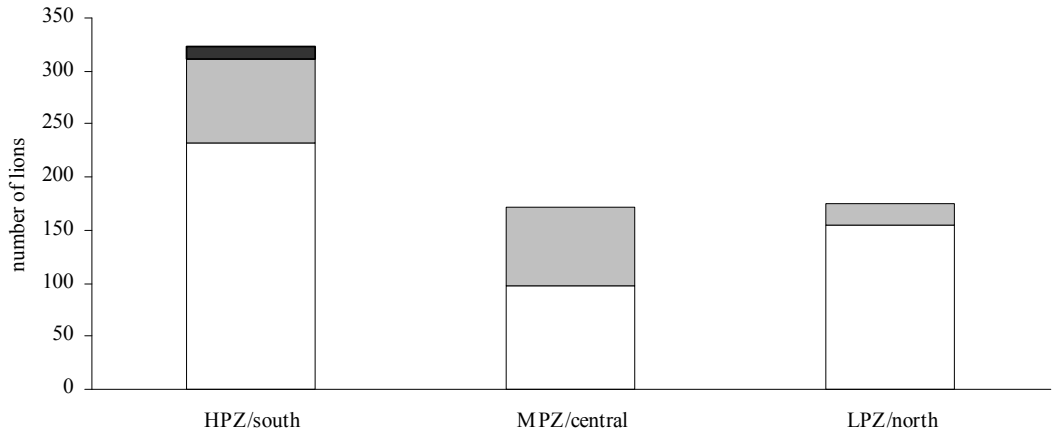
To give insight in the relative proportions of the lions that were either captured at call-up stations or had been brought to the veterinary station, the data set was described with different subdivisions in the tables and graphs, regardless of FIV_{ple} and BTB status. Results are listed in tables S1 to S4 and figures S1 to S4. Table S5 and figure S5 describe the age distribution in the different areas.

Supplementary table and figure S1: area

(a) Numbers of study lions in the three parts of the Kruger National Park (KNP) and adjacent game reserves. HPZ= high prevalence zone. MPZ= medium prevalence zone. LPZ= low prevalence zone.

	Area (n=669)			
	HPZ/south	MPZ/central	LPZ/north	Total
Call-up station	233	97	154	484
Veterinary station	79	74	21	174
Unknown	11	0	0	11
Total	323	171	175	669

(a)



(b)

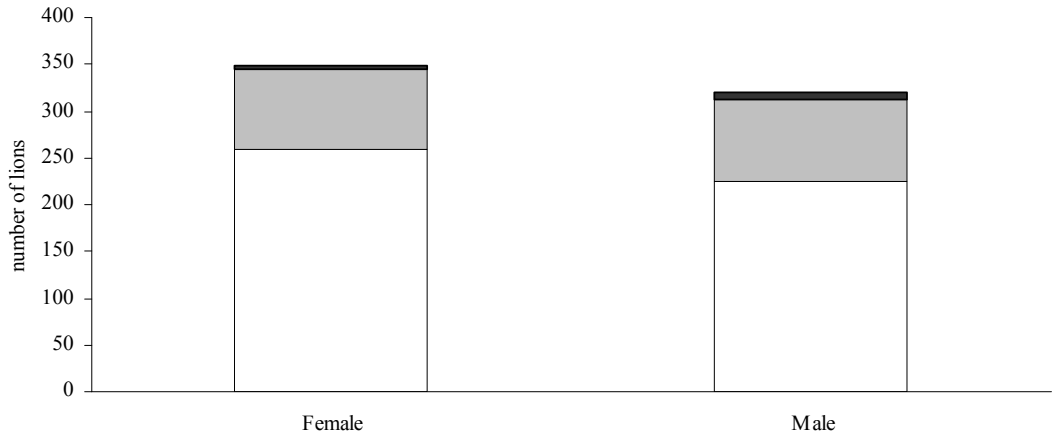
(b) Numbers of study lions in the three parts of the KNP and from a private game reserve (n=669). White bars= lions captured at a call-up station. Grey bars= lions brought to the veterinary station. Black bars= Unknown background.

Supplementary table and figure S2: sex

(a) Description of the sex of the lions in the data set.

	Sex (n=669)	
	Female	Male
Call-up station	260	224
Veterinary station	85	89
Unknown	4	7
Total	349	320

(a)



(b)

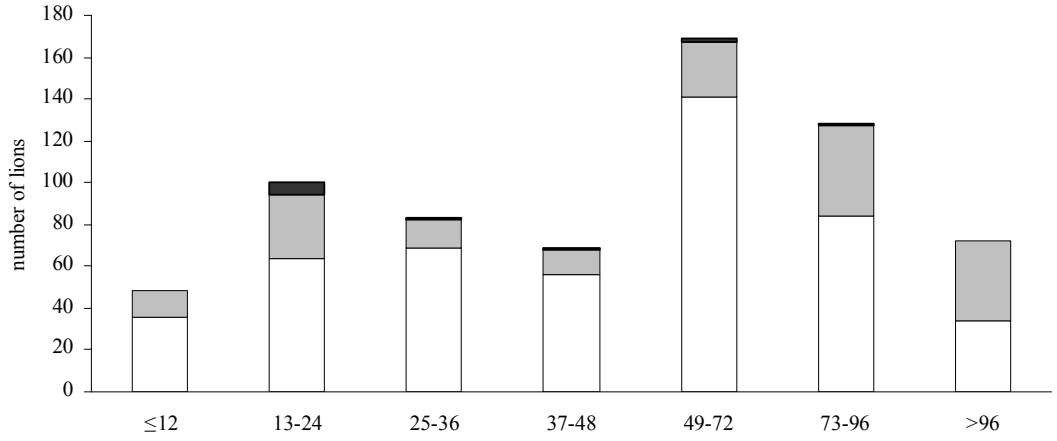
(b) Relative proportion of males and females in the data set (n=669). White bars= lions captured at a call-up station. Grey bars= lions brought to the veterinary station. Black bars= Unknown background.

Supplementary table and figure S3: age

(a) Numbers of lions per age class in the data set, with the age in months. A one-month interval was chosen for ages <36 months, a two-month interval was chosen for the higher ages, as age becomes increasingly difficult to estimate.

	Age classes (n=669)						
	≤12	13-24	25-36	37-48	49-72	73-96	>96
Call-up station	36	64	69	56	141	84	34
Veterinary station	12	30	13	12	26	43	38
Unknown	0	6	1	1	2	1	0
Total	48	100	83	69	169	128	72

(a)



(b)

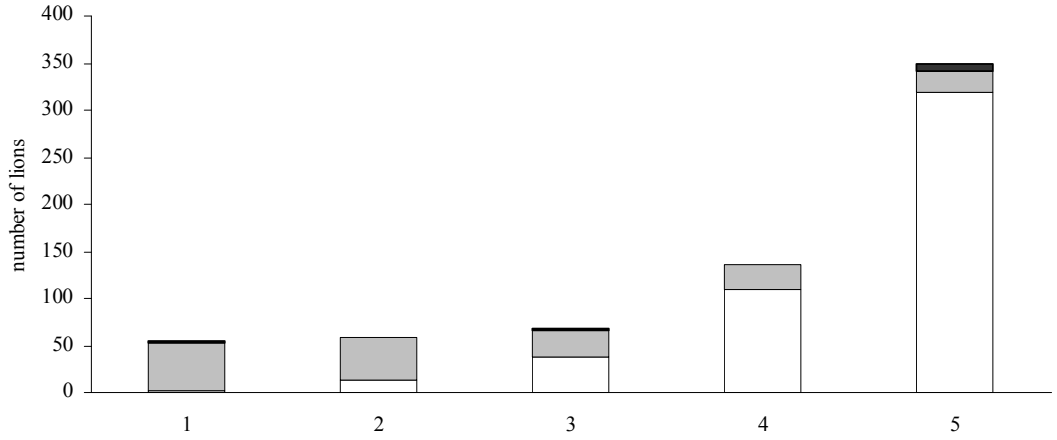
(b) Number of lions per age class, age in months (n=669). White bars= lions captured at a call-up station. Grey bars= lions brought to the veterinary station. Black bars= Unknown background.

Supplementary table and figure S4: BCS

(a) Description of the numbers of lions per body condition score (BCS) in the data set. BCS ranked from 1 (very poor) to 5 (excellent).

	Body condition score (n=666)				
	1	2	3	4	5
Call-up station	2	13	37	110	319
Veterinary station	51	46	29	25	23
Unknown	2	0	1	1	7
Total	55	59	67	136	349

(a)



(b)

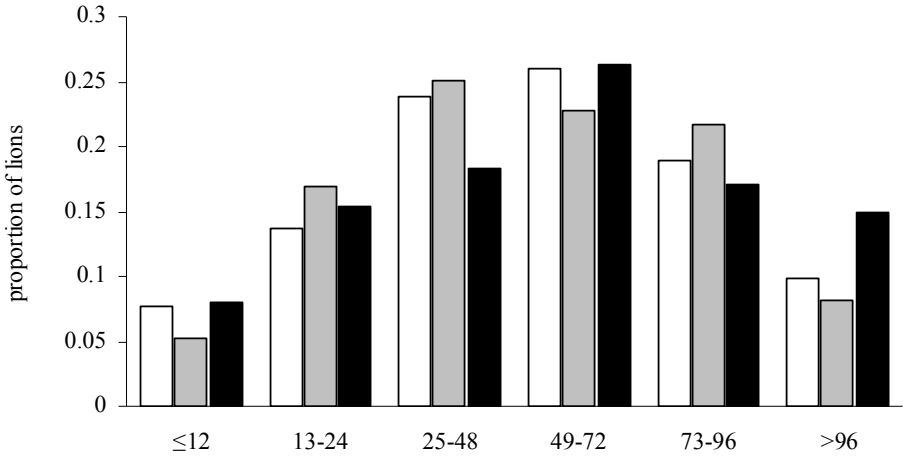
(b) Number of study lions per body condition score. BCS ranked from 1 (very poor) to 5 (excellent) (n=666). White bars= lions captured at a call-up station. Grey bars= lions brought to the veterinary station. Black bars= Unknown background.

Supplementary table and figure S5: Population age distribution per KNP area

(a) Number of study lions in the three different areas in the Kruger National Park divided in six age classes and the mean and median values per area.

	Area (n=669)		
	HPZ/south	MPZ/central	LPZ/north
≤12	25	9	14
13-24	44	29	27
25-48	77	43	32
49-72	84	39	46
73-96	61	37	30
>96	32	14	26
n total	323	171	175
Mean	58.2	58.6	63.5
Median	60	56	66

(a)



(b)

(b) Proportions of study lions per age class per area. White bars= HPZ/south, grey bars= MPZ/central, black bars= LPZ/north. Visual inspection shows similar population age distributions for the three areas.

Description of the data set for lions with an FIV_{ple} or BTB result

All study lions with a test result for FIV_{ple} and/or BTB, were used for descriptive analysis of FIV_{ple} and BTB in the Kruger National Park regarding area, period, sex, age and BCS. Temporal changes in the areas were assessed for three periods, 1993 to 1998, 1999 to 2002 and 2003 to 2008 (resp. 152, 337, 157 lions; 25 lions had an unknown capture date), based on attaining comparable numbers of observations.

Supplementary table S6: number of lions with FIV_{ple}, BTB, with both infections or no infections

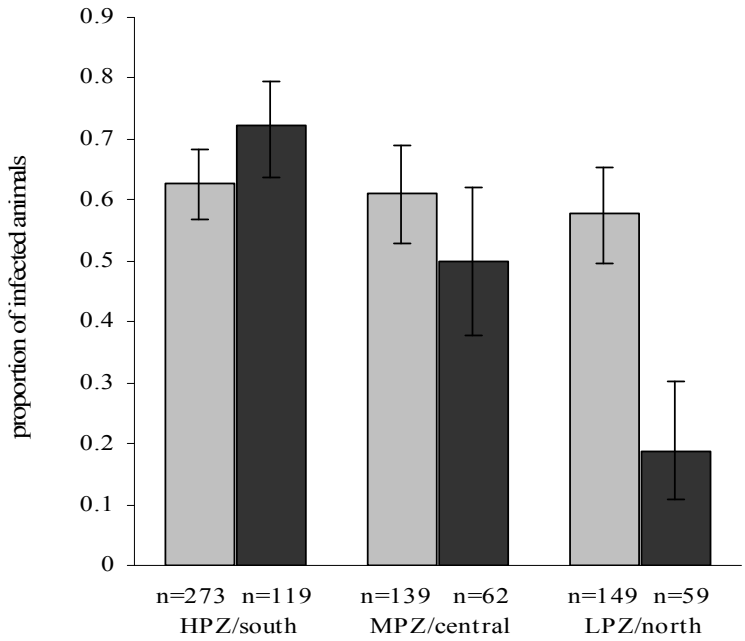
	BTB pos	BTB neg	Total
FIV_{ple} pos	63	53	116 (57%)
HPZ/south	*37*	*15*	
MPZ/central	*19*	*12*	
LPZ/north	*7*	*26*	
FIV_{ple} neg	44	45	89 (43%)
HPZ/south	*33*	*9*	
MPZ/central	*7*	*17*	
LPZ/north	*4*	*19*	
Total	107 (52%)	98 (48%)	205

Supplementary tables and figure S7: area

(a) The proportions of FIV_{ple} and BTB positive study lions over the total study period in the different parts of the KNP and their 95% confidence intervals (95% CI). The prevalence of FIV_{ple} in the three areas in the KNP found in this study compares well to smaller studies that were executed in the past [1,2].

	FIV_{ple} (n=561)			BTB (n=240)		
	n	FIV_{ple} pos (proportion)	95% CI	n	BTB pos (proportion)	95% CI
HPZ/south	273	0.63	0.57–0.68	119	0.72	0.64–0.80
MPZ/central	139	0.61	0.53–0.69	62	0.50	0.38–0.62
LPZ/north	149	0.58	0.50–0.65	59	0.19	0.11–0.30

(a)



(b)

(b) Proportions of FIV_{ple} and BTB infected study lions in the three different KNP areas over the total study period. Grey bars= FIV_{ple} positive lions. Black bars= BTB positive lions. Line segments indicate the 95% confidence interval.

(c) Proportions of FIV_{ple} and BTB positive study lions in the HPZ and the LPZ in three time periods. The central area was not assessed, because for the last time period, data of only three lions could be collected.

	HPZ/south		LPZ/north	
	FIV_{ple}	BTB	FIV_{ple}	BTB
Period 1993-1998	0.63 (n=64)	0.77 (n=31)	0.48 (n=21)	0 (n=11)
Period 1999-2002	0.66 (n=108)	0.66 (n=69)	0.64 (n=76)	0.13 (n=31)
Period 2003-2008	0.62 (n=81)	0.84 (n=19)	0.52 (n=52)	0.41 (n=17)

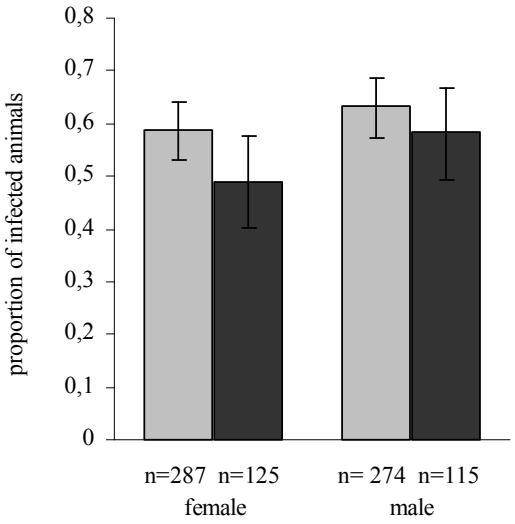
(c)

Supplementary table and figure S8: sex

(a) Proportions of FIV_{ple} and BTB positive study lions for the different sexes. No significant differences were found between males and females for either of the infections using the chi-square test.

	FIV_{ple} (n=561)			BTB (n=240)		
	n	FIV_{ple} pos (proportion)	95% CI	n	BTB pos (proportion)	95% CI
Female	287	0.59	0.53–0.64	125	0.49	0.40–0.57
Male	274	0.63	0.57–0.68	115	0.58	0.49–0.67

(a)



(b)

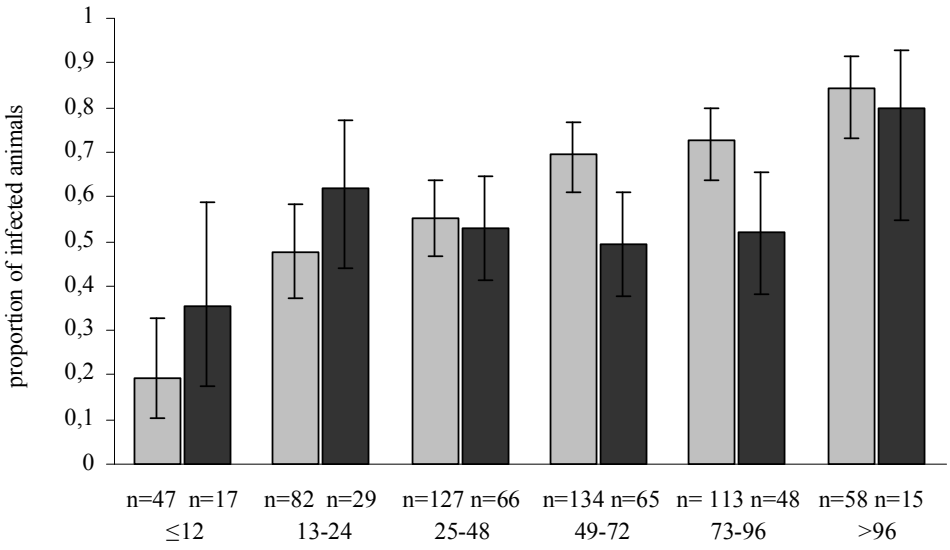
(b) Proportions of FIV_{ple} and BTB infected study lions per sex. Grey bars= FIV_{ple} positive lions, black bars= BTB positive lions. Line segments indicate the 95% confidence interval.

Supplementary table and figure S9: age

(a) Proportions of FIV_{ple} and BTB study positive lions in the different age classes. The youngest lions that were FIV_{ple} positive were 5 months old (4 cases). The two youngest BTB cases were 5 and 7 months old.

	FIV_{ple} (n= 561)			BTB (n=240)		
	n	FIV_{ple} pos (proportion)	95% CI	n	BTB pos (proportion)	95% CI
≤12	47	0.19	0.10–0.33	17	0.35	0.17–0.59
13-24	82	0.48	0.37–0.58	29	0.62	0.44–0.77
25-48	127	0.55	0.46–0.63	66	0.53	0.41–0.65
49-72	134	0.69	0.61–0.77	65	0.49	0.37–0.61
73-96	113	0.73	0.64–0.80	48	0.52	0.38–0.66
>96	58	0.84	0.73–0.92	15	0.80	0.55–0.93

(a)



(b)

(b) Proportions of FIV_{ple} and BTB infected study lions per age class. A general increase with age of the prevalences of FIV_{ple} and *M. bovis* was found. Grey bars=

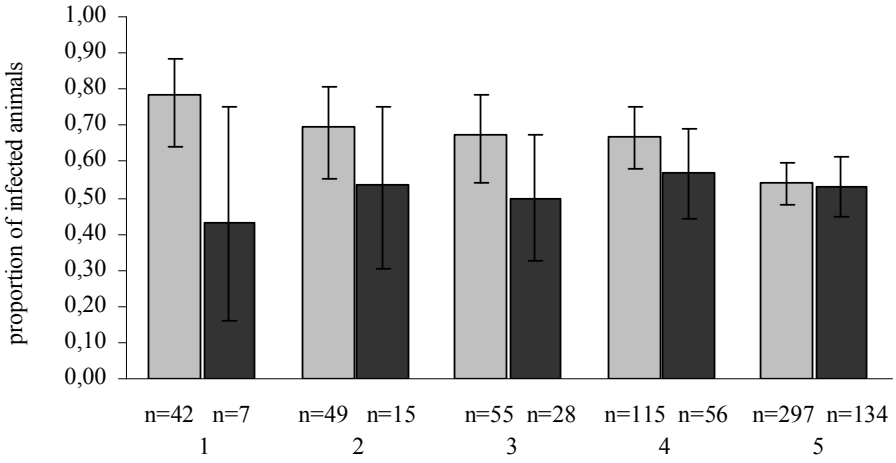
FIV_{ple} positive lions, black bars= BTB positive lions. Line segments indicate the 95% confidence interval. Little information exists about the course of infection of FIV_{ple} and *M. bovis* in a lion, and the common belief is that both infections are life long in lions. Thus, the prevalence of both infections shows an increase with age.

Supplementary table and figure S10: BCS

(a) Proportions of FIV_{ple} and BTB positive study lions in the different BCS classes.

	FIV_{ple} (n=558)			BTB (n=240)		
	n	FIV_{ple} pos (proportion)	95% CI	n	BTB pos (proportion)	95% CI
BCS 1	42	0.79	0.64–0.88	7	0.43	0.16–0.75
BCS 2	49	0.69	0.55–0.80	15	0.53	0.30–0.75
BCS 3	55	0.67	0.54–0.78	28	0.50	0.33–0.67
BCS 4	115	0.67	0.58–0.75	56	0.57	0.44–0.69
BCS 5	297	0.54	0.48–0.59	134	0.53	0.45–0.61

(a)



(b)

(b) Proportion of FIV_{ple} and BTB infected study lions per BCS class. Grey bars= FIV_{ple} positive lions. Black bars= BTB positive lions. Line segments indicate the 95% confidence interval.

Supplementary table S11

Comparison of the calculated lion blood parameter values from the data set with the values of the International Species Information System (ISIS) for lions [3]. These values have been collected using zoo lions worldwide and the means and standard deviations have been given. A difference has been made between lions younger than 3 years old, and lions older than 3 years. Sample size is the number of samples, which comes from the same or from a smaller number of individuals lions (indicate as "animals"). The proportion of lions used for the calculations of the mean values for lions infected with FIV_{ple}, BTB or both, was comparable with overall infection rates. NA=Not available.

	Dependent variables						
Explanatory variables	**Ht (%)**	**WBC (*10^9/L)**	**TSP (g/L)**	**Alb (g/L)**	**Glob (g/L)**	**Gamma glob (g/L)**	**A:G ratio**
Data set							
Mean total	34.3	18.6	84.0	28.3	55.7	24.9	0.5
Animals (n)	164	163	172	172	172	172	172
Mean FIV^+	33.0	19.6	85.5	25.7	59.7	25.9	0.4
Mean BTB^+	33.7	19.4	84.6	27.7	56.9	26.0	0.5
Mean FIV^+ and BTB^+	32.9	19.1	84.7	26.5	58.2	26.0	0.5
ISIS values							
Age <3							
Mean	35.9	12.4	67	34	33	NA	NA
St. Deviation	5.2	4.8	7	5	7	NA	NA
Sample size (n)	135	131	105	101	98	NA	NA
Animals (n)	90	91	71	69	67	NA	NA
Age >3							
Mean	39.4	13.6	75	33	42	28	NA
St. Deviation	5	4.2	6	4	7	10	NA
Sample size (n)	538	514	481	424	417	6	NA
Animals (n)	248	241	215	193	186	6	NA

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[2] Adams, H., van Vuuren, M., Bosman, A. M., Keet, D., New, J. & Kennedy, M. 2009 The epidemiology of lion lentivirus infection among a population of free-ranging lions (Panthera leo) in the Kruger National Park, South Africa*. J. S. Afr. Vet. Assoc.* **80**, 151-156.

[3] Teare, J. A. 1999 *International Species Information System, Physiological Data Reference Values*. Apple Valley, USA.