FELINE LENTIVIRUS AND FELINE ONCOVIRUS STATUS OF FREE-RANGING LIONS (PANTHERA LEO), LEOPARDS (PANTHERA PARDUS), AND CHEETAHS (ACINONYX JUBATUS) IN BOTSWANA: A REGIONAL PERSPECTIVE

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Abstract: Subpopulations of large felids in southern Africa exhibit a range of lentivirus prevalence, with some subpopulations showing no evidence of infection. Botswana lions (Panthera leo), leopards (Panthera pardus), and cheetahs (Acinonyx jubatus) were evaluated for evidence of feline lentivirus infection by assaying for antibodies against test antigens derived from a puma lentivirus isolate (PLV hurd) and a domestic cat feline immunodeficiency virus (FIV) and for oncovirus (feline leukemia virus [FeLV]) infection using an enzyme-linked immunosorbent assay (ELISA) for detection of antigen. Blood collection filter paper kits were distributed countrywide to safari hunters and Department of Wildlife and National Parks field officers involved in problem predator management. All sampling (n = 53) was opportunistic; no cats were captured, anesthetized, or killed for this project. Five different assays for lentivirus were utilized on most samples: PLV hurd indirect immunofluorescence assay (IFA), PLV hurd western immunoblot (WB), FIV ELISA, FIV IFA, and FIV WB. One test was used for detection of oncovirus antigen: domestic cat FeLV ELISA.

None of the cats tested positive for FeLV infection. There are specific characteristics and sensitivities among the lentivirus assays being applied to nondomestic felids, suggesting that assay choice is important and that caution is warranted in interpreting data. Evidence of current lentivirus infection (defined as a positive result on at least the PLV hurd WB) was found in all three species: eight of 31 lions (25.8%), three of 18 leopards (16.7%), and one of four cheetahs (25%). In domestic cats and other mammals so far investigated, lentivirus seropositivity is directly correlated with lentivirus infection. Seropositive cats were found in geographically diverse parts of the country. Although this study is not a comprehensive virologic evaluation of the lion, leopard, and cheetah populations of Botswana, it does reveal wild feline lentivirus infection in a previously unexamined portion of sub-Saharan Africa. With more information on PLV-like lentivirus and FeLV, scientists and managers in southern Africa can make more informed decisions regarding the movement of large cats locally or internationally for research, management, or commercial purposes.

Keywords: Lion, Panthera leo, leopard, Panthera pardus, cheetah, Acinonyx jubatus, lentivirus, feline immunodeficiency virus, oncovirus, feline leukemia virus, free-ranging.

INTRODUCTION

The Republic of Botswana, situated between Namibia, Zambia, Zimbabwe, and South Africa in southern Africa (Fig. 1), has one of the most valuable free-ranging wildlife resources left on the continent. The large felids are considered priority species for the establishment of disease databases because of their economic contributions to the safari industry, their ecologic importance, and their aesthetic value. Concerns over the retrovirus status of free-ranging Botswana felids, especially in regards to feline lentiviruses similar to the domestic cat feline immunodeficiency virus (FIV), have grown out of recent findings that a high percentage of lions (Panthera leo) living in Kruger National Park in eastern South Africa are feline lentivirus positive, whereas lions in Namibia's Etosha National Park appear to be feline lentivirus negative. Recently, three wild lions in
northeastern Namibia’s Mahango Game Reserve were found to be lentivirus positive (Marker-Kraus and Kraus, pers. comm.). This reserve, in Namibia’s Caprivi strip, abuts northwestern Botswana. South Africa’s Umfolozi Game Reserve had 27 lions that tested lentivirus negative (Spencer, pers. comm.). One positive lion, which had been translocated from the eastern Transvaal near Kruger National Park, was found in a private reserve bordering Umfolozi (Spencer, pers. comm.). The presence of antibodies to a lentivirus is evidence of persistent infection in domestic cats and in other mammalian species known to harbor lentiviruses. Antibody presence does not simply represent a postexposure protective titer but correlates with the presence of virus. Serosurveys are vital if contamination of currently lentivirus-free populations is to be avoided.

Cheetahs (*Acinonyx jubatus*) in Namibia’s Etosha Pan National Park have tested lentivirus negative. Elsewhere in Namibia, at least 122 cheetahs were evaluated and were lentivirus negative.27 Sera from seven Kruger National Park cheetahs collected between 1986 and 1991 showed no evidence of lentivirus exposure.46 Six cheetahs from Natal’s Umfolozi Game Reserve tested lentivirus negative (Spencer, pers. comm.). Lentivirus tests of 45 captive cheetahs in South Africa, descendants of wild animals caught in Kruger National Park and Namibia in the 1970s, were also negative.27 A subsequent sample of 56 captive South African cheetahs did contain one positive animal, but in a survey by a different laboratory of two captive populations (*N = 39*) in South Africa, all cheetahs were lentivirus negative (Spencer, pers. comm.).40

Kruger National Park’s first known lentivirus-positive leopard (*Panthera pardus*) was identified during 1992 when the Botswana Department of Wildlife and National Parks (D.W.N.P.) also identified its first free-ranging lentivirus-positive leopard, using the same laboratory in South Africa.49,40 In a 1993 study, there was a 71% prevalence of lentivirus antibodies in seven Kruger National Park leopards evaluated.6 One leopard from Umfolozi Game Reserve tested for lentivirus was negative (Spencer, pers. comm.). Very little is known about the lentivirus status of free-ranging Namibian leopards, although four animals tested were negative (Spencer, pers. comm.).6

Captive and free-ranging individuals of a wide variety of felid species from around the world have lentivirus antibodies,3,6,8,14,15,20,21,22,23,27,31,32,33,15,45 and feline lentiviruses have been isolated from some captive specimens.45 Lentiviruses are endemic in several East African lion and cheetah populations.6,7,27

No free-ranging African cats have been found to be infected with the feline leukemia virus (FeLV). Three captive Namibian cheetahs have tested positive for FeLV, and at least one died from a lymphoproliferative disorder (Spencer, pers. comm.). Captive and free-ranging individuals of a variety of felid species from around the world have been reported as infected with FeLV,4,5,8,15,20,21,22,23,33,34,45 with confirmation by virus isolation (from a leopard cat, *Felis bengalensis*) reported in 1981.33 However, it was not until 1989 that commercial enzyme-linked immunosorbent assay (ELISA) kits were recognized to yield a small proportion of false-positive results because of anti-mouse antibodies in feline sera reacting
with monoclonal antibodies of murine origin incorporated into the test kits themselves. This cross-reactivity complicates interpretation of some earlier reports.

The Kalahari semidesert ecosystem covers >80% of Botswana and extends into Namibia and South Africa (Fig. 1). The absence of serologic evidence of FIV infection in Namibian wild felids except in the northeastern finger of the country, concurrent with widespread evidence of infection in southeastern and eastern Africa, suggested that the Kalahari may be an important barrier to the movements of animals and their pathogens. Unaided movement of various mammals across Botswana’s international borders, despite the prevalence of fences, occurs. In addition, translocation is used as a management tool in problem predator control programs and to stock state and private game reserves, which are becoming increasingly popular in southern Africa as ecotourism grows. A thorough understanding of feline retrovirus epidemiology in southern Africa requires data from Botswana.

MATERIALS AND METHODS

Sample collection procedures in the field

Blood samples were solicited by countrywide distribution of 490 survey kits prepared by the Wildlife Veterinary Unit (W.V.U.) of the Botswana D.W.N.P. All sampling was opportunistic; no cats were captured, anesthetized, or killed for this project. Logistic constraints necessitated a simple and cost-effective technique of maintaining sample integrity pending analyses, which would take place later at a distant location.

Each kit consisted of a data form (Fig. 2) labeled with a preassigned identification number, a 7.5- × 2.5-cm blood collection paper (903 paper, Schleicher and Schuell, Keene, New Hampshire 03431, USA) attached to the data form and labeled with the corresponding identification number, an explanatory cover letter with instructions for use of the filter papers, and a preaddressed postage-paid envelope. Each filter paper has an outlined 3- × 1.5-cm target area to which the blood sample is applied until it soaks through to the other side. A properly prepared filter paper target area absorbs approximately 0.2 ml of fluid: whole blood, serum, or plasma. Blood from any part of the animal was accepted, with the bullet wound a common sample site in categories 1 and 2 (below).

Animals sampled for this study fell into four main categories allowing opportunistic sampling: 1) 25 animals shot by clients of legal commercial safari hunting operations; 2) 20 animals lawfully shot by D.W.N.P. officers or private farmers because they were suspected of or known to be taking livestock prey; 3) six animals captured by the W.V.U. as part of D.W.N.P. problem predator translocation exercises; and 4) one animal found dead from injuries and one found dead from unknown causes. No incentive, financial or otherwise, was provided to encourage the killing of animals or the collection of samples.

Kit users were instructed to air-dry the filter paper for 5–10 min prior to sealing it in its envelope and to keep the packet out of direct sunlight. Data forms were reviewed for completeness at D.W.N.P. Headquarters, and the filter papers were individually frozen at approximately −18°C. The amount of elapsed time between the field collection date and the date of freezing was recorded when known.

Sample evaluation in the United States

Upon completion of the survey, samples were sent by air courier to the National Veterinary Laboratory (Franklin Lakes, New Jersey 07417, USA) for analysis. Shipment of samples was in accordance with a CITES import permit issued to Dr. William D. Hardy, Jr. by the United States Fish and Wildlife Service, Department of the Interior. Each sample was evaluated by up to five different tests for evidence of feline lentivirus infection: puma lentivirus (PLV, rod)
BLOOD COLLECTION FILTER PAPER DATA FORM
Botswana Department of Wildlife And National Parks

DATE: ____________________       TIME: ____________________

Species: ____________________

Sex: ____________________       Age: ____________________

ID Number: ____________________

LOCATION (as PRECISELY as possible): ____________________

WAS THE ANIMAL SICK? (if so, describe) ____________________

WHY WAS IT KILLED? (hunting, problem predator, etc.) ____________________

ANY COMMENTS ON THE CONDITION OF THE ANIMAL (i.e., thin, fat, normal), etc. ____________________

Signed, ____________________

Printed Name: ____________________

Phone Number: ____________________

[ ] DWNP Officer, rank: ____________________

[ ] Safari Company: ____________________

[ ] Other: ____________________

FILL OUT ONE OF THESE FORMS FOR EACH ANIMAL SAMPLED.

FILTER PAPER(S) AND FORM(S) SHOULD BE POSTED TO:
Dr. Steve Osofsky
Wildlife Veterinary Officer
Department of Wildlife and National Parks
P.O. Box 131 (Tsholetsa House)
Gaborone

Figure 2. Blood Collection Filter Paper Data Form used by hunters and wildlife officers to record information on each cat sampled. Each form was given a unique ID number corresponding to a numbered filter paper stapled to the lower left hand corner.
indirect immunofluorescence assay (IFA), PLV_eto western immunoblot (WB), FIV ELISA (Petchek FIV Antibody Test Kit, Idexx Laboratories, USA), FIV IFA, and FIV WB. In addition, samples were tested for FeLV antigen using a domestic cat FeLV ELISA (Petchek FeLV Antigen Test Kit, Idexx Laboratories).

Isolation of puma lentivirus

The PLV_eto isolate was obtained from a captive-bred 8-mo-old female puma with lymphadenopathy at the Calgary Zoo, Alberta, Canada (Hardy, Zuckerman, and Cooper, unpubl.). Mononuclear cells were isolated from heparinized whole blood on lymphocyte separation medium gradient (Organon Teknika Corp., Durham, North Carolina 27704, USA). A total of $15 \times 10^6$ mononuclear cells were harvested and resuspended in 3 ml of McCoy’s-L15 medium with 20% fetal calf serum (FCS). One-milliliter aliquots of this suspension (containing $5 \times 10^6$ puma mononuclear cells) were cocultured with three different cell lines: 1) $5 \times 10^6$ 3201B FeLV-negative feline lymphosarcoma cells* in 5 ml of McCoy’s-L15 medium with 20% FCS; 2) $5 \times 10^6$ 3191 FeLV-negative feline lymphosarcoma cells* in 5 ml of McCoy’s-L15 medium with 20% FCS; and 3) 20% confluent freshly passaged Crandell feline kidney (CRFK) monolayer cells in Dulbecco’s modified Eagle’s (DME) medium with 10% FCS. The cultures were incubated at 5% CO₂ in a humidified incubator at 37°C and were subcultured weekly at a ratio of 1:3 for the 3201B and 3191 suspension cells and at a ratio of 1:5 for the CRFK monolayer cultures. Three weeks after the initiation of the cultures, the cells were tested by IFA for the presence of FIV-like antigens using a known MIV antibody-positive domestic cat’s serum and the puma’s own serum; approximately 1% of the cells were positive. The cultures were continued as described above, and eventually 100% of the cells were infected with the puma lentivirus.

Domestic cat FIV isolate

The Petaluma strain* of FIV growing in CRFK cells was obtained (Dr. F de Noronha, Cornell University, Ithaca, New York 14853, USA) and adapted to grow in 3201B cells in our laboratory.

Domestic cat FIV ELISA

The Idexx ELISAs** were performed and interpreted according to the manufacturer’s specifications. Sample sera were eluted from the dried blood on the filter papers by cutting a 6.4-mm disk, containing the equivalent of 10 μl of whole blood (5 μl of serum), with a paper punch and placing the disks in 125 μl of phosphate-buffered saline (PBS) (dilution 1:25) for 1 hr at room temperature with constant rocking.

Indirect immunofluorescent assays

Preparation of target cells: All uninfected 3201B suspension cells and PLV- or FIV-infected 3201B cell cultures were grown in McCoy’s-L15 medium with 20% FCS, 1% glutamine, 1% penicillin/streptomycin, and 0.04% fungizone and were passaged twice weekly by splitting the cultures 1:3.

Preparation of IFA slides: Standard 12-well (5-mm-diameter wells) IFA slides (Cel-Line Associates, Newfield, New Jersey 08344, USA) were used. Live cells were harvested from a lymphocyte separation medium gradient, washed twice in PBS, and resuspended to a concentration of $2 \times 10^7$/ml in PBS. One drop (0.05 ml) of the target cell mixture consisting of 50% PLV- or FIV-infected cells and 50% uninfected 3201B cells was placed in each well. The drops containing the target cell suspensions were removed after 1 min, and the cells remaining in the wells were air-dried, fixed in acetone for 5 min at room temperature, and then stored at −70°C until use.

Performance of IFAs: Each 12-well IFA slide was used to test 10 sera, one PLV-positive (1:500) control serum, and one FIV-positive (1:25) control serum. Sera were eluted from the dried blood on the fil-
ter papers as described for FIV ELISA testing. Fifty microliters of each test sample and 50 µl of the control sera were placed in respective wells, and the slides were incubated at 37° C for 1 hr in a moist chamber. The slides were then washed twice in PBS for 5 min, dipped in distilled water, and air-dried. Fifty microliters of a fluorescein isothiocyanate-conjugated goat anti-cat IgG (H & L chain) serum (ICN Biomedicals, Costa Mesa, California 92626, USA) was used at a dilution of 1:60 in PBS in each well, and the slides were then incubated at 37° C for 1 hr in a moist chamber. Following the incubation, the slides were washed twice for 5 min each in PBS, dipped in distilled water, counterstained with 0.05% Evans blue in PBS for 7 min at room temperature, washed as previously described, and air-dried. A drop of 50% glycerol in PBS was placed on each well, and a coverslip was applied. The slides were evaluated using an epiluminated UV microscope. The presence of apple green cytoplasmic fluorescence in the 50% of the cells known to be expressing viral antigen was interpreted as a positive test, whereas the lack of fluorescence was interpreted as a negative test.

**Western immunoblots**

*Preparation of PLV and FIV antigens:* Unlike other studies that have used purified lentiviral antigens, we used PLV- and FIV-infected cell lysates as a source of viral antigens. This procedure allows for the isolation of greater quantities of viral antigens and unprocessed viral precursor polyproteins, which are absent or greatly reduced in purified viral preparations. The PLV- and FIV-infected 3201B cells, which were 100% antigen positive by the corresponding IFA tests, were harvested and washed three times in Tris buffer (Tris 1.436 g, NaCl 8.766 g, MgCl₂ 6H₂O 0.305 g in 1 L distilled H₂O, pH 7.4). The cell pellets were resuspended to a concentration of 10⁶ cells/ml in lysing buffer (Tris buffer plus 0.5% Nonidet P-40) for 30 min on ice. Following lysis, the cells were centrifuged at 500 x g for 20 min in a refrigerated centrifuge, and the supernatant was removed and saved.

The PLV or FIV cell lysate supernatants were mixed with equal volumes of sample buffer (2 ml of 0.5 M Tris HCl [pH 6.8], 2 ml 10% stock sodium dodecyl sulfate [SDS], 2 ml glycerol, 0.2 ml 2-mercaptoethanol, 0.2 ml 0.1% pyronin-Y [Sigma Chemical Co., St. Louis, Missouri 63103, USA]) and heated in a water bath at 90°C for 10 min. This antigen preparation was stored at -70°C until use.

*SDS–polyacrylamide gel electrophoresis and transblotting:* PLV and FIV cell lysate antigens (equivalent to 10⁷ cells in 0.2 ml of sample buffer) were electrophoresed on 11% SDS-polyacrylamide gels in a Mini-Protean II cell apparatus and transblotted onto nitrocellulose membranes in a Mini-Trans-Blot cell (Bio-Rad Laboratories, Hercules, California 94547, USA). The nitrocellulose membranes were blocked in 5% nonfat milk in PBS with 5% normal goat serum for 18 hr at 4°C. Following blocking, the membranes were washed three times in PBS for 10 min each, cut into 3-mm-wide strips, and frozen at -70°C until use.

*Performance of WB assays:* The assays were performed using sera eluted from the blood collection filter papers by placing four 6.4-mm disks from each sample (1:50 dilution) into a WB trytough containing 1 ml of 5% nonfat milk in PBS with 5% normal goat serum and nitrocellulose strips containing the PLV or FIV antigens. The strips were incubated at room temperature for 18 hr with constant rocking. Following incubation, the strips were washed twice for 10 min each time in PBS-Tween 20 (0.05%) then incubated for 1 hr at room temperature with constant rocking with 1 ml of a 1:1,000 dilution of biotinylated goat anti-cat IgG (H & L chain) (Vector Laboratories, Burlingame, California 94010, USA) in 5% nonfat milk in PBS. The strips were then washed twice for 10 min each...
time in PBS–Tween 20 and incubated for 1 hr at room temperature with constant rocking with 1 ml of a 1:1,000 dilution of horseradish peroxidase avidin D (Vector Laboratories). The strips were again washed twice as above and developed for 7 min in 1 ml of developing solution (2 ml 0.3% 4-chloro-1-naphthol stock solution [0.3 g 4-chloro-1-naphthol in 100 ml methanol] plus 10 ml PBS to which 0.004 ml of H$_2$O$_2$ [30%] was freshly added). The presence of a p24 band and at least two other viral bands was interpreted as a positive test, whereas the presence of only a single band was interpreted as indeterminate.

**FeLV ELISA**

Unlike tests for PLV or FIV infection, which detect antibodies against the viruses, the tests for FeLV infection detect viral antigens in the peripheral blood leukocytes by IFA or as soluble antigens in the plasma or serum by ELISA. Because the blood samples were collected on filter paper, an FeLV ELISA was applied. The Idexx FeLV ELISAs were performed and interpreted ac-
Table 1. Lentivirus status of 53 lions, leopards, and cheetahs in Botswana. PLV<sub>cat</sub> = puma lentivirus isolate; FIV = domestic cat feline immunodeficiency virus; IFA = indirect immunofluorescence assay; WB = western immunoblot; ELISA = enzyme-linked immunosorbent assay. Animals deemed positive, defined as having a positive result on at least the PLV<sub>cat</sub> WB, are grouped together within each species for ease of comparison.

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*Age, if provided, is very approximate based on assessments made in the field by safari hunters or wildlife officers with a diverse range of experience. Adult = fully grown animal but numerical age estimate not provided.

RESULTS

Fifty-three Botswana cats were sampled: 31 lions, 18 leopards, and four cheetahs. The length of time between collection of a sample and freezing ranged from <1 day to 46 days. The geographic distribution of the sampled cats is illustrated in Figure 3.

All 52 cats tested for FeLV with ELISA were negative. One cat did not have sufficient sample material for FeLV testing. Results of lentivirus assays are summarized in Table 1.

Twelve of 53 cats (22.6%) were considered lentivirus infected (defined as a positive result on at least the PLV\textsubscript{CZ} WB): eight of 31 sampled lions (25.8%), three of 18 leopards (16.7%), and one of four cheetahs (25%). Only four of these cats were positive on the FIV WB. Antibody bands corresponding to viral proteins were darker and better defined for the PLV\textsubscript{CZ} WB (Fig. 4) than for the FIV WB. Of the 12 PLV\textsubscript{CZ} WB-positive cats, three were positive on the PLV\textsubscript{CZ} IFA (Fig. 5), seven were positive on the FIV ELISA, and one was positive on the FIV IFA. One cat, lion no. 302, tested positive twice on the FIV ELISA but was negative on the PLV\textsubscript{CZ} WB, PLV\textsubscript{CZ} IFA, FIV IFA, and FIV WB. Thus, this lion was classified as a false positive on the FIV ELISA.

Safari hunters and wildlife officers were asked to comment on their perception of each animal’s health prior to their intervention (Fig. 2). Comments from them were generally based on body condition, and were often limited to “starving,” “thin,” “very fat,” etc. The six animals captured for live removal by the W.V.U. underwent comprehensive physical examinations and biomedical evaluations. Of the 53 cats sampled for this study, six were reported to be in less than “normal” condition: leopard nos. 3, 294, and 407 and lion nos. 227, 237, and 353. None of these six animals were infected with lentivirus. The only animal in this study found dead of unknown causes, leopard no. 322, was also lentivirus negative. This type of evaluation is extremely subjective and merely implies that there was no clinical illness in lentivirus-infected cats. Most of the cats appeared to be healthy, free-ranging animals.

All 12 lentivirus-infected animals were adult males. However, the majority (77.4%) of the animals sampled were adult males (Table 1), and there was no significant cor-
relation between gender and lentivirus status ($\chi^2 = 3.02, P < 0.01$).

DISCUSSION AND CONCLUSIONS

Collection of blood on filter paper has been previously validated for human immunodeficiency virus testing.\(^{16}\) Filter paper samples have been stored at room temperature for up to 8 wk prior to analysis without detrimental effects on the results of ELISA or WB.\(^{16}\) In addition, high temperature (37°C), high humidity, and very dry conditions had no effect on human test results for filter paper specimens stored for 3 and 7 days.\(^{10}\) Studies of domestic cat blood have shown that the sensitivity and specificity of ELISA, IFA, and WB are similar with fresh whole blood or dried blood collected on filter paper (Hardy and Zucker-

man, unpubl.). Whole cat blood collected on filter paper is stable for at least 3 mo at room temperature, and freezing soon after collection (as in this study) extends the preservation of the samples (Hardy and Zuckerman, unpubl.). Samples collected by hunters in particular often did not arrive at D.W.N.P. Headquarters for 3 wk or more, and positive lentivirus results were still obtained. This study could, however, underestimate the prevalence of lentivirus infection in the study population because of the multitude of variables related to sample handling and transport in the field.

Lions and leopards are legal trophy animals in Botswana, which has a set hunting season and quotas. Hunter compliance with the survey was good, with over 20% of legally killed lions and leopards sampled dur-
Figure 5. Indirect immunofluorescence assay for PLV_{cat} antibodies performed on a mixture of 50% uninfected and 50% infected PLV_{cat}-infected cat lymphoid cells. **a.** A negative test result is indicated by the lack of apple green fluorescence in the PLV_{cat}-infected cells in the mixture. **b.** A positive test result for PLV_{cat} antibodies is indicated by the strong fluorescence in the PLV_{cat}-infected cells in the test mixture.
ing the 1993 hunting season. The methods
used to opportunistically obtain specimens
precluded a representative sampling of the
whole population. Most lions and leopards
sampled were hunted on safari or killed as
problem predators. Because of their endan-
gered status, cheetahs are not legally hunted
for sport in Botswana: all four cheetahs
sampled were either captured or killed as
problem predators. Adult males are obvi-
ously preferred by trophy hunters, and all
25 safari-hunted lions and leopards sampled
were males. Of the remaining 28 animals,
26 were problem (livestock taking) predat-
ers. Most problem lions,12 leopards,12 and
cheetahs (Marker-Kraus and Kraus, pers.
comm.) are males. Among domestic cats,
older males with access to the outdoors are
at greater risk of FIV infection, most likely
because of bite wounds incurred while
fighting; the virus is shed in saliva.46 With
sampling methods biased towards males, it
is not possible to comment on the possi-
bility of a correlation between gender and len-
tivirus infection at the population level. One
study of Kruger National Park lions found
no correlation between gender and lentivi-
rus status, with both sexes having a sero-
positivity rate of >80%.40 Further study of
this issue, taking into account modes of vi-
ral transmission in wild African felid spe-
cies exhibiting a variety of social systems,
is needed.

Samples came from all over Botswana
(Fig. 3). The 12 cats with lentivirus infec-
tion were found near the eastern border
with South Africa (leopard nos. 1, 2, 372),
in the northern part of the country in the
hunting areas surrounding Moremi Game
Reserve (lion nos. 15, 25, 44, 57, 61, 135,
281, 301), and near the western border with
Namibia (cheetah no. 2). Sample sites were
biased toward popular hunting areas and ar-
 eas where problem predator control officers
were more active. Nevertheless, large felid
exposure to lentivirus is not limited to a
particular portion of Botswana. In addition,
lentivirus strain identity and species speci-
ficity were not studied.

Although this study was not a comprehen-
sive evaluation of the lion, leopard, and
cheetah populations of Botswana, it did re-
veal wild felid lentivirus infection in a pre-
viously unexamined portion of sub-Saharan
Africa. Botswana is considered a critical
range country for lion, leopard, and cheet-
ah, although adequate in-country census
data are currently lacking for all three spe-
cies. It may have the second largest re-
maining free-ranging cheetah population in
the world, after Namibia (Marker and
Kraus, pers. comm.). With evidence of
lentivirus infection in free-ranging South
African lions and leopards (but not cheetahs
thus far),27,46 no evidence of lentivirus in
Namibian cheetahs,37 and recent evidence of
infection in wild Namibian lions (Mark-
er-Kraus and Kraus, pers. comm.),27,40 these
Botswana findings have important
management implications.

We recommend that no large cats be
moved locally or internationally without
prior evaluation for lentivirus infection. Na-
mibian authorities have already institut-
ed such a policy for lions, whereby only se-
ronegative lions are allowed to be moved
within the country or internationally
(Scheepers, pers. comm.). This policy, how-
ever, relies on a domestic cat FIV ELISA
and not a more sensitive assay such as the
PLV_t WB and may be inadequate. Al-
though there have been no published re-
ports of immunologic compromise or clin-
ical illness in nondomestic felids definitively
 correlated to FIV-like lentiviruses,27,35
preliminary findings of inverted CD4:CD8
lymphocyte ratios, polyclonal gammopathy,
and retinopathy in several captive lentivi-
rus-positive African lions suggest that in-
fec tion in lions may not be benign.37 A clin-
ically ill FIV-seropositive captive African
lion in Italy was recently reported to have
had FIV gag p24–sequences isolated from
multiple lymphoid tissues via polymerase
chain reaction amplification.32 Results from
a recent survey of North American zoos
also suggest that lentivirus-infected non-
domestic felids may exhibit clinical signs
similar to those accompanying FIV infection of domestic cats, including enlarged cervical lymph nodes, periodic behavior changes, chronic gingivitis, ocular lesions, myeloproliferative disease, and renal involvement.\textsuperscript{6,10}

There is still a great deal that is not known about the epidemiology of FIV-like lentiviruses.\textsuperscript{6} Retrospective analyses of banked domestic cat sera have identified FIV antibodies in samples collected as far back as 1967 (Hardy and Zuckerman, unpubl.) and 1968.\textsuperscript{30} Retrospective analyses of lion sera banked in South Africa's Kruger National Park have identified FIV antibodies in samples obtained as far back as 1977.\textsuperscript{30} Lentiviruses related to FIV have been found in nondomestic felids worldwide, and FIV and its relatives may be millions of years old.\textsuperscript{47} Lentiviruses are well known for their propensity to mutate.\textsuperscript{6} Thus, even without current direct evidence of clinical implications, vigilance regarding free-living wild felid populations seems prudent.

Different lentivirus assays applied to free-ranging felid populations sometimes yield markedly different prevalence figures. Two recent studies of free-ranging California pumas illustrate the point: one immunoblot-based project (n = 16) yielded a 56\% seropositivity rate;\textsuperscript{33} and the other study (n = 58) using an IFA and an antigen-detecting ELISA indicated a 0\% seroprevalence.\textsuperscript{33}

For domestic cats, the false-positive rate for the FIV ELISA used on the Botswana felids is 14–26\%, when the immunoblot technique is used as the standard (Hardy and Zuckerman, unpubl.).\textsuperscript{34} The use of the PLV\textsubscript{esu} WB, which is more sensitive than an IFA, as the standard in this study seems prudent. The lentiviruses that infect wild cats appear antigenically different from domestic cat FIV. The lentivirus-positive Botswanaan cats generally reacted more strongly on the PLV\textsubscript{esu} WB than on the FIV WB, as demonstrated by darker, more-defined antibody bands corresponding to the viral proteins (Fig. 4). There are different specificities and sensitivities among the lentivirus assays being applied to nondomestic felids, suggesting that assay choice is important and that caution is warranted in interpreting data. More African felid populations should be evaluated using the PLV\textsubscript{esu} WB, and prevalence data from other regions where only the domestic cat FIV isolate was used for serologic assays should be reexamined.

Translocation and reintroduction of large carnivores in Africa are complex issues.\textsuperscript{35} The importance of monitoring disease and the potential influence of disease on the success of translocation and/or reintroduction programs has started to receive long-overdue attention.\textsuperscript{31} Subpopulations of large felids in southern Africa exhibit a range of lentivirus prevalence, with some subpopulations thus far showing no evidence of infection. This survey of free-ranging lions, leopards, and cheetahs in Botswana found evidence of lentivirus infection in all three species. Seropositive cats were found in geographically diverse parts of the country, including parts of the Kalahari ecosystem, which covers >80\% of Botswana. The historical effectiveness of the Kalahari desert as a barrier to the movement of animals and their pathogens\textsuperscript{67} is uncertain. With more information on PLV-like lentivirus and FeLV, scientists and managers in southern Africa can make more informed decisions regarding the movement of large cats for research, management, or commercial purposes.

Acknowledgments: We thank all of the safari hunters and D.W.N.P. officers who participated in sample collection. We also thank Valerie Sellen, Mary Beiter, Judy Perry, Nancy Westphal, and Gloria Longo for valuable technical assistance. This project was officially organized and sanctioned by the Botswana D.W.N.P., the Governmental body responsible for the monitoring and enforcement of all wildlife legislation in the Republic of Botswana, including regulations put forth by CITES, of which the Republic of Botswana is a member.
LITERATURE CITED


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