

## Seroprevalences to Viral Pathogens in Free-Ranging and Captive Cheetahs (*Acinonyx jubatus*) on Namibian Farmland<sup>∇</sup>

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**Cheetah populations are diminishing rapidly in their natural habitat. One reason for their decline is thought to be a high susceptibility to (infectious) diseases because cheetahs in zoos suffer from high disease-induced mortality. Data on the health status of free-ranging cheetahs are scarce, and little is known about their exposure and susceptibility to infectious diseases. We determined seroprevalences to nine key viruses (feline herpesvirus 1, feline calicivirus, feline parvovirus, feline coronavirus, canine distemper virus, feline immunodeficiency virus [FIV], puma lentivirus, feline leukemia virus, and rabies virus) in 68 free-ranging cheetahs on east-central Namibian farmland, 24 nonvaccinated Namibian captive cheetahs, and several other wild carnivore species and conducted necropsies of cheetahs and other wild carnivores. Eight of 11 other wild carnivores were seropositive for at least one of the viruses, including the first record of an FIV-like infection in a wild felid west of the Kalahari, the caracal (*Felis caracal*). Seroprevalences of the free-ranging cheetahs were below 5% for all nine viruses, which is significantly lower than seroprevalences in nonvaccinated captive cheetahs and those for five of seven viruses in previously studied free-ranging cheetahs from north-central Namibia (L. Munson, L. Marker, E. Dubovi, J. A. Spencer, J. F. Evermann, and S. J. O'Brien, *J. Wildl. Dis.* 40:23–31, 2004). There was no clinical or pathological evidence of infectious diseases in living or dead cheetahs. The results suggest that while free-ranging wild carnivores may be a source of pathogens, the distribution of seroprevalences across studies mirrored local human population density and factors associated with human habitation, probably reflecting contact opportunities with (nonvaccinated) domestic and feral cats and dogs. They also suggest that Namibian cheetahs respond effectively to viral challenges, encouraging consistent and sustainable conservation efforts.**

Knowledge of the health status and disease susceptibility of threatened and endangered species is fundamental for understanding the population dynamics of such species and for planning truly sustainable and successful conservation strategies. The global cheetah (*Acinonyx jubatus*) population has diminished drastically during the last century (31), yet the health status and disease susceptibility of cheetahs have been studied predominantly in captive cheetahs. Cheetahs kept in various breeding facilities and zoos can suffer from infectious and chronic degenerative diseases, with subsequent mortality (4, 11, 12, 15, 22, 40, 42, 49). The high mortality from infectious diseases in captive cheetahs was suggested to be a consequence of a lack of genetic variability at the class I loci of the major histocompatibility complex (MHC) in this species (44, 45, 67),

because the MHC class I genes encode peptides that mediate the immune response to viral infections (3). These studies imply that free-ranging cheetahs should also show a high level of mortality from infectious diseases.

Today, the largest free-ranging cheetah population lives in Namibia, with most of them roaming on commercial farmland, not in protected areas (35). Little is known about the exposure and susceptibility of this cheetah population to infectious diseases (41). Lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*), the cheetah's main competitors and predators (8) and potential sources of viral infections, are absent on Namibian farmland. Other carnivore species that do live on Namibian farmland and could potentially transmit viral diseases to cheetahs include leopards (*Panthera pardus*) and smaller wild carnivores as well as domestic or feral cats and dogs. Not all domestic cats and dogs on Namibian farms, and hardly any feral ones, are vaccinated. Because both cats and dogs can carry viral pathogens transferable to cheetahs (55), free-ranging cheetahs that come into contact with nonvaccinated cats and dogs may become exposed to viral pathogens. The risk of cheetahs becoming infected with a virus is expected to be higher in areas with high human density, since in these areas contact with nonvaccinated cats and dogs is likely to be increased.

In this study, we determined seroprevalences in free-ranging cheetahs and nonvaccinated cheetahs kept on private farms in east-central Namibia for nine key viruses: feline herpesvirus 1

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(FHV1), feline calicivirus (FCV), feline parvovirus (FPV), feline coronavirus (FCoV), canine distemper virus (CDV), feline immunodeficiency virus (FIV), puma lentivirus (PLV), feline leukemia virus (FeLV), and rabies virus. We also screened sera of various carnivore species on Namibian farmland for antibodies against the same nine viruses. To examine incidences of infectious diseases in cheetahs and other carnivores, we checked all animals for the presence of clinical symptoms related to viral infections and opportunistically conducted necropsies on carnivore carcasses.

Because in east-central Namibia there are fewer and smaller human centers and a lower human density on farmland than in north-central Namibia, which has several major human centers and a higher human density on farmland (29), we compared the seroprevalences from this study with those for free-ranging cheetahs previously studied in north-central Namibia (41). If cats and dogs play an important role in the transmission of pathogens to cheetahs, then seroprevalences in free-ranging east-central cheetahs (our study) should be significantly lower than those in north-central cheetahs. For nonvaccinated captive cheetahs kept in the vicinity of farmhouses and lodges, we also expected a higher seroprevalence than that for free-ranging east-central cheetahs, because rates of contact with nonvaccinated cats and dogs are likely to be higher than those for free-ranging cheetahs and because pathogens are likely to accumulate in the enclosure and facilitate the infection of captive group members.

#### MATERIALS AND METHODS

**Study animals and sample collection.** Between June 2002 and October 2004, 62 cheetahs ranging freely on commercially used farmland in east-central Namibia ( $-21^{\circ}45'S$  to  $-22^{\circ}45'S$  and  $16^{\circ}30'E$  to  $18^{\circ}30'E$ ) were trapped, immobilized, examined, sampled, and released again. Study animals included 35 adult males (17 solitary and 18 in groups of 2 [ $n = 6$ ] or 3 [ $n = 2$ ]), 8 adult females (3 solitary and 5 accompanied by their cubs), 11 cubs, and 8 independent juveniles (1 solitary and 7 in groups, of 3 and 4). Juveniles were assessed to be between 1 and 2 years old. We additionally trapped, examined, and sampled four adult leopards, three adult caracals (*Felis caracal*), and one adult black-backed jackal (*Canis mesomelas*). The study area was located approximately 200 km south of the area where most free-ranging cheetahs of a previous study in north-central Namibia were investigated (41). We further examined and sampled 24 adult cheetahs that were kept in large enclosures in their natural habitat on seven farms and lodges in central, southern, and northern Namibia. Seven of these captive cheetahs in three facilities were vaccinated against FHV, FCV, and FPV with a combined vaccine (FHV and FCV live, attenuated viruses and FPV inactivated virus; Pfizer, Sandton, Republic of South Africa) and against rabies virus (Meril South Africa Ltd., Halfway House, Republic of South Africa). For these cheetahs, only serology results for viruses that they were not vaccinated against were included in the analyses. Four free-ranging and one nonvaccinated captive cheetah were sampled and tested a second time, after periods of 1, 2, 3, and 13 months, and one free-ranging cheetah was tested a total of three times, the initial time and after periods of 1.5 and 4.5 months.

Most free-ranging cheetahs (49/62 cheetahs), all captive cheetahs, and all leopards were immobilized with Hellabrunn mixture (100 mg/ml ketamine [Kyron Laboratories, Benrose, Republic of South Africa] and 125 mg/ml xylazine [Bayer, Isando, Republic of South Africa]), with a dosage of 0.04 ml/kg of body weight corresponding to 4.0 mg/kg ketamine and 5.0 mg/kg xylazine. For the remaining cheetahs, a mixture of ketamine (4.5 mg/kg) and medetomidine (0.08 mg/kg; Novartis, Spartan, Republic of South Africa) was used. Caracals were immobilized with 6.0 mg/kg ketamine plus 0.08 mg/kg medetomidine, and the jackal was immobilized with 3.0 mg/kg ketamine plus 0.05 mg/kg medetomidine. Anesthesia of animals immobilized with Hellabrunn mixture was reversed with yohimbine (0.1 mg/kg; Kyron Laboratories, Benrose, Republic of South Africa), whereas animals immobilized with ketamine and medetomidine were reversed with atipamezole (0.25 mg/kg for cheetahs, leopards, and caracals and 0.2 mg/kg

for the jackal; Novartis, Spartan, Republic of South Africa). All drugs were administered intramuscularly.

Anesthetized cheetahs were checked for symptoms that might be related to viral infections, such as diarrhea, fever, ocular or nasal discharge, and cachexia. Venous blood was collected into serum blood tubes (BD Vacutainer Systems, Plymouth, United Kingdom). Blood samples were kept at 4°C during transport to the field station and were centrifuged at 5,000 rpm for 15 min. Serum was stored at  $-196^{\circ}C$  in a liquid nitrogen container and then transported and stored at  $-80^{\circ}C$  until serology was performed.

Necropsies were conducted on 1 captive and 15 free-ranging cheetahs, 8 free-ranging leopards, 2 black-backed jackals, 1 African wild cat (*Felis libyca*), 1 bat-eared fox (*Otocyon megalotis*), 1 honey badger (*Mellivora capensis*), and 1 aardwolf (*Proteles cristatus*). Eight of the free-ranging cheetahs were shot by farmers as "problem animals," two were shot as trophies, two were found dead on the road, and three were found dead in the field after they had been dead for a few days. The captive cheetah was thin, had not fed well, and died 2 days after immobilization for purposes other than for this study. This animal and two of the free-ranging cheetahs were study animals previously sampled serologically. All eight leopards were shot as trophies; the other six carnivores were found dead on the road. Postmortem blood of six cheetahs not previously sampled serologically and of three leopards was gently aspirated into a 5-ml syringe after cutting of a large blood vessel and then transferred to a serum tube and processed as described above. Tissue samples from cheetahs (3 brains, 6 hearts, 5 lungs, 12 stomachs, 7 pancreases, 14 livers, 14 spleens, 4 lymph nodes, 13 kidneys, and 13 adrenal glands), leopards (3 hearts, 2 lungs, 8 stomachs, 5 pancreases, 7 livers, 8 spleens, 5 lymph nodes, 6 kidneys, and 7 adrenal glands), jackals (1 lung, 1 stomach, 2 livers, 1 lymph node, and 2 kidneys), the wild cat (heart, spleen, kidney, and adrenal gland), and the bat-eared fox (heart, lung, liver, and spleen) were stored and transported in 10% or 4% buffered formalin solution for histopathological examination. Brain or spinal cord samples of seven cheetahs, three leopards, one black-backed jackal, one honey badger, and one aardwolf were stored and transported either at  $-196^{\circ}C$  or in phosphate-buffered 50% glycerol solution until tested against rabies virus antigen.

**Testing for antibodies against FHV, FCV, FPV, FCoV, and CDV.** Immunofluorescence assays (IFAs) were conducted as described previously (19, 26), using the following as antigens: a Swiss isolate obtained from a cat suffering from herpes keratitis (Zurich 5-04) for FHV, the F9 strain (Veterinaria AG, Zurich, Switzerland) for FCV, the FPL/01 strain (Veterinaria AG, Zurich, Switzerland) for FPV, a transmissible gastroenteritis virus, the Purdue strain (48), for FCoV, and the Onderstepoort strain (Veterinaria AG, Zurich, Switzerland) for CDV. The result was considered positive if specific fluorescence was detected in infected cells (19, 26) and seen at a titer dilution of at least 1:20 (19). This dilution allows for detection of antibodies specific for the antigens of interest (19) and usually also for nonspecific reactions in vaccinated cats. Since serology tests were conducted only for nonvaccinated cheetahs, a titer dilution of 1:20 allowed specific detection of antibodies. All positive sera were titrated in twofold serial dilutions until fluorescence was no longer detected.

**Quality control.** All antigens used for the IFA were tested by PCR or reverse transcription-PCR (RT-PCR) for the absence of possible contaminating agents following previously described protocols for FHV (63), FPV (51), FCoV (16), FIV (25), FeLV (20), and CDV (38). For FCV, primer and probe sequences were derived from those published previously (18) and kindly provided by C. Helps: forward primer, 5'-GTTGGATGAACCTACCCGCCAATC-3'; reverse primer, 5'-CATATGCGGCTCTGATGGCTTGAAACTG-3'; and probe, 5'-TCGGTGGTTTGATTGGCCTG-3'.

**Testing for FeLV antigen and antibodies against FIV.** Enzyme-linked immunosorbent assays (ELISAs) were used to detect FeLV p27 antigen, the major core protein of the virus, as described previously (27). Sera that produced an optical density (OD) of  $>25\%$  of a defined positive control were considered positive (28). Because it was shown that the detection of antibodies against FIV for free-ranging felids is likely to be more sensitive using PLV than FIV antigens (23, 62), two ELISAs were conducted: one using a recombinant FIV-Z2 transmembrane glycoprotein developed in the laboratory as described previously (7) and one using a synthetic peptide derived from the transmembrane glycoprotein of PLV (23). Sera of an FIV-infected domestic cat and of a lion naturally infected by a lentivirus were used as positive controls under previously described conditions (61).

**Testing for antibodies against rabies virus and rabies antigen.** Sera were tested for the presence of rabies-specific virus-neutralizing antibody by the rapid fluorescent-focus inhibition test (RFFIT), using a standard challenge virus as described previously (10). WHO reference serum was included to determine the international units/milliliter (IU/ml), and titers of  $\geq 0.5$  IU/ml were considered positive (66). Brain and spine samples were tested by RT-PCR for the presence

TABLE 1. Prevalence of antibodies and titer levels against eight viruses and presence of FeLV antigens in free-ranging and nonvaccinated captive cheetahs in Namibia

Virus	Free-ranging, east-central Namibian cheetahs		Captive, nonvaccinated cheetahs		<i>P</i> <sup>e</sup>	Prevalence (no. of positive cheetahs/total no. of cheetahs [%]) in free-ranging, north-central Namibian cheetahs <sup>c</sup>	<i>P</i>
	Prevalence (no. of positive cheetahs/total no. of cheetahs [%])	Positive results <sup>a</sup>	Prevalence (no. of positive cheetahs/total no. of cheetahs [%])	Positive result(s) <sup>a</sup>			
FHV	2/67 (3.0)	1:20, 1:40	1/13 (7.7)	1:160	NS	9/74 (12.2)	0.059
FCV	3/67 (4.5)	2 × 1:20, 1:40	0/13 (0.0)		NS	32/49 (65.3)	<0.0001 <sup>d</sup>
FPV	2/67 (3.0)	2 × 1:20	3/13 (23.1)	1:20, 1:40, 1:160	0.028 <sup>b</sup>	24/50 (48.0)	<0.0001 <sup>d</sup>
FCoV	2/66 (3.0)	1:20, 1:80	1/22 (4.5)	1:160	NS	21/72 (29.2)	<0.0001 <sup>d</sup>
CDV	3/67 (4.5)	1:20, 1:40, 1:80	5/22 (22.7)	2 × 1:20, 1:40, 2 × 1:160	0.020 <sup>b</sup>	17/70 (24.3)	0.0012 <sup>d</sup>
FeLV	0/66 (0.0)		0/22 (0.0)			0/69 (0.0)	
FIV	0/48 (0.0)		0/19 (0.0)			0/39 (0.0)	
PLV	0/63 (0.0)		0/22 (0.0)			Not tested	
Rabies virus	2/42 (4.9)	2 × 0.5	5/13 (38.5)	3 × 0.5, 2 × 4.2	0.0058 <sup>b</sup>	Not tested	

<sup>a</sup> Positive results are expressed as dilution titer levels for FHV, FCV, FPV, FCoV, and CDV and as IU/ml for rabies virus.

<sup>b</sup> Seroprevalence was higher in captive Namibian cheetahs than in free-ranging east-central Namibian cheetahs.

<sup>c</sup> Data are from reference 36.

<sup>d</sup> Seroprevalence was higher in free-ranging north-central cheetahs than in free-ranging east-central Namibian cheetahs.

<sup>e</sup> NS, not significant.

of viral antigen, using murine neuroblastoma cell cultures as described previously (10). Samples were tested at the Federal Research Centre for Virus Diseases of Animals, Tübingen, Germany, and the National Rabies Reference Laboratory, Wusterhausen, Germany.

**Histopathological examination.** Tissue samples stored in formalin solution were paraffin embedded, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Samples of heart, liver, and kidney were additionally stained with van Gieson stain, and stomach samples were stained with Warthin Starry's silver stain to detect *Helicobacter* bacteria.

**Data and statistical analysis.** Differences in seroprevalences were tested for significance with Fisher's exact test, using SYSTAT 12.0. *P* values of ≤0.05 were considered significant. Seroprevalence test protocols used in this study and in the study conducted in north-central Namibia (41) differed for some viruses. The validity of comparison is assessed in detail in Discussion.

## RESULTS

**Prevalence of antibodies in cheetahs.** Seroprevalences of free-ranging cheetahs varied between 0 and 4.9% for the tested viral antibodies and FeLV antigens (Table 1). Seroprevalences of captive, nonvaccinated cheetahs ranged between 0 and 38.5% (Table 1). Antibody prevalences were lower for free-ranging than captive cheetahs for FPV (*P* = 0.028; *n* = 80), CDV (*P* = 0.020; *n* = 89), and rabies virus (*P* = 0.0058; *n* = 55).

Free-ranging cheetahs in this study had lower seroprevalences than free-ranging cheetahs in north-central Namibia (41) for FCV (*P* < 0.0001; *n* = 116), FPV (*P* < 0.0001; *n* = 117), FCoV (*P* < 0.0001; *n* = 138), and CDV (*P* = 0.0012; *n* = 137), and there was a trend toward lower seroprevalence for FHV (*P* = 0.059; *n* = 141) (Table 1). All free-ranging cheetahs in our study tested seronegative for FeLV (*n* = 66) and FIV (*n* = 48), consistent with previous findings in north-central Namibia (41) (Table 1).

Five of the 12 free-ranging cheetahs of this study that were seropositive for FHV, FCV, FPV, FCoV, CDV, or rabies virus were solitary, whereas seven were part of a group. The seven cheetahs that were part of a group were members from six different groups. Only in one group did more than one member (a lactating mother and one of her two cubs) test seropositive for the same virus (FCV). Within nonvaccinated captive chee-

tahs, four of six groups with seropositive members contained more than one individual positive for a specific pathogen. There was no difference in the probability of exposure for a specific virus between group members of free-ranging and nonvaccinated captive cheetahs if one member was infected with this virus (*P* = 0.24; *n* = 12).

One free-ranging cheetah and one nonvaccinated captive cheetah were seropositive for more than one virus. The free-ranging cheetah (the lactating female mentioned above) was seropositive for FCV (titer, 1:40) and CDV (titer, 1:80), and the captive cheetah was seropositive for FHV, FPV, FCoV, and CDV (all titers were 1:160).

Four of the six cheetahs that were sampled repeatedly were seronegative for all five viruses tested at all time periods. One free-ranging cheetah was seronegative in the first tests but seropositive for FHV (titer, 1:20) 1 month later. The other free-ranging cheetah tested positive for FCoV (titer, 1:80) when first examined but was negative for this virus 2 months later. The same animal tested seronegative for CDV when first examined but was seropositive (titer, 1:20) 2 months later, at the second examination.

Two free-ranging and five nonvaccinated captive cheetahs showed neutralizing activity against rabies virus in RFFIT, with titers of 0.5 IU/ml and 4.2 IU/ml (Table 1). One of the seropositive free-ranging males (titer, 0.5 IU/ml) died 10 months after sampling, when he and the two other males of his group (both with titers of <0.5 IU/ml) were shot by a farmer. The other seropositive free-ranging male (titer, 0.5 IU/ml) lived for 7 months after sampling before his carcass was found in the field. Three of the five seropositive captive cheetahs were observed after sampling. Two (both with a titer of 0.5 IU/ml) lived until the end of the study period (28 months after sampling), and one (titer, 4.2 IU/ml) died 22 months after sampling. The latter was the cheetah that died 2 days following immobilization. No information on the fate of the remaining two cheetahs was available after sampling.



TABLE 2. Prevalence of antibodies and titer levels against eight viruses and presence of FeLV antigens among free-ranging leopards and caracals

Virus	Leopards		Caracals	
	Prevalence (no. of positive leopards/total no. of leopards [%])	Positive results <sup>a</sup>	Prevalence (no. of positive caracals/total no. of caracals [%])	Positive results <sup>a</sup>
FHV	0/7 (0.0)		2/3 (66.7)	1:20, 1:40
FCV	0/7 (0.0)		2/3 (66.7)	2 × 1:20
FPV	0/7 (0.0)		2/3 (66.7)	1:20, 1:1,280
FCoV	0/7 (0.0)		3/3 (100.0)	1:320, 1:640, 1:1,280
CDV	4/7 (57.1)	1:20, 1:80, 1:160, 1:640	2/3 (66.7)	2 × 1:320
FeLV	0/6 (0.0)		0/2 (0.0)	
FIV	0/3 (0.0)		0/3 (0.0)	
PLV	0/6 (0.0)		3/3 (100.0)	43, 96, 105
Rabies virus	0/2 (0.0)		Not tested	

<sup>a</sup> Positive results are expressed as dilution titer levels for FHV, FCV, FPV, FCoV, and CDV and as optical densities (%) for PLV.

**Serology in free-ranging carnivores other than cheetahs.** Leopards were seropositive for CDV only, whereas caracals were seropositive for FHV, FCV, FPV, FCoV, CDV, and/or PLV, but not FIV (Table 2). One of the three caracals was positive for six viruses, and all three caracals were positive for FCoV (Table 2). The black-backed jackal was tested for FHV, FCV, FPV, FCoV, CDV, FeLV, and PLV and was seropositive only for FCoV (titer, 1:20).

**Symptoms of viral infections.** None of the 62 free-ranging and 24 captive cheetahs, 4 leopards, 3 caracals, and 1 black-backed jackal showed typical signs of an infectious viral disease, such as fever, anorexia, or ocular or nasal discharge.

**Necropsies.** None of the tissue samples obtained from cheetahs, leopards, jackals, the wild cat, and the bat-eared fox during necropsies showed lesions related to viruses for which serological analyses were conducted. Serum samples from six cheetahs and three leopards were analyzed, and antibodies against FCV were detected in one cheetah (titer, 1:20; result included in Table 1), with antibodies against CDV detected in two leopards (titers, 1:20 and 1:640; results included in Table 2). Some minor lesions were nevertheless observed: changes in 5 of 14 examined cheetah livers consisted of Ito-cell activation ( $n = 3$ ), minimal centrilobular perivenular fibrosis ( $n = 1$ ), and a focal minimal granulomatous lesion ( $n = 1$ ). The splenic corpuscles were slightly activated in the spleens of 10 of 14 examined cheetahs, all 8 leopards, the wild cat, and the bat-eared fox. In cheetahs and the wild cat, the corpuscle germinal center diameters did not exceed the width of the corona, whereas in leopards and the bat-eared fox, the germinal centers stood out and their diameters exceeded the width of the corona. Furthermore, in the adrenal glands of 9 of 13 examined cheetahs, the cortical cells in the zona glomerulosa and/or zona fascicularis were vacuolated, whereas no such vacuolization was found in seven leopard samples. Five of 12 cheetah stomach samples showed mild lymphoplasmatic infiltration in the basal mucosa, with one sample (from the captive animal that died 2 days after immobilization) being associated with the presence of *Helicobacter*. Similar mild lymphoplasmatic infiltrations in the basal stomach mucosa were found in three of eight examined leopard samples.

All 13 brain and spinal cord samples from the cheetahs, leopards, jackal, honey badger, and aardwolf tested negative

for rabies virus antigen, including the brain sample of a free-ranging cheetah male who tested positive for rabies virus antibodies and was found dead 7 months later.

## DISCUSSION

**Seroprevalence and sources of transmission.** The prevalence of antibodies against FHV, FCV, FPV, FCoV, CDV, FIV, and PLV and the occurrence of rabies virus and FeLV antigens in free-ranging cheetahs were generally low, with the highest prevalence being 4.9%, for rabies virus. In only one of seven free-ranging cheetah groups was more than one individual seropositive for a specific virus. Since this was a lactating mother and one of her cubs, it is likely that the antibodies were transferred from the mother to the cub via maternal milk and were not the consequence of an infection with the virus. Thus, intraspecific contacts or encounters might not be sufficiently frequent or intense to facilitate viral transmission and to maintain infections at a high level within groups or in the population.

As expected, seroprevalence among cheetahs living in areas with a lower density of people (0.1 to 1.0 person/km<sup>2</sup> [39]), and therefore a lower density of domestic and feral cats and dogs, was lower than that among cheetahs living in an area with a higher density of people (1 to 5 people/km<sup>2</sup> [39]) and therefore a higher density of nonvaccinated domestic and feral animals. It is unlikely that the difference in seroprevalence between the cheetahs of the two areas was due to differences in intraspecific contact rates, because cheetah densities are similar in the two areas (17). Differences in interspecific contact rates with other wild carnivores are possible, but the densities of leopards are similar in the two areas (17), and densities of other carnivores are also likely to be similar. FPV, FCoV, and CDV—for which some of the seven leopards, three caracals, and one jackal tested positive—can also be transmitted through contact with infected feces; thus, other carnivores may be potential infection sources for cheetahs via fecal-oral transmission (9, 37, 65).

Are differences in seroprevalence between the cheetahs in the two areas likely to be a consequence of differences in test protocols, cutoff levels to determine positive results, or antigen strains in the two studies? The previous study investigating cheetahs in the area of higher human density (41) applied IFA

and serum neutralization tests (1992–1993 and 1993–1998, respectively) to detect FHV and FCV antibodies (this study used IFA), IFA and hemagglutination inhibition assays (1992–1993 and 1993–1998, respectively) to detect FPV antibodies (this study used IFA), serum neutralization tests to detect CDV antibodies (this study used IFA), and Western blotting to detect FIV antibodies (this study used ELISA). Only FCoV antibodies and FeLV antigens were tested with the same tests in both studies (IFA and ELISA, respectively). Comparable cut-off levels were specified only for FCoV (titer of 1:25 in reference 13 of reference 41), and antigen strains used for antibody detection were mentioned for only two viruses. For CDV, the previous study used the Onderstepoort strain, the same strain used in this study, and for FIV, the Petaluma strain (46) was used, which differs from the strain used in this study. However, the use of different antigens and protocols is likely to lead to different results only if the investigated virus is highly variable in antigenicity (46, 62), as is likely the case with FCV and FCoV (24, 50), but not if it is antigenically conserved, as is likely with FHV, FPV, and CDV (14, 21, 36). Also, serum neutralization tests are more specific than IFA because in the former tests antibodies are detected only when they bind to relatively small areas on the viral surface, which results in the inhibition of infectivity (54). In contrast, IFA detects antibodies directed to a broader array of epitopes on the viral surface.

We concluded that the higher seroprevalences in north-central than east-central Namibian cheetahs for FHV, FCV, FPV, and CDV are likely to reflect genuine differences in seroprevalence, because these viruses are conserved and/or their antibodies in north-central Namibian cheetahs were tested with the serum neutralization test. We therefore suggest that the significant difference in seroprevalences between the two study areas is a consequence of one or several biological causes that effectively change transmission opportunities for pathogens. We consider the likely difference in densities of nonvaccinated domestic and feral cats and dogs to be one factor likely to promote virus transmission to cheetahs. For CDV, the high seroprevalence of 24% between 1992 and 1998 in north-central Namibia (41) might also have been a consequence of a CDV pandemic in sub-Saharan Africa in the mid-1990s (1, 41, 52).

**Comparison of free-ranging and captive populations.** Nonvaccinated captive cheetahs on farms and lodges had higher seroprevalences for FPV, CDV, and rabies virus than did free-ranging cheetahs in east-central Namibia. This provides additional support that nonvaccinated domestic cats and dogs may transfer viral antigens to cheetahs. In a reported case of a captive cheetah that died of infection with FeLV, a domestic cat was traced to have been the source of infection (32). In the case of antibodies against CDV, transmission of human morbillivirus to captive cheetahs might also have been possible, leading to transient infection without clinical signs and inducing antibodies cross-reacting with CDV (58).

There was no difference in the probability of group members among nonvaccinated captive and free-ranging cheetahs becoming infected with a specific virus if one member was infected with this virus. Thus, pathogens do not appear to accumulate and facilitate the infection of group members in enclosures. Seropositive nonvaccinated captive cheetahs, like free-ranging cheetahs, showed no evidence of disease susceptibility in terms of external clinical signs, and the owners of the

farms where the captive cheetahs were housed did not report any signs before or after blood sampling.

**FIV and FIV-like exposure and infection.** None of the carnivores tested with FIV-ELISA had antibodies against FIV. This is consistent with results of previous studies in Namibia, which also did not find seropositivity (5, 41, 46, 57). Since free-ranging felids in other parts of southern Africa and eastern Africa were shown to be FIV positive (5, 46, 47, 57), it was suggested that the Kalahari desert represents a faunal barrier isolating the Namibian free-ranging felid populations from populations further east (5).

Whereas cheetahs and leopards tested with PLV-ELISA in this study were seronegative for PLV, the three tested caracals were seropositive for PLV. This is the first report, to our knowledge, of an FIV-like infection in a free-ranging felid in Namibia and suggests that an FIV-like infection was present in the area but was not detected with the FIV-ELISA protocol developed for domestic cats. It has previously been shown that the FIV transmembrane protein carries immunodominant epitopes which do not cross-react with those of lentiviruses of lions and pumas (6, 7). The results for caracals suggest that it might be useful to apply PLV-ELISA to test nondomestic felids and that actual infections in the wild may remain undetected using the FIV-ELISA developed for domestic cats. Since the immunodeficiency virus is transmitted primarily through intense physical contact, such as biting, and since such contact between caracals and cheetahs can be assumed to be rare in the wild, it might be unlikely that this virus is transmitted from caracals to cheetahs. Nevertheless, it is important to continue testing free-ranging Namibian cheetahs with PLV-ELISA, since currently this population appears to be free of FIV and FIV-like infections and any change in seroprevalence should be detected as early as possible.

**Exposure to rabies virus.** The low neutralizing activity against rabies virus in the seven seropositive cheetahs, with titers of 0.5 IU/ml (threshold of positivity) and 4.2 IU/ml, is difficult to interpret, as the threshold for positivity is arbitrarily defined and specific reactions cannot be distinguished from unspecific reactions. The negative rabies virus antigen result for the brain sample of one of these seropositive animals indicates, however, that the viral load, if present, was low. All rabies virus-positive cheetahs lived for many months after blood sampling without expressing clinical signs of virulent rabies virus infection. This contrasts with the common perception that rabies virus is an aggressive pathogen, usually leading to death within a few days or weeks after incubation (53), but is consistent with studies on spotted hyenas (*Crocuta crocuta*) (10) and bats (*Myotis myotis*) (2). In spotted hyenas, 50% of 37 seropositive animals survived for more than 4.4 years after blood sampling, and there was no association between longevity and exposure to the virus (10). Similarly, in *Myotis myotis* bats, all 37 seropositive animals that were recaptured survived for at least 1 year and for up to 8 years, and mortality did not increase after episodes of viral infection (2). Cheetahs in Namibia might become infected with the virus through bites by other carnivores. Consumption of rabies virus-infected prey species might be another possibility for interspecific transmission of rabies to cheetahs, because rabies virus regularly causes serious disease outbreaks among kudu in Namibia (30), and kudu are a common prey of cheetahs (33, 64). Contact with a

low viral load via mucous membranes may lead to abortive infection and induction of the immune response.

**Vulnerability to pathogens and stress.** Recently, a new explanation for increased susceptibility to infectious diseases in cheetahs kept in zoos was suggested. Captive cheetahs in North American zoos had higher fecal glucocorticoid concentrations and a larger adrenal corticomedullary ratio, indicative of chronic stress, than those of free-ranging Namibian cheetahs (59), suggesting that a hormone-based suppression of the immune response may negatively affect health in captive cheetahs (59). Free-ranging Namibian and captive North American cheetahs investigated in previous studies originated from the same gene pool (34), and thus the development of diseases in cheetahs might be modulated by stress levels rather than genetic predisposition (56). Since free-ranging and captive Namibian cheetahs have similar fecal corticoids (59, 60) and similar adrenal gland sizes, as measured by ultrasonography (61), glucocorticoid influences on viral infections should be similar under both study conditions, and these cheetahs should not be highly susceptible to infectious diseases, as was found in this study. These findings are in line with a previous study that demonstrated that free-ranging cheetah females reproduce well and that the low genetic variability of cheetahs is unlikely to negatively affect the reproductive performance of cheetah females (61).

If short-term stress increases the probability of disease outbreaks in infected cheetahs, as was suggested for long-term stress in zoos (59), translocation and similar potentially stressful handling should be conducted with caution, as this may compromise the successful immune response to viruses an individual may have been exposed to, especially in areas with high levels of seroprevalence, where the chance of handling a seropositive animal is high. Translocation of cheetahs is conducted regularly in Namibia, by authorized organizations, when farmers have trapped a cheetah and want to have it removed from their farm to decrease the chance of livestock being killed by it (35). Translocated cheetahs on Namibian farmland are rarely monitored after release, and thus cheetahs that develop virulent infection after translocation are unlikely to be recorded. It seems reasonable to suggest that translocation from areas with high infection levels to areas with low infection levels should be avoided to avoid the risk of exposure for seronegative cheetahs. Also, translocation from areas with low infection levels to areas with high infection levels should be avoided because it might increase the risk of viral exposure for seronegative, naïve cheetahs.

The minor lesions found in necropsied cheetah organs were similar to previously described lesions (43). The observed differences in the morphology of splenic corpuscles in cheetahs and the wild cat and that in the bat-eared fox and leopards could, however, not be interpreted. Nor is it currently known whether the vacuolization of cheetah adrenal cortical cells that was absent in leopard samples might reflect a functional difference. Future studies that also include hormonal measurements for these species might shed light on these results.

**Conclusions.** This study suggests that free-ranging and captive Namibian cheetahs from the same population are in good health, despite reports of low genetic variability (44, 45). This result is encouraging for conservation plans concerning free-

ranging cheetahs and is useful for studies on cheetah population dynamics.

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