4. Good health status of free-ranging Namibian cheetahs and low prevalence of viral infections in the population

4.1 Introduction

The health of captive cheetahs has been studied intensively in the past. This was mainly due to the high occurrence of diseases and mortality of cheetahs kept in zoos and breeding facilities (e.g. Evermann 1986; Junge et al. 1991; Munson 1993; Eaton et al. 1993; Munson et al. 1999). In the early 1980s a lack of genetic variability was found in the isozymes and soluble proteins of the cheetah (O'Brien et al. 1983, O'Brien et al 1985) and later also at the major histocompatibility complex (MHC) class I loci (Yuhki & O'Brien 1990). The MHC class I genes encode peptides that mediate the immune response to viral infections, and therefore it was repeatedly proposed that the cheetah is particularly vulnerable to infectious diseases (O'Brien et al. 1983; O'Brien et al. 1985; Evermann et al. 1988; Yuhki & O'Brien 1990; Grisham & Killmar 1997; O'Brien et al. 1998; Marker 2000; Marker et al. 2003a). An outbreak of feline infectious peritonitis (FIP) in a captive cheetah population in a North American zoo caused the death of 60% of the population and is commonly cited as a support for the limited immunological competence of cheetahs (O'Brien et al. 1983; O'Brien et al. 1985; Evermann 1986; Evermann et al. 1988; O'Brien & Evermann 1988; Heeney et al. 1990; Yuhki & O'Brien 1990; Evermann et al. 1993; Munson 1993; Munson et al. 2004). Other authors have proposed that rather the artificially increased density in zoos and breeding facilities led to a higher incidence of infectious disease in this solitary living species (Merola 1994; May 1995) or that stressful husbandry conditions are responsible for pathological changes (Caro & Laurenson 1994).

Whether cheetahs are vulnerable to infectious diseases has not only implications for husbandry management but also for the conservation of free-ranging cheetahs. It is important to have information on the general health status and the susceptibility to infectious diseases of free-ranging cheetahs, especially when cheetahs range outside protected areas and potentially get into contact with un-vaccinated domestic dogs and cats carrying viral diseases that cheetahs might be susceptible to. To investigate the susceptibility to infectious diseases in more detail and to examine the general health status, a comprehensive survey was carried out on free-ranging cheetahs inhabiting commercial farmland in central Namibia and on captive cheetahs kept by Namibian farmers in large enclosures in their natural habitat. Several free-ranging and captive study animals were repeatedly examined, providing information on their infectious status over several months.

If there is a genetically based elevated susceptibility to infectious diseases in this species, a considerable number of free-ranging Namibian cheetahs

should show clinical symptoms of these viral diseases. Alternatively, if the immunocompetence of cheetahs is not limited in its capacity to effectively respond to viral challenges, wild-caught cheetahs should be in good general health and show little or no symptoms of acute viral infections.

Until todate, little information has been available on the health status, e.g. the nutritional and physical status, the occurrence of injuries and the presence of external parasites of free-ranging male and female cheetahs (Caro et al. 1987; Marker & Dickman 2003). Males are likely to invest their energy in the search for females and competition over oestrous females. For females, the highest energy turnover is likely to be associated with gestation and lactation (Caro 1994). In the cheetah population in the Serengeti National Park in Tanzania there were no sex differences amongst adult animals in nutritional status, scored as the ease with which the dorsal spinous process vertebrae could be palpated, or in physical status, scored as the degree of mange that could be observed (Caro et al. 1987). Also, no difference between the sexes was found in terms of body mass index (BMI) when calculating this parameter using the data of Caro et al. (1987). Therefore, total energy expenses might be similar for males and females in Tanzania.

In Tanzania, females raise litters with an average size of 1.8 cubs (Laurenson 1994), whereas cheetah females in Namibia raise substantially larger litters with an average of 3.0 cubs (Chapter 2). Since energetic costs of raising offspring increases with litter size (Laurenson 1995), it is likely that Namibian females have higher energetic costs than Tanzanian females. It is therefore possible that energy costs between males and females in Namibia differ and that Namibian females have a lower nutritional status than males, i.e. were more often in a bad nutritional status and also have a lower BMI. Concerning sub-adult cheetahs, no difference between the sexes in nutritional status and BMI were expected. The only previous study of Namibian cheetahs by Marker & Dickmann (2003) found no sex differences in nutritional and physical status for free-ranging Namibian animals. However, Marker & Dickmann (2003) used a combined assessment of nutritional and physical status, including musculature, body fat, coat condition, injuries and external parasites and included all age classes from newborn to adult animals in their assessment.

Caro (1994) reported that intraspecific fights usually occur between males, less often between males and females, and were not observed between females. Therefore, males were expected to show a higher incidence of (severe) injuries than females. Free-ranging cheetah males roam either alone or in groups of 2 to 4 males (Caro 1994; Marker et al. 2003). General health status was therefore compared between single males and group-living males.

Captive and free-ranging cheetahs on Namibian farmland derive from the same source population and thus the same genetic stock. Once a viral antigen has entered an enclosure and infected animals start to shed the virus,

it is likely that the pathogen accumulates within the enclosure. Through repeated direct or indirect contact with viral antigen viral load might increase over time and lead to more infections in captive than free-ranging cheetahs. Furthermore, captive cheetahs are kept in the vicinity of the farmhouses or lodges and therefore are more frequently exposed to domestic animals acting as possible pathogen transmitters than free-ranging cheetahs. For captive cheetahs kept in artificially high densities, higher contact rates and - in case of infection – higher transmission rates of pathogens as compared to free-ranging cheetahs might be expected. Thus, it was expected that captive individuals show a higher prevalence of antibodies against pathogens than free-ranging ones.

Animals were tested for the prevalence of antibodies against feline herpes virus 1 (FHV-1), feline calici virus (FCV), feline parvovirus (FPV), feline corona virus (FCoV), canine distemper virus (CDV), feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and rabies virus.

4.2 Material and Methods

4.2.1 Study animals

A list of the animals included in this study is given in Appendix II. Briefly, between June 2002 and October 2004, 61 free-ranging cheetahs were caught, immobilised, sampled and aged as described in Chapter 2. These cheetahs consisted of 35 adult males, 8 adult females, 6 sub-adult males, 6 sub-adult females, 3 male cubs and 3 female cubs. During the study, also 3 adult leopards (*Panthera pardus*), 3 adult caracals (*Felis caracal*) and 1 adult black-backed jackal (*Canis mesomelas*) were caught, immobilised and sampled. In addition, 22 adult captive cheetahs were immobilised on several farms; information on their life history and vaccination status was provided by the farmers. 7 captive cheetahs were vaccinated against FHV, FCV, FPV and rabies virus. Furthermore, serum and/or brain samples were collected from 9 dead cheetahs, 6 dead leopards and one dead black-backed jackal. Identification codes of free-ranging and captive cheetahs consisted of a letter and three numbers (e.g. A001), with the letters A, K, P and W used for free-ranging and Z used for captive animals.

4.2.2 Repeat sampling

Five free-ranging and two captive cheetahs were sampled more than once. P009, P011, W015, W016, Z030 and Z038 were sampled for the second time after 1, 13, 2, 2, 12 and 3 months, respectively, whereas P023 was sampled again after 1.5 months and a third time after a further 3 months.

4.2.3 Evaluation of overall health status

Anaesthetised free-ranging cheetahs were inspected for their general health status. This included visual examination of the physiological orifices (anus, genitals, eyes, nostrils and oral cavity including teeth), rectal measurement of body temperature, inspection and palpation of thorax, abdomen and muscles. Claws were inspected for injuries. Heart frequency, respiration rate and partial oxygen pressure were checked with an oxymeter (Nellcor N-20PA, Nellcor Puritan Bennett Inc., Pleasanton, U.S.A.) and recorded every 5 minutes. Examination focussed on symptoms that might be related to viral infections such as diarrhoea, fever, ocular or nasal discharge and anorexia.

Nutritional status was estimated for 50 adult and sub-adult free-ranging cheetahs by assessing the amount of body fat and muscle mass present, and classifying the animals in three categories: (1) very good (bones palpable only with pressure, a lot of muscle mass); (2) fair to good (bones palpable with slight pressure, fair to good muscle mass); (3) bad (bones palpable without pressure, only little muscle mass, bone protuberantia clearly visible*).* Belly size was not considered since a full stomach reflects the last meal rather than the general nutritional status of the individual.

Physical status was evaluated in 53 adult and sub-adult free-ranging cheetahs as a combination of coat appearance, condition of body orifices and teeth. Physical status was recorded as: (1) very good (soft and shiny coat, clean orifices, intact teeth); (2) fair to good (fair coat, some irritations of the orifices and teeth); (3) bad (coarse coat, several to many irritations of the orifices and teeth).

In order to standardise the assessment of nutritional and physical status, examination and classification was always done by the same person.

4.2.4 Ectoparasites

The coat of free-ranging cheetahs was checked for ectoparasites and number and location of them were recorded. Ears and surrounding skin were checked for mange symptoms, and in case of suspicion (N = 6), skin scraps were taken and investigated under the microscope.

4.2.5 Injuries

Free-ranging cheetahs were examined for the presence of injuries. Of any visible injury the quality, localisation, extension, possible cause and consequences were noted. According to extent and possible consequence for the animal, injuries were classified as: (0) no injuries; (1) superficial injuries of small extent (skin abrasions); (2) injuries of larger extent and possibly restraining the individual's normal behaviour (e.g. bite wounds, parts of

tongue missing); (3) deep and obviously painful injuries (e.g. deep bite wounds, fractures of limbs). Injuries that were acquainted in the box traps (fresh skin abrasions, freshly broken claws) were excluded from the assessment because they did not reflect consequences of natural events.

4.2.6 Body mass, body length and body mass index

Body mass of free-ranging cheetahs was determined by calculating the difference between the two weights of a person standing on a balance with and without carrying a cheetah. Weights were recorded to an accuracy of 0.1 kg. Body length was determined by measuring the animal from the tip of the nose to the sacro-coccygeal joint. Measurements were made with a flexible measuring tape to an accuracy of 0.5 cm.

A body mass index (BMI) was calculated by dividing body mass [kg] with the squared body length in meter:

$$BMI = \frac{bodymass(kg)}{bodylength(m)^2}$$

To calculate whether the BMI differs between females and males of the Serengeti population, body masses and body lengths were extracted from Table 1 in Caro et al. (1987, see Table 4.1 below).

Table 4.1 Body mass, body length and BMI for females and males in the Serengeti National Park in Tanzania. Values for body masses and body lengths extracted from table 1 in Caro et al. (1987).

Females	Mass (kg)	Bogy length (m)	BMI	Males	Mass (kg)	Bogy length (m)	BMI
A	42.0	-		H	52.0	1.22	34.9
B	40.0	1.90	11.1	I	39.5	1.25	25.3
C	33.0	1.40	16.8	J	45.5	1.27	28.2
D	34.0	1.22	22.8	K	40.5	1.23	26.8
E	43.0	1.25	27.5	L	46.5	1.29	27.9
F	31.5	1.13	24.7	M	-	1.21	-
G	41.5	1.22	27.9	N	33.5	1.08	28.7
				O	45.0	1.28	27.5
				P	36.5	1.26	23.0
				Q	41.5	1.24	27.0

The body length of female B was assumed to be a clearly unbiological value (typing error in Caro et al. 1987) and thus not used in the analysis below. A comparison between females and males then revealed no difference in the BMI between the sexes (Mann-Whitney U-Test, U=11, N_1=5, N_2=9, p=0.12).

4.2.7 Blood sampling and processing

In immobilised animals venous blood was taken from the *V. brachyocephalica* or *V. saphena* for serological analysis. Blood was collected into serum blood tubes (BD Vacutainer Systems, Plymouth, UK) at volumes of 2 - 5 ml per tube. During necropsies full blood was gently aspired into a 5 ml syringe after cutting a large blood vessel and then filled into a serum tube. Samples were kept cool at 4°C during transport to the field station, where they were processed. Collected blood in the serum tubes was centrifuged at 5'000 rpm for 15 min. The components were separately filled into 1.8 or 2.0 ml cryo vials (Cryovial®, Simport, Quebec, Canada) and stored at –196°C in a liquid nitrogen container.

Samples were transported on dry ice (-80°C) or in a dry shipper (-196°C) to Europe, where they were stored in a –80°C freezer until serological tests were

carried out. Tests for antibodies to FHV, FCV, FPV, FCoV, CDV, FIV and FeLV were conducted at the Clinical Laboratory of the Vetsuisse Faculty in Zurich, Switzerland. Tests on antibodies to rabies virus and for rabies virus antigen were conducted at the Federal Research Centre for Virus Diseases of Animals, Tübingen, Germany and at the National Rabies Reference Laboratory at the Friedrich-Loeffler Institute at Wusterhausen/Dosse, Germany.

4.2.8 Immunofluorescence assays

Detection of antibodies to FHV, FCV and FPV

Immunofluorescence assay tests (IFA) were conducted on sera of 67 free-ranging cheetahs, seven free-ranging leopards, three free-ranging caracals, one free-ranging black-backed jackal and 15 non-vaccinated captive cheetahs. Antibodies to FHV, FCV and FPV were detected as described in Hofmann-Lehmann et al. (1996) using slides masked with Teflon coating, leaving open 10 wells where the antigen containing cells were attached. To prepare such virus infected cells, CRFK cell monolayers were grown in 75 cm^2 cell culture flasks in RPMI-1640 medium (Sigma-Aldrichs Inc., Buchs, Switzerland) with 10% fetal calf serum (Bio Concept, Allschwil, Switzerland), 1% L-glutamine 200 MM (Gibco, Fisher Scientific, Basel, Switzerland) and 1% antibiotic/antimycotic solution (Gibco, Fisher Scientific, Basel, Switzerland) at 37°C and 5% CO_2. Cells were infected with the respective virus when the monolayers showed 90% confluence. When the cytopathic effect (i.e. when the cells rounded up and detached from the bottom of the flask) reached 60% of the cells, they were harvested. The cells were removed with the medium and washed twice with Hanks balanced salt solution (HBSS; Invitrogen AG, Basel, Switzerland). Simultaneously, mock-infected cells were grown and handled under identical conditions and mixed with the infected cells. Then, a ratio of 80% infected and 20% non-infected cells was generated.

Cells were infected with: FHV (Zurich 5-04, a Swiss isolate obtained from a cat suffering from a herpes keratitis), FCV (F9 strain, Veterinaria AG, Zurich, Switzerland) or FPV (FPL/01, Veterinaria AG, Zurich, Switzerland Intervet Ltd, UK). All viruses were tested for the absence of contamination by other viruses and agents (see below). Then, 7 µl of a suspension of 1.5 X 10^6 cells per ml were placed on each well of the IFA slides. The slides were air-dried, fixed in acetone for 10 min at -20°C, and stored at -20°C.

For analysis, the sera were screened at a dilution of 1:20 in phosphate buffered saline (PBS; 2 mM NaH_2PO_4, 8 mM $NaHPO_4$, 0.15 M NaCl, pH 7.2). Incubation was carried out at 37°C for 60 min, then the slides were washed in PBS solution 3 times for 5 min and air dried. Fluorescent FITC conjugate (rabbit anti cat IgG, H+L chain, Nordic Immunology, Tillburg, The Netherlands)

diluted in PBS 1:40 was added and the slides were again incubated for 60 min at 37°C. After final rinsing of 3 times for 5 min in PBS, the slides were examined under a fluorescence microscope. The result was considered positive when specific fluorescence was detected in infected cells (Hoffmann-Lehmann et al. 1996). All positive sera were titrated on two-fold serial dilutions and tested under conditions described above.

Detection of antibodies to FCoV

The preparation of virus infected cells and IFA slides was done following the same protocol as described above. Cells were infected with transmissible gastroenteritis virus (Purdue Strain, Osterhaus et al 1977), and antibodies to FCoV in the sera were detected by IFA using fluorescent FITC conjugate (rabbit anti cat IgG, H+L chain, Nordic Immunology, Tillburg, The Netherlands) as described in Lutz et al. (1984). Sera of 67 free-ranging cheetahs, seven free-ranging leopards, three free-ranging caracals, one free-ranging black-backed jackal and 22 non-vaccinated captive cheetahs were tested with this assay. Figure 4.1. shows examples of a negative and a positive result of an IFA for antibodies against FCoV.

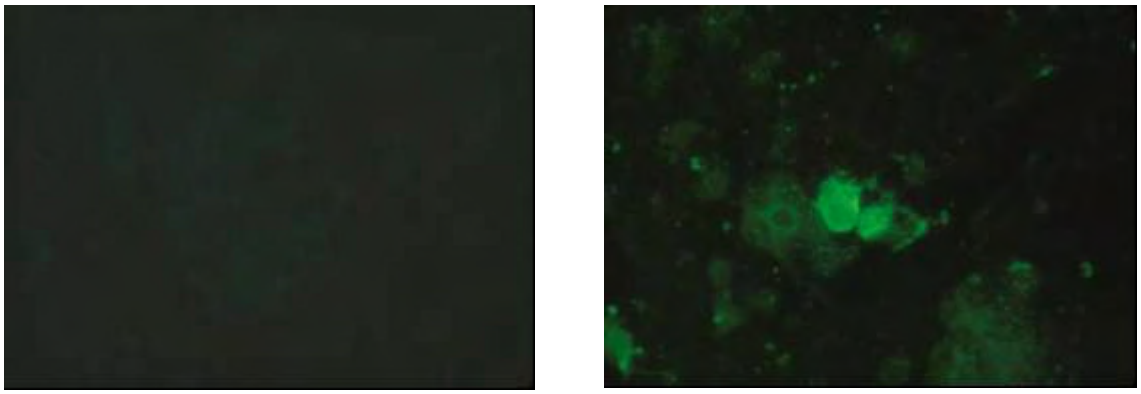

Figure 4.1. Pictures of a negative (left) and a positive (right) result of an IFA for antibodies against FCoV (kindly provided by H. Lutz). Fluorescent structures represent cells replicating the coronavirus. Antibodies bind to the coronavirus antigen and are made visible by addition of a rabbit-anti-cat IgG antibody conjugated with a fluorescent marker (FITC).

Detection of antibodies to CDV

Confluent VERO cell monolayers were infected with CDV (Onderstepoort strain). When 30% of the cells were infected the cells were removed from the

bottom of the 75 cm^2 cell culture flask using 1 ml of Trypsin-EDTA (Gibco Fisher Scientific, Basel, Switzerland), 2.5 g/L of Trypsin and 0.38 g/L of EDTA•4Na in HBSS. These cells were used to prepare the IFA as described above. IFA tests were conducted for serum samples of 67 free-ranging cheetahs, seven free-ranging leopards, three free-ranging caracals, one free-ranging black-backed jackal and 22 non-vaccinated captive cheetahs as described in Lutz et al. (1984) and Hofmann-Lehmann et al. (1996). Additionally, every second well was covered with 100% non-infected cells in order to have an external negative control. All sera were screened at a dilution of 1:20. Fluorescence typical for this test was considered a positive result. Positive serum samples were titrated up to 1:640 and screened at these dilutions.

4.2.9 Quality control of antigen preparation, total nucleic acid extraction and PCR

All antigens used for the IFA were tested by PCR or RT-PCR, respectively, for absence of possible contaminating antigens. Aliquots of the cell cultures or scrapings from the slides were tested for presence of antigen of FCV, FHV, FPV, FCoV, CDV, FIV and FeLV.

For the extraction of nucleic acids from the aliquots, the MagNA Pure LC® instrument (Roche Diagnostics AG, Rotkreuz, Switzerland) using the Total Nucleic Acid Isolation Kit (Roche Diagnostics AG, Rotkreuz, Switzerland) was used. Briefly, 140 µl of the aliquots or the scrapings from the slides were incubated at 40°C for 10 min in 300 µl lysis buffer. Then the MagNA Pure® instrument was started using the Total NA External lysis protocol. The purified nucleic acids were eluted in a final volume of 100 µl. The extracted total nucleic acid samples were analyzed by one-tube real-time PCR using an automated fluorometer (TaqMan®, ABI 7700, Applied Biosystems, Rotkreuz, Switzerland) for the presence of the agents of interest and following the protocols for FHV (Vögtlin et al. 2002), FPV (Ramsauer et al. 2007), FCoV (Gut et al. 1999; Meli et al. 2004), FIV (Leutenegger et al. 1999), FeLV (Hofmann-Lehmann et al. 2001) and CDV (Filoni 2005). For FCV, primers and probe sequences were derived from those published (Helps et al. 2005) and kindly provided by C. Helps:

primer forward=5'-GTTGGATGAACTACCCGCCAATC-3';

primer reverse=5'-CATATGCGGCTCTGATGGCTTGAAACTG-3',

probe=5'-TCGGTGTTTGATTTGGCCTG-3'.

4.2.10 Enzyme-Linked Immunoabsorbent Assay

General method

All ELISA techniques used in this study detected antibodies to specific proteins of the viruses. For this purpose, ELISA plates were coated with viral antigens and subsequently, sera were added. If antibodies to the respective antigens were present, they bound to the antigen. Bound antibodies were quantitated by addition of rabbit-anti-cat IgG antibodies conjugated to horseradish. Addition of the substrate {2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)} (Fluka AG, Buchs, St. Gallen, Switzerland) led to a colour change. The more antibodies were present in a serum sample the higher its optometric density (OD) became. On all ELISA plates (Micro ELISA plates, Integra Bioseciences AG, Chur, Switzerland) positive (FeLV infected domestic cat) and negative control sera were tested with the OD of the positive control set at 100% and the negative control set at 0%. The OD of each sample was measured in an ELISA reader (Micro ELISA reader, BD Bioseciences, Basel, Switzerland) and given as percentage of the positive control. This procedure revealed also OD values of samples below zero. For graphical presentation the OD values were grouped into 5%-intervals and visual cut-offs were used to define positive, questionable and negative samples in the population (Figures 4.2 – 4.6).

Detection of antibodies to FeLV with three different tests

FeLV p45 ELISA. The presence of antibodies to FeLV p45, i.e. to the unglycosylated form of the antigen section gp70 (Bachmann et al. 1997), was measured in serum samples from 50 free-ranging cheetahs, three free-ranging leopards and 18 captive cheetahs as described in Lehmann et al. (1991). According to the analysis described above, samples with an OD between 10% and 20% of the positive control (set at 100%) were considered questionable, and samples with an OD higher than 20% of the positive control as positive (Figure 4.2).

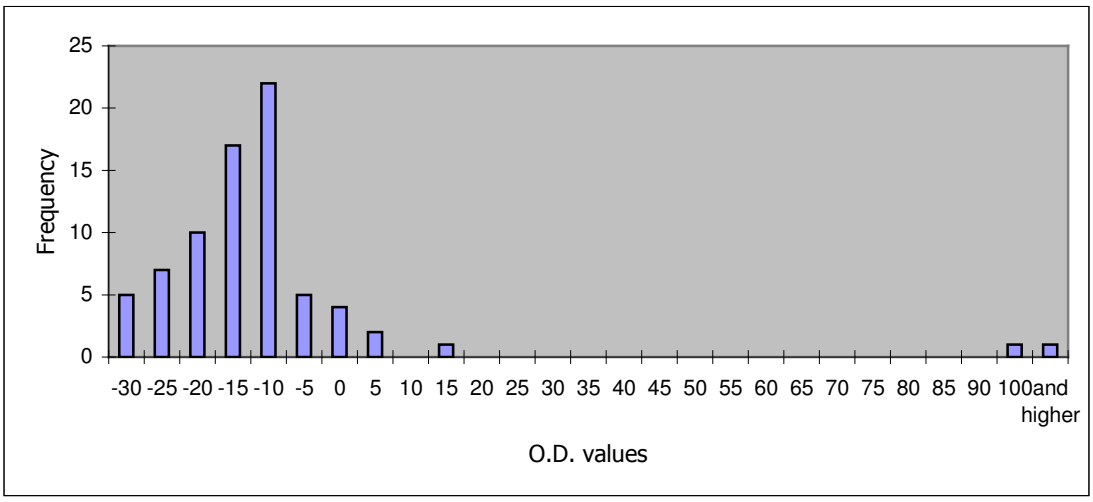

Figure 4.2 Frequency of the samples tested for antibodies to FeLV p45 allocated to the different OD-categories given as percentage of the positive control which was set at 100%. Samples with OD between 10% and 20% of the positive control were considered questionable and with OD higher than 20% of the positive control as positive.

FeLV FL-74 ELISA. With an ELISA using FL-74-Virus as antigen, antibodies against the three FeLV subtypes A, B and C were detected (Lutz et al. 1980). This protocol was followed to test serum samples for antibodies against FL-74-virus. For presence of these antibodies, serum from 67 free-ranging cheetahs, six free-ranging leopards, two free-ranging caracals, one free-ranging black-backed jackal and 22 captive cheetahs were tested. According to the distribution of OD values, animals with an OD higher than 40% of the positive control were considered positive (Figure 4.3).

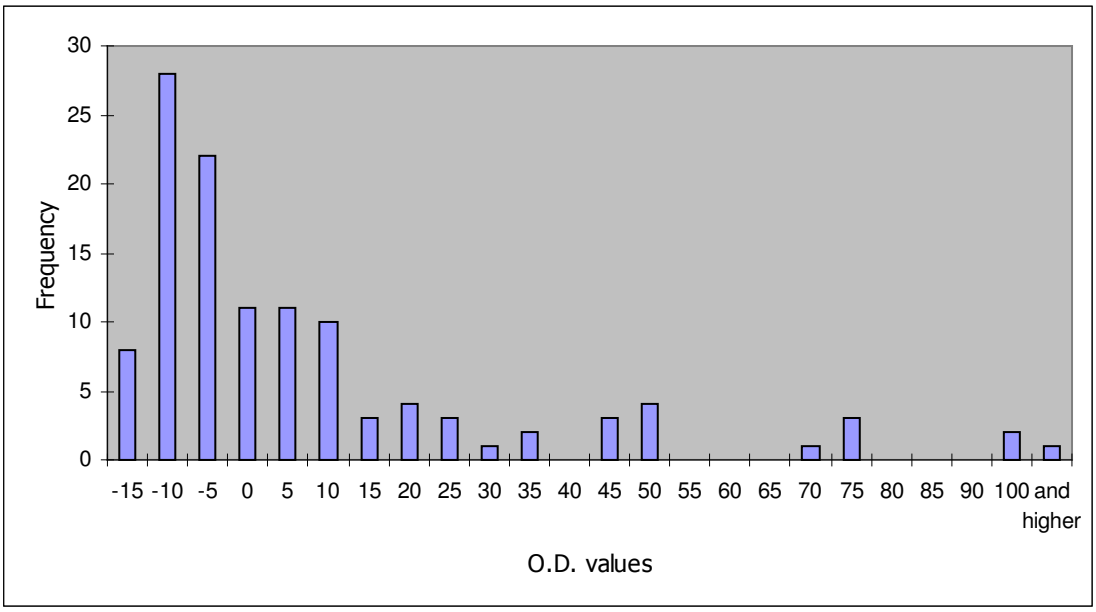

Figure 4.3 Frequency of the samples tested for antibodies to FeLV subtypes A, B and C allocated to the different OD-categories given as percentage of the positive control which was set at 100%. Samples with OD higher than 40% of the positive control were considered positive.

FeLV p27 ELISA. Serum samples from 67 free-ranging cheetahs, six free-ranging leopards, three free-ranging caracals and 22 captive cheetahs were tested for presence of p27, the major core protein of FeLV, as described by Lutz et al. (1983a). Presence of p27 is a marker of FeLV viremia and therefore of FeLV infection (Lutz et al. 1983b). Animals with OD values higher than 25% of the positive control were considered positive (Figure 4.4).

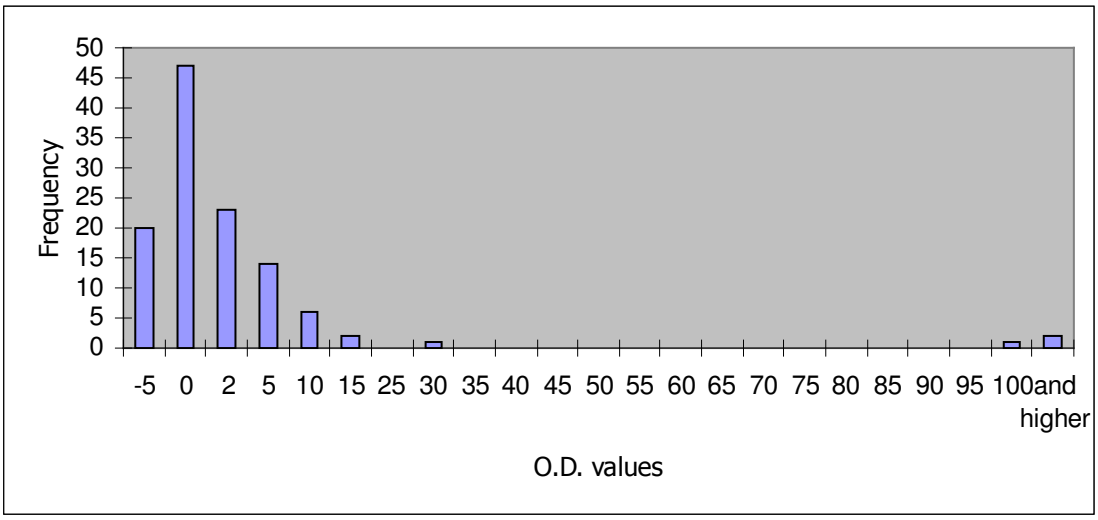

Figure 4.4 Frequency of the samples tested for antibodies to FeLV p27 allocated to the different OD-categories given as percentage of the positive control which was set at 100%. Samples with OD higher than 25% of the positive control were considered positive.

Detection of antibodies to FIV

FIV-ELISA. 49 free-ranging cheetah, three free-ranging leopard, three free-ranging caracal and 19 captive cheetah sera were tested for antibodies to FIV. The sera were tested by ELISA using recombinant FIV-Z2 transmembrane glycoprotein developed in the laboratory as described by Calzolari et al. (1995). Animals with OD values higher than 5% of the positive control (set at 100%) were considered positive (Figure 4.5). Serum of a FIV-infected domestic cat was used for the positive control.

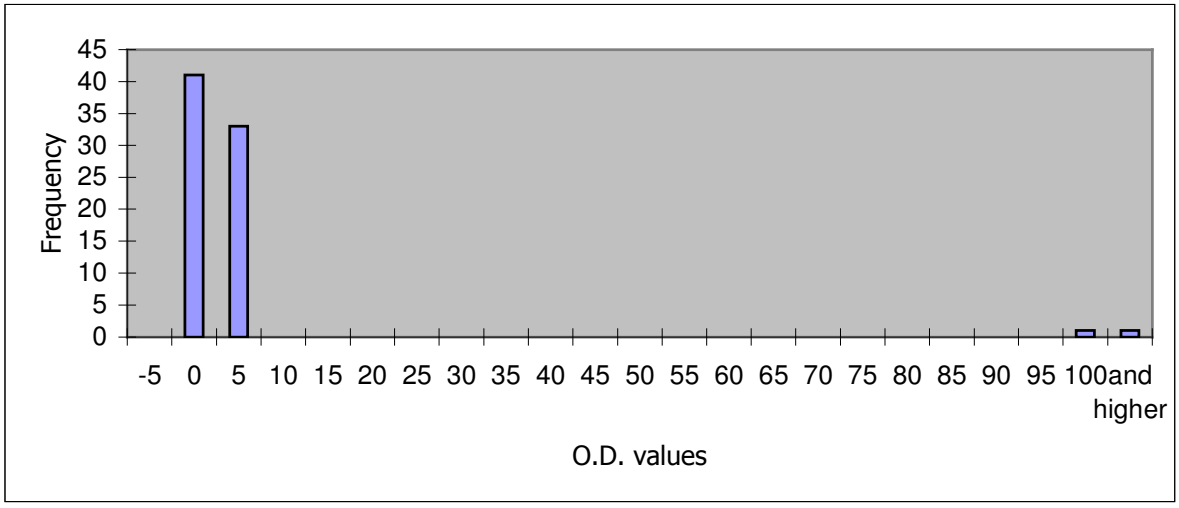

Figure 4.5 Frequency of the samples tested for antibodies to FIV allocated to the different OD-categories given as percentage of the positive control which was set at 100%. Samples with OD higher than 5% of the positive control were considered positive.

PLV-ELISA. It was shown for wild felids that the detection of antibodies against immunodeficiency virus might be more sensitive when using puma lenti virus (PLV) antigen (Van Vuuren et al. 2003). PLV is related to FIV and therefore antibodies against immunodeficiency viruses show cross-reactivity to both antigens. Thus, a second ELISA technique was applied to detect antibodies against immunodeficiency virus. For antibodies against PLV, 65 free-ranging cheetahs, six free-ranging leopard, three free-ranging caracal and 22 captive cheetah sera were screened. To detect antibodies against PLV, a synthetic peptide derived from the transmembrane glycoprotein of PLV was used (Kania et al. 1997). The ELISA was conducted by using flat-bottomed micro titre plates (MicroELISA plates, Integra Bioseciences AG, Chur, Switzerland) coated with 100 µl coating buffer (0.1 M Na_2CO_3, pH 9.6) containing 1 µg PLV peptide antigen. The plates were kept at 37°C for 3 h and washed three times with wash solution (0.9% NaCl, 0.1% Tween 20). The sera were incubated in duplicate at 37°C for 60 min at a dilution of 1:200 in buffer 3X (0.15 NaCl, 1 mM Na_2-EDTA, 50 mM Tris-Base, 0.1% Tween 20, and 0.1% bovine serumalbumin) in the antigen-coated wells. After incubation, the plates were washed as described and 100 µl of a peroxidase-conjugated goat anti-cat IgG preparation (Jackson ImmunoResearch Laboratories®, West Baltimore Pike, Pennsylvania, USA) was added at a dilution of 1:1000 in PBS-Buffer 3X. After washing thoroughly, the substrate {2,2'-azino-bis(3-ehtylbenz-thiazoline-6-sulfonic acid)} was added and the plates placed for 20 min on a rocker at room temperature. Sera that gave an OD of more than 40% of the positive control serum (set at 100%) were considered as positive (Figure 4.6).

Serum of a lion (*Panthera leo*) naturally infected by lentivirus was used for the positive control.

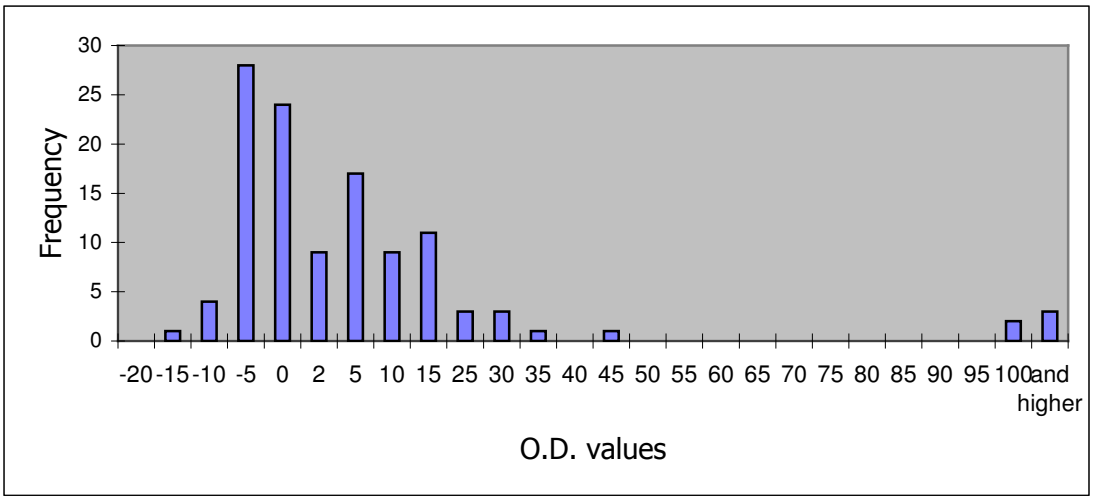

Figure 4.6 Frequency of the samples tested for antibodies to PLV allocated to the different OD-categories given as percentage of the positive control which was set at 100%. Samples with OD higher than 40% of the positive control were considered positive.

4.2.11 Western Blot

Additionally to ELISA tests to detect antibodies against FeLV, sera from 23 free-ranging and 11 captive cheetahs were also examined for the presence of antibodies against FeLV proteins by using the Western blot technique described by Lutz et al. (1988). Samples containing antibodies at least to the proteins p15 (E) and p27 were considered positive (Lutz et al. 1980). Figure 4.7. shows the results of nine sera tested for antibodies against FeLV in a Western blot.

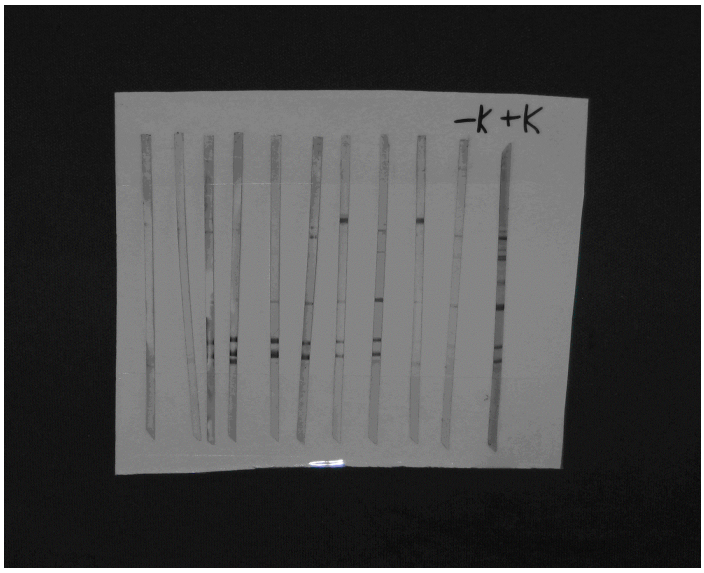

Figure 4.7 A FeLV Western blot with nine tested sera samples and a negative and positive control (-K, +K). Six of the samples (strips 3, 4, 5, 6, 7 and 8) were considered positive, three of the samples (strips 1, 2 and 9) were considered negative.

4.2.12 Rapid Fluorescent Focus Inhibition Test (RFFIT)

Serum samples from 41 free-ranging and 20 captive cheetahs (11 vaccinated and 9 non-vaccinated), as well as from two free-ranging leopards were tested for the presence of rabies-specific virus-neutralizing antibody using challenge virus standard virus as described in East et al. (2001). World Health Organization reference serum was included to determine international units (IU/ml) and titres equal or higher 0.5 IU/ml were considered positive.

4.2.13 Reverse Transcriptase PCR

Brain samples of seven free-ranging cheetahs, three free-ranging leopards and one free-ranging jackal were tested for the presence of viral antigen using murine neuroblastoma cell cultures as described in East et al. (2001).

4.2.14 Statistics

Statistical analysis was performed on a personal computer using SYSTAT 11.0 following recommendations by Engel (1997). All means are given with standard deviations and p-values are for two-tailed tests.

4.3. Results

4.3.1 Symptoms of acute viral infections

None of the 61 examined free-ranging cheetahs showed signs of an acute infectious disease such as fever, anorexia, ocular or nasal discharge. Only one sub-adult free-ranging male cheetah (P034) had diarrhoea. Similarly, none of the captive cheetahs (vaccinated or non-vaccinated) showed any symptoms of a viral infection.

4.3.2 Nutritional and physical status

Five of the eight adult free-ranging females were classified to have a bad nutritional status (63%) and 5 of the 30 adult free-ranging males were classified to have a bad nutritional status (17%, Table 4.2a). None of the females was in a very good nutritional status, but two (6%) of the males were (Table 4.2a). This resulted in females being in a significantly worse nutritional status than males, when testing category 3 (bad nutritional status) against categories 1 and 2 (very good and fair to good nutritional status, Fisher's exact test, p=0.019, *N*=38). No such difference was found for physical status (Fisher's exact test, *p*=1.0, *N*=41, Table 4.2b), where all eight females and 33 males were in very good or fair to good physical condition.

Table 4.2 Numbers of adult free-ranging females and males categorised into very good (category 1), fair to good (category 2) or bad (category 3) nutritional status (4.2a) and physical status (4.2b).

Table 4.2a Nutritional status

	Category		
	1	2	3
Adult ♀	0	3	5
Adult ♂	2	23	5

Table 4.2b Physical status

	Category		
	1	2	3
Adult ♀	1	7	0
Adult ♂	6	27	0

None of the sub-adult animals were in a very good nutritional status (Table 4.3a). A comparison between the 6 sub-adult females and 6 sub-adult males showed no difference in nutritional status (Fisher's exact test, *p*=1.0, *N*=12). The same result applied to physical status (Fisher's exact test, *p*=1.0, *N*=12), with the majority of animals categorised to be in a fair to good status (Table 4.3b).

Table 4.3 Numbers of sub-adult free-ranging females and males categorised into very good (category 1), fair to good (category 2) or bad (category 3) nutritional status (4.3a) and physical status (4.3b).

Table 4.3a Nutritional status

	Category		
	1	2	3
Subadult ♀	0	4	2
Subadult ♂	0	5	1

Table 4.3b Physical status

	Category		
	1	2	3
Subadult ♀	1	4	1
Subadult ♂	0	6	0

When comparing the nutritional status of group-living males (*N*=16) and single males (*N*=14), social males were significantly more often in a very good or fair to good nutritional status than single males (Fisher's exact test, *p*=0.014, *N*=30, Table 4.4a). No such difference was found when comparing the physical status of group-living males (N=19) and single males (N=14, Fisher's exact test, *p*=1.0, *N*=33, Table 4.4b).

Table 4.4 Numbers of adult group living males and single males categorised into very good (category 1), fair to good (category 2) or bad (category 3) nutritional status (4.4a.) and physical status (4.4b).

Table 4.4a Nutritional status

	Category		
	1	2	3
Group ♂	2	14	0
Single ♂	0	9	5

Table 4.4b Physical status

	Category		
	1	2	3
Group ♂	3	16	0
Single ♂	3	11	0

4.3.3 Body mass index (BMI)

The mean BMI of seven adult free-ranging females was with 23.3 ± 0.8 significantly lower than the mean BMI of 34 males with 27.1 ± 2.1 (Mann-Whitney U-Test, U=11.0, N_1=7, N_2=34, p<0.001). This is consistent with the result that females are in a significantly worse nutritional status than males (see above). The four sub-adult females also had a significantly lower BMI at 20.0 ± 1.4 than the 6 sub-adult males at 24.1 ± 1.2 (Mann-Whitney U-Test, U=0.0, N_1=4, N_2=6, p=0.011).

Group-living males (N=18) had a significantly higher BMI at 28.0 ± 1.8 than single males (N=16) at 26.2 ± 2.0 (Mann-Whitney U-Test, N_1=18, N_2=16, U=77.5, p=0.022). This result is consistent with the finding that group-living males were in a better nutritional status than single males (see above).

4.3.4 Injuries

None of 5 adult females and 6 of 32 adult males (19%) had severe injuries, not a significant difference between the sexes (Fisher's exact test, p=0.57, N=37). Figure 4.8 shows the distribution of the injuries for adult females and males. None of the 6 sub-adult females and only one of the 6 sub-adult males had injuries when examined.

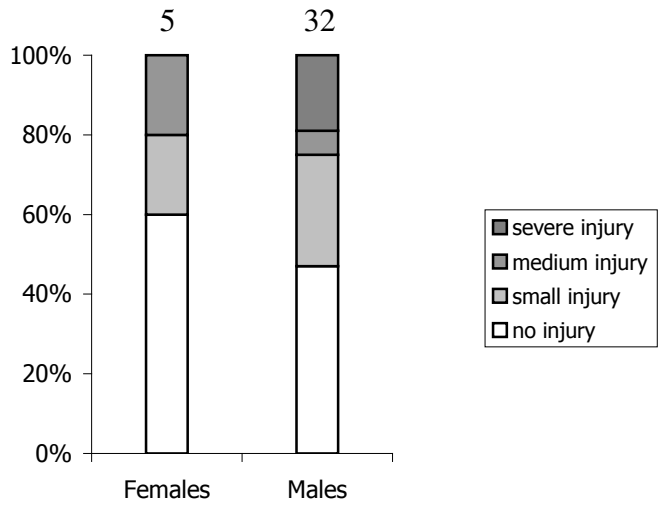

Figure 4.8 Percentage of occurrence of different injury categories in adult female and male cheetahs. The numbers above the bars represent sample sizes.

There was no difference in the occurrence of injuries between 17 group-living males at 53% and 15 single males also at 53% (Fisher's exact test, p=1.0, N=32).

4.3.5 Ectoparasites

Ectoparasites were ticks (*Hyalomna sp.*) and flies (*Hippobosca sp.*). Ticks were usually located around the anal region and on the neck and face whereas flies were mostly found at the inside of limbs and on the belly. Numbers of ectoparasites varied highly between individuals, with numbers of ticks ranging between 0 and 50 and for flies between 0 and 55 per individual.

The mean number of ticks on adult and sub-adult females (N=10) of 1.4±1.7 did not significantly differ from the mean number on adult and sub-adult males (N=38) of 2.5±5.6 (Mann-Whitney U-Test, N_1=10, N_2=38, U=178.0, p=0.74). Similarly, there was no difference in the mean number of flies between females (N=10) of 11.9±16.5 and males (N=40) of 11.1±10.9 ticks (Mann-Whitney U-Test, N_1=10, N_2=40, U=181.5, p=0.65).

Cheetahs roaming with other cheetahs (N=31), i.e. males in groups, mothers with sub-adult offspring or independent sub-adult sibling groups, had on average 1.2±2.9 ticks whereas single animals (N=17), i.e. single males or single females, had 4.3±7.1 ticks. Thus, social cheetahs had significantly fewer ticks than solitary animals (Mann-Whitney U-Test, N_1=31, N_2=17, U=153.5, p=0.0095). Also, group-living males (N=17) had significantly fewer ticks at 0.6±1.5 than single males (N=15) at 4.6±7.5 (Mann-Whitney U-Test, N_1=17, N_2=15, U=62, p=0.0078).

There was no such difference in the number of flies of cheetahs roaming in groups at 11.3±13.3 (N=33) and of single cheetahs (N=17) at 11.0±9.4 (Mann-Whitney U-Test, N_1=33, N_2=17, U=260.5, p=0.68), nor between group-living males at 9.8±8.3 (N=19) and single males (N=15) at 10.9±9.2 (Mann-Whitney U-Test, N_1=19, N_2=15,U=132, p=0.71).

None of the examined cheetahs showed symptoms of mange. Also, in none of the skin scraps taken from suspicious animals, mange causing agents as *Sarcoptes* sp., *Otodectes* sp. or *Notoedres* sp. were diagnosed.

4.3.6 Serology by immunofluorescence assay

Detection of antibodies to FHV, FCV, FPV, FCoV and CDV

Free-ranging and non-vaccinated captive cheetahs. Of the 67 free-ranging cheetahs tested for antibodies to FHV, FCV, FPV, FCoV and CDV two each (3.0%) were positive for FHV, FPV and FCoV and three each (4.5%) were positive for FCV and CDV (Table 4.5a). Of the 15 captive cheetahs not vaccinated against FHV, FCV and FPV four (26.7%) were tested positive for FHV, none (0.0%) were tested positive for FCV and three (20.0%) were tested positive for FPV (Table 4.5b). Of the 22 captive cheetahs not vaccinated against FCoV and CDV, one (4.5%) and five (22.7%) animals, respectively, had antibodies against these viruses (Table 4.5c). Antibody prevalences were significantly higher in captive than in free-ranging cheetahs for FHV (Fisher's exact test p=0.0092, N=82), FPV (Fisher's exact test p=0.04, N=82) and CDV (Fisher's exact test, p=0.02, N=89).

Table 4.5 Prevalence of antibodies to FHV, FCV, FPV, FCoV and CDV in free-ranging (4.5a), captive cheetahs not vaccinated against FHV, FCV and FPV (4.5b) and captive cheetahs not vaccinated against FCoV and CDV (4.5c). Only animals with at least one positive result are listed. Positive results are listed with the highest dilution titre at which antibodies could still be detected; negative results are denoted as not present (neg). Numbers in brackets in the ID-column represent results for specific examinations of individuals sampled more than once.

Table 4.5a Free-ranging cheetahs

ID	FHV	FCV	FPV	FCoV	CDV
E002	neg	neg	1:20	neg	neg
K001	neg	neg	1:20	neg	neg
P009(2)	1:20	neg	neg	neg	neg
P017	neg	1:20	neg	neg	neg
P019	neg	1:40	neg	neg	1:80
P021	1:40	neg	neg	neg	neg
P033	neg	neg	neg	1:20	neg
W005	neg	1:20	neg	neg	neg
W006	neg	neg	neg	neg	1:40
W016(1)	neg	neg	neg	1:80	neg
W016(2)	neg	neg	neg	neg	1:20
Percentage Positive animals	3.0 % 2 of 67	4.5 % 3 of 67	3.0 % 2 of 67	3.0 % 2 of 67	4.5 % 3 of 67

Table 4.5b Captive cheetahs not vaccinated against FHV, FCV and FPV

ID	FHV	FCV	FPV
Z001	neg	neg	1:20
Z002	1:160	neg	1:160
Z028	1:80	neg	neg
Z030(2)	1:20	neg	neg
Z037	1:20	neg	1:40
Percentage	26.7 %	0.0 %	20.0 %
Positive animals	4 of 15	0 of 15	3 of 15

Table 4.5c Captive cheetahs not vaccinated against FCoV and CDV

ID	FCoV	CDV
Z002	1:160	1:160
Z004	neg	1:160
Z005	neg	1:20
Z006	neg	1:40
Z014	neg	1:20
Percentage	4.5 %	22.7 %
Positive animals	1 of 22	5 of 22

Several of the sero-positive free-ranging cheetahs roamed with other cheetahs, i.e. lived in a male group, were part of a mother-offspring group or an independent sub-adult sibling group (see Appendix II), but there was only one case (P019 and P017, mother and one of her offspring) of two animals in the same group being sero-positive for the same virus (FCV, Table 4.5a). All other group members of sero-positive cheetahs were found to be negative for all tested viruses. Within captive cheetahs, several groups contained more than one individual positive for the same pathogen. In the group of Z001 and Z002, both animals were sero-positive for FPV, in the group of Z003, Z004 and Z005, the latter two individuals were sero-positive for CDV and in the group of Z028, Z029 and Z030, the first and third animal were sero-positive for FHV (Table 4.5b, 4.5c).

Seven cheetahs were sampled and tested more than once. P011, W015, P023 and Z038 were tested negative for all five viruses in all tests. P009, W016 and Z030 were positive for FHV, CDV and FHV, respectively, on their second examination (Table 4.5a, 4.5b). W016 was tested positive for FCoV when first examined but was negative on the second occasion 2 months later (Table 4.5a).

In two free-ranging (P019, W016) and two captive cheetahs (Z002, Z037) antibodies against more than one virus were found (Table 4.5a, 4.5b, 4.5c). P019 was positive for FCV and CDV, W016 for FCoV and CDV, Z002 for FHV, FPV, FCoV and CDV and Z037 for FHV and FPV. P009 and Z030 were also positive for FeLV when tested with the FL-74 ELISA (see below).

Vaccinated captive cheetahs. In five of seven (71%) captive cheetahs vaccinated against FHV, FPV and FCV, antibodies against FHV and FPV were found whereas antibodies to FCV were found only in one cheetah (14%, Table 4.6). Two individuals kept in the same enclosure (Z014, Z015) showed very high antibody titres of 1:1280 to FHV. The two individuals that were negative for any antibodies (Z003, Z005) belonged to the same group (Z003, Z004, Z005).

Table 4.6 Prevalence of antibodies to FHV, FCV and FPV in 7 captive cheetahs vaccinated against FHV, FCV and FPV. Positive results are listed with the highest dilution at which antibodies could still be detected, negative results with a not present (neg).

ID	FHV	FCV	FPV
Z003	neg	neg	neg
Z004	1:20	1:20	1:80
Z005	neg	neg	neg
Z009	1:40	neg	1:40
Z010	1:20	neg	1:80
Z014	1:1280	neg	1:160
Z015	1:1280	neg	1:80

Other free-ranging carnivores. Four of seven leopards had antibodies against CDV and the one black-backed jackal was positive for FCoV (Table 4.7). One of the three caracals was positive for all five viruses and all three caracals were positive for FCoV (Table 4.7).

Table 4.7 Prevalence of antibodies to FHV, FCV, FPV, FCoV and CDV in free-ranging leopard, caracal and black-backed jackal. Positive results are listed with the highest dilution at which antibodies could still be detected, negative results with a not present (neg).

	FHV	FCV	FPV	FCoV	CDV
Leopard 1	neg	neg	neg	neg	1:20
Leopard 2	neg	neg	neg	neg	1:160
Leopard 3	neg	neg	neg	neg	1:80
Leopard 4	neg	neg	neg	neg	1:640
Leopard 5	neg	neg	neg	neg	neg
Leopard 6	neg	neg	neg	neg	neg
Leopard 7	neg	neg	neg	neg	neg
Caracal 1	neg	neg	1:1280	1:640	neg
Caracal 2	1:20	1:20	neg	1:1280	1:320
Caracal 3	1:40	1:20	1:20	1:320	1:320
Black-backed jackal	neg	neg	neg	1:20	neg

4.3.7 Serology with ELISA

Detection of antibodies to FIV and PLV

None of the cheetahs and leopards had antibodies against FIV or PLV. In contrast, all three tested caracals were negative in FIV-ELISA but positive in PLV-ELISA, i.e. had OD values higher than 40% of the positive control OD. The OD values were 43%, 96% and 104.5%.

Detection of antibodies to FeLV

FeLV FL-74 ELISA. To detect FeLV, three ELISA-test were conducted. In the ELISA using FL-74 virus as antigen, seven of 67 free-ranging (10%) and four of 22 captive cheetahs (18%) had OD values higher than 40% of the positive control and were considered positive (Table 4.8). There was no difference in FeLV prevalence between free-ranging and captive cheetahs (Fisher's exact test, p=0.26, N=89). P009 was tested negative at the second examination. None of the tested leopards, caracals and the black-backed jackal were positive in this test.

Table 4.8 OD values (%) of free-ranging and captive cheetahs reacting positive in the FL-74-ELISA. The number in brackets represents the first of two examinations of P009 and the second examination of Z030.

Free-ranging	OD %	Captive	OD %
A011	41.9	Z023	70.8
A021	70.2	Z025	74.2
P009 (1)	45.8	Z026	44.7
P021	47.1	Z030 (2)	47.0
P027	41.7		
P028	45.3		
W013	67.8		

FeLV p45 and FeLV p27 ELISA. None of the tested cheetahs, leopards and caracals were positive in the ELISA testing for antibodies against the unglycolisated form of gp 70 (p45 ELISA) or testing for p27 antigen. The result of one captive cheetah (Z001) was questionable when tested with the p45 ELISA.

4.3.8 Serology with Western Blot

Detection of antibodies to FeLV

To verify the results obtained with the ELISA tests, samples of 23 free-ranging and 11 captive cheetahs were additionally tested in Western blot. The results, however, did not correspond clearly with the ELISA results (Table 4.9).

Table 4.9 Results of different ELISAs (p27 ELISA, p45 ELISA, FL74 ELISA) and Western blot for the diagnosis of FeLV antibodies (p45 ELISA, FL74 ELISA, p15(E) Western blot, p27 Western blot) and antigens (p27 ELISA) in 23 free-ranging and 11 captive cheetahs. Samples containing antibodies to p15(E) or p27 are marked with ‚x'. Samples containing antibodies to p15(E) and p27 were considered positive. Samples not tested for p45 ELISA are marked with '-'. The number in brackets represents the first or second examination of the respective individual.

	ELISA results			Western blot results		Western blot
ID	p27 ELISA	FL74 ELISA	p45 ELISA	p15 (E)	p27	interpretation
A001	neg	neg	neg		x	neg
A002	neg	neg	neg			neg
A003	neg	neg	neg	x	x	positive
A006	neg	neg	neg		.	neg
A007	neg	neg	neg	x	x	positive
A008	neg	neg	neg	x	x	positive
A009	neg	neg	neg	x	x	positive
A011	neg	positive	neg	x	x	positive
E001	neg	neg	neg			neg
P001	neg	neg	neg	x	x	positive
P002	neg	neg	neg	x	x	positive
P003	neg	neg	neg	x	x	positive
P008	neg	neg	neg	x	x	positive
P009(1)	neg	positive	neg	x	x	positive
P009(2)	neg	neg	neg	x	.	neg
P010	neg	neg	neg	x	x	positive
P011(1)	neg	neg	neg	x	x	positive
P017	neg	neg	neg	x		neg
P020	neg	neg	neg	x	x	positive
P021	neg	positive	neg	x	x	positive
P036	neg	neg	-	x		neg
W002	neg	neg	neg	x	x	positive
W015(2)	neg	neg	-	x		neg
W016(2)	neg	neg	-	x		neg
Z006	neg	neg	neg		x	neg

Z015	neg	neg	neg	x		neg
Z017	neg	neg	neg	x	x	positive
Z023	neg	positive	neg	x	x	positive
Z025	neg	positive	neg	x	x	positive
Z026	neg	positive	neg		x	neg
Z028	neg	neg	-	x		neg
Z029	neg	neg	-	x	.	neg
Z030(2)	neg	positive	-	x	x	positive
Z035	neg	neg	-	x		neg
Z038(1)	neg	neg	-		.	neg

4.3.9 Serology with Rapid Fluorescent Focus Inhibition Test (RFFIT)

Of the 41 free-ranging cheetahs that were tested for antibodies to rabies virus, 39 were negative and two (A009, P007) had a positive titre of 0.5 IU/ml. All of the eleven captive cheetahs previously being vaccinated against rabies were positive for antibodies. Titres varied highly at 0.5, 0.5, 0.8, 12.5, 62.5 and 104.2 IU/ml, respectively. Nine non-vaccinated captive cheetahs and two free-ranging leopards were negative for rabies antibodies.

4.3.10 Reverse Transcriptase (RT)-PCR

None of the seven free-ranging cheetahs, three free-ranging leopards and one free-ranging jackal from which brain tissue was obtained tested positive for rabies virus RNA in the RT-PCR.

4.4 Discussion

4.4.1 Absence of symptoms of acute viral infections

Visible external symptoms of viral infections were virtually absent in the study population of free-ranging cheetahs on commercial farmland. Only one of the examined 61 free-ranging cheetahs showed diarrhoea that might have been related to a viral infection. However, this animal was negative for all antibodies tested. When being examined, the animal might have incubated a

viral infection at an early stage in which case the blood sample was taken before detectable production of antibodies occurred.

If, as suggested by some authors, cheetahs are highly susceptible to infectious agents because of their limited immunocompetence, a substantial proportion of animals should have shown symptoms to viral infections. Since 98.4 % of the examined animals did not show such symptoms, it is likely that the importance of genetic monomorphism for the effectiveness of immunocompetence has been overstated. The results from this study suggest that it was most likely extrinsic factors that led to a serious outbreak of feline infectious peritonitis (FIP) in a captive cheetah population in a North American zoo (O'Brien et al. 1985; Evermann et al. 1986) and also to the aggravated symptoms described for some infections in captive cheetahs (Junge et al. 1991). The results of the present study are also consistent with the results of Munson et al. (2005) who did not find severe inflammatory reactions in 49 necropsies of free-ranging Namibian cheetahs.

4.4.2 General health status

Most free-ranging Namibian cheetahs were in a fair to good nutritional and physical status. However, adult females were in a worse nutritional status and had a clearly lower BMI than adult males. This is different from the Serengeti population in Tanzania, where no difference in the BMI was found between the sexes (see methods). Litter sizes and cub survival in Namibia are substantially higher than in Tanzania (Chapter 2), thus Namibian females might have a higher energetic expenditure when raising more offspring and also when raising offspring more often to independence, i.e. supporting them over a longer period of high food demand. Such potentially high energetic expenditure for Namibian females might also account for the worse nutritional status and lower BMI in females than in Namibian males. For sub-adult females and males no such differences in energetic costs were expected. While there was no difference between the sexes in the nutritional status, sub-adult females did have a lower BMI than sub-adult males. Since five of the six sub-adult females were still in their mixed-sex sibling group with or without their mothers, it might be that their BMI is a consequence of lower social status and obtaining less food than their brothers.

Marker & Dickman (2003) found no difference in body condition between females and males in their study population of free-ranging Namibian cheetahs. They used a combined assessment of nutritional and physical status and did not distinguish between adult and sub-adult animals as in this study. In this study, only a difference in nutritional status - likely to be linked to energetic costs - but not in physical status was found, and this difference applied only to adult animals. It is therefore conceivable that the data of

Marker & Dickman (2003) would reveal similar results as in this study if they were analysed in the same way as here.

For adult males the formation of permanent groups appears to be advantageous, since group males were in a better nutritional status and had a higher BMI than single males. One of the advantages of living in a group might be a higher rate of hunting success and/or a higher food intake. In the Serengeti, there was no evidence of a higher rate of hunting success as an advantage of staying with other males but it was demonstrated that members of a group had a higher food intake than single males because groups were able to kill significantly larger prey than single males (Caro 1994). This might also be valid for group-living males on Namibian farmland and explain the differences in nutritional status and BMI between group-living and single males.

4.4.3 Injuries

Severe injuries occurred only in adult male cheetahs. Overall there was no significant difference in the incidence of noticeable injuries between the sexes. Little is known about aggressive encounters between cheetahs and conspecifics or other predators in Namibia. In Tanzania, such encounters have been observed amongst males, mainly aggression of group males against single territory intruders, and less intensively between males and females (Caro 1994). Thus, the severe injuries found in males in this study are most likely the result from aggressive encounters with other males.

4.4.4 Ectoparasites

Females and males had an equal ectoparasite load. Cheetahs roaming with other cheetahs (males in groups, mothers with sub-adult offspring or independent sub-adult sibling groups) had significantly lower numbers of ticks than single animals. This is the first study on cheetahs to show such a difference. The result suggests that social grooming (allo-grooming) is more effective than auto-grooming.

The inspection of ears and fur revealed no cases of infection with mange causing ectoparasites. This is in contrast to the Serengeti cheetahs, where 86% of females (N=7) and 70% of the males (N=10) had mange (Caro et al. 1987). Thus, this ectoparasitosis is less important in Namibia than in Tanzania.

4.4.5 Viral antibodies in free-ranging cheetahs

Prevalence of antibodies to FHV, FCV, FPV, FCoV, CDV

Overall, the prevalence of antibodies against FHV, FCV, FPV, FCoV and CDV in free-ranging cheetahs was relatively low, with 3.0% for FHV, FPV and FCoV and 4.5% for FCV and CDV. Also, titres of antibodies were in all positively tested cases considerably low. This might reflect a previous but not recent exposure to the virus and/or low viral load. This result is consistent with the finding that no external symptoms of viral infections were found (see above).

The results on sero-prevalence from this study differ substantially to the results of Munson et al. (2004) for free-ranging Namibian cheetahs sampled at a different time. Munson et al. (2004) found 12% sero-prevalence for FHV, 24% for CDV, 29% for FCoV, 48% for FPV and 65% for FCV. The 81 cheetahs in their study were sampled between 1992 and 1998 and inhabited an area about 100 km north of the area used in this study. The difference may be partly explained that the present study and the study of Munson (2004) did not use exactly the same assay conditions and may have used different cut-offs. The difference may, however, also reflect a high exposure to these viruses in the 1990s and/or a high re-infection rate, which would explain such a high prevalence.

Of interest is the absence of any visual external symptoms of viral infection in 240 free-ranging cheetahs in the same study area during approximately the same study period (1991 – 1999, Marker & Dickman 2003) and no findings of inflammatory reactions to viral infections in 49 necropsies conducted between 1986 and 2003 (Munson et al. 2005) in the area. While the high levels of antibodies against CDV might have been a consequence of a CDV pandemic in sub-Saharan Africa in the mid 1990s (Roelke-Parker et al. 1996; Alexander et al. 1996; Munson et al. 2004), the high antibody prevalence against the other viruses appears more difficult to explain. Perhaps large differences in contact rates within the cheetah populations or between cheetahs and other carnivores, including domestic dogs and cats, exist between the two study sites. Alternatively, the study area of Munson et al. (2005) might have a much higher density of marking trees, i.e. trees where cheetahs deposit faeces. At least FPV, FCoV and CDV can be transmitted through contact with infected faeces. At present, however, no comparable information on contact rates, domestic animal density or the density of marking trees is available.

Prevalence within social groups

Transmission of the viruses tested in this study is possible through biting, licking and slapping between animals (Rolle & Mayr 1993). However, the contact must fall into the time period of virus shedding / excretion or viremia, which usually happens only for a limited period of time. In only one of the cheetah groups in this study more than one individual was found to be sero-positive for a specific virus. This was P019 and one of her cubs (P017), both

tested positive for FCV. Since the cub was about three to four months old at time of sampling, i.e. was still suckling, it is likely that the antibodies were transferred from the mother via the milk and did not develop after an intense body contact or an infection with this virus. Thus, intraspecific contacts or encounters might not occur frequently and intensely enough to facilitate viral transmission and to maintain infections in a group or in the population at a high level.

Transmission of FPV, FCoV and CDV is also possible through contact with infected animal faeces. Free-ranging cheetahs can get into contact with faeces of other cheetahs at marking trees. However, under the relatively harsh environmental conditions in Namibia with daily temperatures varying by 20-40°C, high UV-radiation for most days of the year and very low humidity (Mendelsohn et al. 2002), only FCoV and CDV might remain active and thus infectious, and probably only for a short period of time (Rolle & Mayr 1993).

Prevalence of antibodies against several pathogens

Only three free-ranging cheetahs showed antibodies against more than one virus, indicating that these animals came into contact with different pathogens. P019 was sero-positive for FCV and CDV, W016 for FCoV and CDV and P009 for FHV and FeLV. P009 and W016 were tested twice and found to be positive for one virus in the first examination and another virus in the second examination. Examinations were apart one and two months respectively and antibody titres were low, suggesting non-recent exposures to the viruses or a low viral load.

Prevalence of antibodies in other carnivores

Many pathogens can infect multiple host species, resulting in reservoirs of infected hosts with the potential to spill-over to other species. Thus, an important aspect when investigating the sero-prevalence of viruses in free-ranging cheetahs is the presence of potential hosts for viruses that may infect cheetahs. For free-ranging cheetahs on Namibian farmland such host species would primarily be other felid species ranging in the same habitat such as leopard, caracal, African wild cat (*Felis sylvestris*) and serval (*Felis serval*) but also the black-backed jackal, a member of the family Canidae. In this study, caracals were shown to have been exposed to FHV, FCV, FPV, FCoV and CDV. The jackal had antibodies to FCoV and leopards to CDV. Thus, interspecific virus transmission might be possible, if physical interactions between species occur.

4.4.6 Viral antibodies in non-vaccinated captive cheetahs

A comparison between free-ranging and non-vaccinated captive cheetahs revealed a higher incidence of sero-prevalence in captive animals for FHV (26.7 %), FPV (20.0%) and CDV (22.7%). Such a higher incidence might be explained by two mechanisms: (1) accumulation of pathogens within enclosures, and (2) contacts to infected domestic animals or free-ranging carnivores at the enclosure fences.

Pathogen accumulation in enclosures

Once an antigen enters an enclosure, virus shedding by infected animals and subsequent accumulation of the pathogen inside the enclosure can be expected. Through repeated contact with viral antigen (direct or indirect) the virus load increases over time and might lead to a higher infection rate and/or aggravated course of infection. Recent studies of FCoV in cats showed that the shedding of viral antigen depended on the severity of infection and that high FCoV antibody titres were representative of a high viral load (Meli et al. 2004). Considering the clinical outbreak of FIP infection in a North American zoo, Meli et al. (2004) suggested that high viral loads in this zoo would be the basis for the generation of virulent FIPV strains, which derive from mutations of the non-pathogenic FECV.

In contrast to free-ranging cheetah groups, where only one group had more than one individual positive for a virus, several captive groups contained more than one individual positive for the same virus. In the group of Z001 and Z002 both animals were sero-positive for FPV, in the group of Z003, Z004 and Z005, the latter two animals were sero-positive for CDV and in the group of Z028, Z029 and Z030, the first and third animals were sero-positive for FHV. It seems likely that these pathogens accumulated in the enclosure and facilitated the infection of group members.

Contacts to domestic animals or other free-ranging carnivores

Lodges and farms with cheetahs in enclosures regularly have unvaccinated domestic animals on their properties. The higher rate of CDV prevalence in captive cheetahs in this study could be explained by direct or indirect contact between domestic animals and cheetahs through contaminated food or through-fence contact. In addition, transmission of a human morbillivirus to captive cheetahs must be considered, which may lead to transient infection without clinical signs and which may induce antibodies cross-reacting with CDV. In the case of a captive cheetah dying of infection with FeLV, a domestic cat was suggested to have been the source of infection (Marker et al. 2003a).

4.4.7 Viral antibodies in vaccinated captive cheetahs

The antibody titres found in the three captive groups that were vaccinated against FHV, FPV and FCV, differed from each other. The group of Z014 and Z015 showed very high antibody titres of 1:1280 to FHV. In the group of Z003, Z004 and Z005 two individuals did not show antibody titres to any virus whereas the third one had antibodies to all three viruses. It is possible that these differences resulted from variations in the immunisation protocols, i.e. the timing of regular booster vaccines, applied on the different facilities.

4.4.8 Prevalence of antibodies to FIV and FeLV

FIV

None of the cheetahs or leopards had antibodies to feline immunodeficiency virus as tested by FIV-ELISA. This result is consistent with previous studies in Namibia (Munson et al. 2004) and also with the lack of reports of any Namibian free-ranging felids infected with immunodeficiency virus. Since animals in East Africa but also in other parts of southern Africa were shown to be FIV-positive (Olmsted et al. 1992; Brown et al. 1993; Osofsky et al. 1996), it was suggested that the Kalahari represents a faunal barrier (Brown et al. 1993), isolating the Namibian wild felid population from the populations further to the east.

None of the cheetahs and leopards had antibodies to the immunodeficiency virus as tested with a PLV-ELISA. In contrast, the three tested caracals were positive for antibodies against PLV but negative for antibodies against FIV. This suggests that a FIV-like infection is present in the area but that it could not be detected with the FIV-ELISA protocol developed for domestic cats. The PLV-ELISA protocol might be more sensitive to test immunodeficiency virus in caracals and cheetahs), since this protocol was developed for pumas (*Puma concolor*). Pumas are closer related to caracals (and cheetahs) than domestic cats. The results found for caracals suggest that it might be important to apply PLV-ELISA to test non-domestic species and that actual infections in the wild might remain undetected when using FIV-ELISA for domestic cats. Since the immunodeficiency virus is transmitted primarily through intense physical contact (e.g. biting) and contact rates between caracals and cheetahs can be assumed to be low in the wild, it might be unlikely that this virus is transmitted from caracal to cheetahs. Nevertheless, it is worth to continue testing free-ranging Namibian cheetahs with PLV-ELISA.

FeLV

Results of the tests for feline leukemia virus are not straightforward and need further investigation. None of the animals was positive for p27 or had

developed antibodies against p45. However, a number of animals reacted positive when FL-74 antigen was used in the ELISA.

Two interpretations appear possible. Firstly, it might be that FeLV does occur in the cheetah population and that it is also transmitted between individuals. Three of the four captive cheetahs (Z023, Z025, Z026) identified as positive with the FL-74 ELISA were kept together with 16 other cheetahs in a 10 ha enclosure. Since no blood samples were taken from the 16 other cheetahs it is unknown whether they were also positive, but it might be that the unnatural density of these 19 cheetahs led to an increased contact rate and thus to an increased probability of virus transmission between the animals. The fourth sero-positive animal (Z030) was kept with two sero-negative cheetahs (Z028, Z029) in an enclosure. Four of the six free-ranging positive cheetahs were in groups of males (P009) or mothers with cubs (A011, P028, W013), but in each case only one individual of the group was positive. This supports the idea that frequent close contact (as it is likely within an enclosure) might be required to transmit the virus between group members.

Secondly, the inconsistent results of the different ELISA and Western Blot might be explained by cross-reactions between feline leukemia virus and murine leukemia virus, both belonging to the group of type C retroviruses (Rolle & Mayr 1993). Two scenarios for the development of antibodies against murine leukemia virus or a closely related retrovirus in cheetahs are conceivable. In both scenarios the underlying causes would be the consumption of mice by cheetahs. This is possible as mice have been shown to turn up in the scats of cheetahs in the study area (Wachter et al. 2006). In the first scenario RNA from the virus carried by the mouse enters into the blood of the predator through micro-injuries in the predator's gingiva, leading to a development of antibodies against this virus. It has been shown that the transmission of MuLV to felids is the cause for the generation of endogenous FeLVs in cats, a mechanism that might occur repeatedly wherever cats - or other carnivores - predate on mice (Benveniste et al. 1975). In this case, the antibodies detected are not antibodies against feline leukemia virus but against exogenous murine leukemia virus. The second scenario could be explained by a "cheetah-retrovirus" closely related to FeLV. So far, no information is available on a cheetah retrovirus. To test this possibility, sequencing of virus antigen is needed.

4.4.9 Prevalence of antibodies against rabies virus

Tests for antibodies against rabies virus in the serum of free-ranging cheetahs were negative in all but two animals. These animals were a solitarily living male and a group living male. After sampling, the latter male lived for another 10 months before the whole group of three males was shot by a farmer. The other male lived for another 7 months before he was found dead in the field.

No viral antigen could be isolated from a brain sample of this animal. The two low antibody titres and the negative result of the brain sample of one of the animals indicate that the viral load was low and did not cause an infection in either of them. This is interesting since rabies is known to be an aggressive pathogen that after incubation usually leads to death within a few days or weeks (Rolle & Mayr 1993). However, long periods of survival of rabies antibody sero-positive animals without disease outbreak have been reported in a previous study on another carnivore species, the spotted hyena (East et al. 2001). In that study, 50% of 37 sero-positive animals survived for more than 4.4 years after blood sampling, and there was no association between longevity and exposure to the virus (East et al. 2001).

Two sources of infection might be available for free-ranging Namibian cheetahs. First, they could get in contact with the virus through bites by other carnivores or other cheetahs. For southern Africa, black-backed jackals, bat eared foxes (*Otocyon megalotis*) and domestic dogs have been described as reservoir hosts for canine rabies strains that are able to maintain the infection (Swanepoel et al. 1993; Thomson & Meredith 1993; Wandeler 1993). In Namibia, rabies in carnivores has been diagnosed in black-backed jackals, bat eared foxes, yellow mongoose (*Cynictis pencillata*), slender mongoose (*Herpestes sanguinea*), banded mongoose (*Mungos mungo*), lions and cheetahs (Swanepoel et al. 1993).

The other possible way of virus transmission is the consumption of rabies-infected animals by susceptible cheetahs. This way of transmission has been proposed for sporadic infections in predators (Swanepoel et al. 1993). For a number of wild and domestic prey species in Namibia, rabies infection has been diagnosed, namely the greater kudu (*Tragelaphus strepsiceros*), oryx (*Oryx gazella*) hartebeest (*Alcelaphus buselaphus*), springbok (*Antidorcas marsipialis*), suricate (*Suricata suricata*), spring hare (*Pedetes capensis*), cattle, goat and sheep (Swanepoel et al. 1993). Especially kudu rabies produced serious outbreaks in Namibia until recently (Berry 1993; Mansfield et al. 2006). All these species, except for suricates, are known to be consumed by Namibian cheetahs as investigations of undigested hair remains in cheetah faeces have shown (Marker et al. 2003b; Wachter et al. 2006). Thus, cheetahs might consume rabies infected prey species and get infected in this way.

All six captive cheetahs that were previously vaccinated against rabies showed significant sero-conversion. Antibody titres of vaccinated animals ranged from 0.5 IU/ml to very high titres of 104.5 IU/ml, indicating a good immunoresponse of the animals. It is questionable whether annual booster vaccines against rabies are strictly necessary, since vaccinations of captive animals require the immobilisation of the animal or the administration of the vaccine by dart gun, both being stressful to the animals.