

INCIDENCE OF FELINE IMMUNODEFICIENCY VIRUS REACTIVE ANTIBODIES IN FREE-RANGING LIONS OF THE KRUGER NATIONAL PARK AND THE ETOSHA NATIONAL PARK IN SOUTHERN AFRICA DETECTED BY RECOMBINANT FIV p24 ANTIGEN

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ABSTRACT

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Lion sera from the Kruger National Park (KNP) dating back to 1977 and from the Etosha National Park (ENP), obtained from 1989 to 1991, have been analysed by ELISA and Western blot analyses using a genetically engineered antigen representing the p24 structural protein of feline immunodeficiency virus (FIV). It was concluded that some 83 % of 98 KNP lion sera reacted with the p24 antigen, while none of 28 ENP lion sera reacted. A few other KNP felids (cheetahs and genets) gave samples that did not react with the FIV p24 antigen. For the KNP lions, apart from a lower prevalence in cubs (50 %), no particular trends were demonstrated in terms of age, sex, date or origins of the samples. In Western blot and radio-immunoprecipitation analyses the lion sera reacted with the engineered p24 antigen, as well as with the p15 and p24 *gag* proteins and the p50 *gag* precursor protein from FIV, indicating that the agent is probably a lentivirus related to FIV. The ELISA with the engineered p24 antigen required less serum and appears to be more sensitive at detecting FIV-reactive antibodies than assays with available commercial kits.

INTRODUCTION

Feline immunodeficiency virus (FIV), a member of the subfamily *Lentivirinae* of the *Retroviridae*, is a pathogen of domestic cats. Infection with FIV is associated with immunodeficiency and opportunistic infections in these animals (Pedersen, Ho, Brown & Yamamoto, 1987; Yamamoto, Hansen, Ho, Morishita, Okuda, Sawa, Nakamura & Pedersen, 1989; Yamamoto, Sparger, Ho, Andersen, O'Connor, Mandell, Lowenstine, Munn & Pedersen, 1988). FIV is regarded as a possible animal model for the human acquired immunodeficiency syndrome (AIDS). It is morphologically similar to the human immunodeficiency viruses (HIV) and exhibits a similar cell tropism (Pedersen *et al.*, 1987; Brunner & Pedersen, 1989; Phillips, Talbot, Lamont, Muir, Lovelace & Elder, 1990). Several independent virus isolates have been made (Pedersen *et al.*, 1987; Harbour, Williams, Gruffydd-Jones, Burbridge & Pearson, 1988; Miyazawa, Furuya, Itagaki, Tohya, Nakano, Takahashi & Mikami, 1989; Morikawa, Lutz, Aubert & Bishop, 1991). The nucleotide sequences of different proviral clones derived from American, European and Asian isolates of FIV have revealed that

the genome organization is similar in complexity to those of other lentiviruses, including HIV (Talbot, Sparger, Lovelace, Fitsch, Pedersen, Luciw & Elder, 1989; Olmsted, Barnes, Yamamoto, Hirsch, Purcell & Johnson, 1989; Olmsted, Hirsch, Purcell & Johnson, 1989; Phillips *et al.*, 1990; Morikawa, Lutz, Aubert & Bishop, 1991).

Epidemiological surveys indicate that FIV is distributed worldwide in domestic and feral cats (Gruffydd-Jones, Hopper, Harbour & Lutz, 1988; Grindem, Corbett, Ammerman & Tomkins, 1989; Ishida, Washizu, Toriyabe, Motoyoshi, Tomoda & Pedersen, 1989; Witt, Moench, Gittelsohn, Bishop & Childs, 1989). FIV antibodies cross-react with group-specific antigens (*gag*) of both caprine arthritis encephalitis and visna viruses (Olmsted, Hirsch, Purcell & Johnson, 1989), and a reciprocal cross-reactivity of the major core proteins of FIV and equine infectious anaemia virus has been detected (Egberink, Ederveen, Montelaro, Pedersen, Horzinek & Koolen, 1990; Steinman, Dombroski, O'Connor, Montelaro, Tonelli, Lawrence, Seymour, Goodness, Pedersen & Andersen, 1990). It has also been reported that FIV *gag* components cross-react with antibodies present in some lion sera. Putative infection in 2 lions (*Panthera leo*) with a virus related to FIV was first reported by Barr and co-workers (Barr, Calle, Roelke & Scott, 1989) who conducted a survey during which FIV-reactive antibodies were also detected in several other captive non-domestic felid species, namely the snow leopard (*P. uncia*), jaguar (*P. onca*), white tiger (*P. tigris*), as well as free-ranging Florida panthers (*Felis concolor coryi*), and a bobcat (*F. rufus floridanus*). For the Florida panther, molecular studies have subsequently shown that the animals were infected with a retrovirus that exhibited some sequence homology to FIV from domestic cats (e.g., 75 % of their reverse transcriptase sequences, O'Brien and associates,

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reported at the International Workshop on Feline Immunology and immunodeficiency, 1991). No data are available on the molecular relationships of the putative lion retrovirus to FIV, or to the Florida panther virus.

Antibodies reactive to FIV have been detected in 30/53 (57 %) African lions surveyed in European zoos (Lutz, Isenbügel, Lehmann, Sabatara & Wolfensberger, in press), and in 16/22 (73 %) of Asian lions residing at the Lincoln Park Zoo, Chicago, USA (Letcher & O'Conner, 1991). A limited survey of lions from zoos in the Pretoria and Johannesburg region in South Africa have provided similar data (see later). In contrast, sera collected from free-ranging lions in the Etosha National Park in Namibia were found to be seronegative to FIV antigens (Spencer & Morkel, 1992).

Assuming that reactivity to FIV p24 reflects infection with FIV or a related virus, this paper presents evidence indicating that a genetically engineered FIV p24 *gag* protein produced in bacteria is a useful reagent for detecting FIV-related antibodies in lion sera. A detailed serological survey has been conducted using this reagent to determine the incidence of FIV-reactive antibodies in free-ranging lions in two game reserves in southern Africa, namely the Kruger National Park (KNP) in the Republic of South Africa and the Etosha National Park (ENP) in Namibia. The reactivities of the sera in relation to their origins (male/female, age, date and site of acquisition) have been investigated. Sera from a few other felids from the KNP were included. These gave no evidence of reactivity to the FIV *gag* antigen. Apart from lions, analyses of captive felids in two zoos in the Republic of South Africa have also given no evidence of antibodies reactive to FIV antigens. Some lion sera were tested in Western blot and radio-immunoprecipitation analyses using cell culture grown FIV as an antigen. They were found to recognize FIV *gag* proteins p15, p24 and the p50 *gag* precursor protein.

MATERIALS AND METHODS

Serum samples

A total of 98 serum samples were obtained from lions in the KNP. Some 28 serum samples came from lions in the ENP. All of the ENP and 73 of the KNP lion sera were received lyophilized. The remaining 25 lion sera from the KNP were collected during 1991. They were kept frozen at -20°C . The KNP lion sera were collected over a 14 year period dating back to 1977, while those from the ENP were collected over a 3 year period (1989–1991). With some exceptions, records were available on the date of collection, sex, estimated age and location where the animals were captured. In addition to the samples from lions, lyophilized serum samples from 7 cheetahs, 1 leopard and 2 genets were obtained from the KNP in the period 1986–1991. Fresh serum samples were also obtained from captive lions and 10 other felid species in two zoos in the Republic of South Africa, namely the National Zoo in Pretoria and the Johannesburg Zoo.

Enzyme-linked immunosorbent assay (ELISA)

Two ELISA formats were used. The first was a commercial combined ELISA kit developed for domestic cats to test simultaneously for the presence of feline leukemia virus (FeLV) antigen and antibody to FIV (CITE Combo FeLV Ag/FIV Ab, IDEXX Corporation, Portland, Maine 04101, USA). The kit was used according to the manufacturer's specifications. Horseradish peroxidase (HRPO)-conjugated antigen from inactivated FIV is used in the kit to detect FIV antibodies. Detection of FeLV antigen utilizes HRPO-conjugated monoclonal antibodies (Ab) to FeLV p27 antigen (Ag). This Ag is diagnostic for FeLV infection. In the CITE Combo FeLV/FIV ELISA kit a concentrated sample serum is required (i.e. 0.2 ml of undiluted serum is mixed with an equal volume of conjugate in the first step of the procedure).

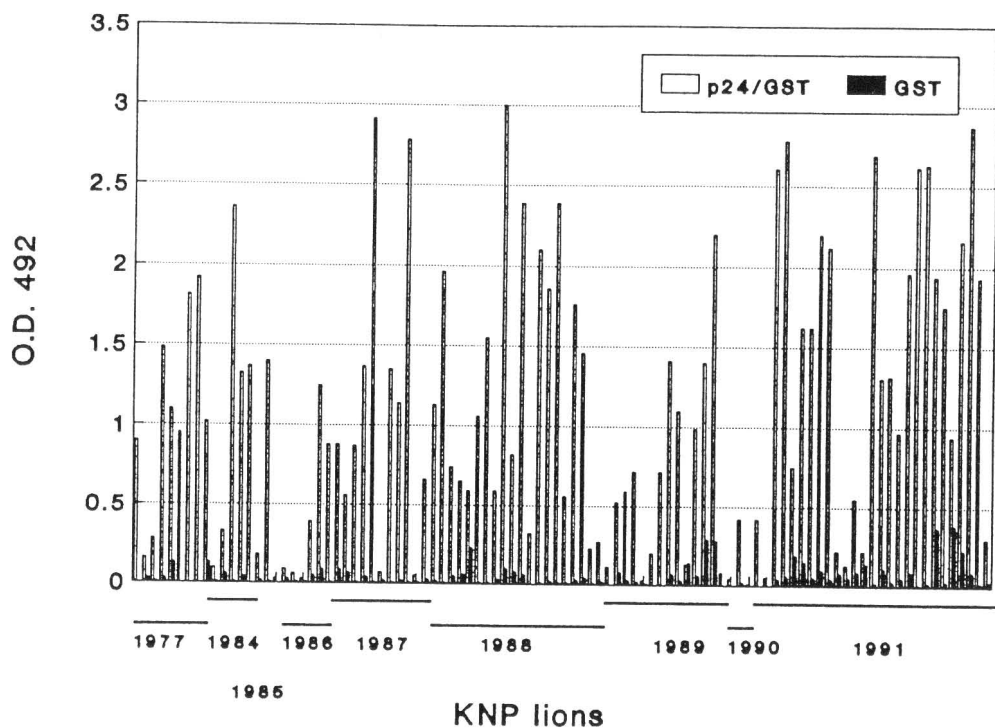
The second ELISA employed a FIV p24 antigen coated plate (ACP) to detect antibodies to the FIV p24 *gag* protein. The p24 gene derived from a cDNA clone to FIV (Morikawa, Booth & Bishop, 1991) was expressed as a 50 kDa fusion protein with glutathione-S-transferase (p24/GST) in bacteria using the pGEX expression system (Smith & Johnson, 1988). The GST gene, which originated from *Schistosoma japonicum* species (Smith & Johnson, 1988), was used to provide the control 26 kDa GST antigen in this assay. Both proteins were purified to homogeneity by binding to and elution from immobilized glutathione (Smith & Johnson, 1988). Stocks of these proteins were kept at -20°C at concentrations of 1 mg/ml.

The ACP ELISA was performed essentially as described by Voller, Bidwell & Bartlett (1976). Wells of polystyrene microtitre plates (Nunc Polysorb, Denmark) were coated with 0.2 μg Ag/well using phosphate-buffered saline (PBS) as diluent. After incubation at 4°C for at least 4 h, the wells were washed three times with PBS/0.05 % Tween-20. Blocking was accomplished using a buffer comprising of 7 % skimmed milkpowder (Nestle's Infant Starter Formula marketed as "Lactogen")/1 % Tween-20/1 % casein hydrolysate at 25°C for 2 h, followed by washes with PBS/0.05 % Tween-20. Application of dilutions of the sample serum (prepared in 3.5 % "Lactogen"/0.5 % Tween-20/0.5 % casein hydrolysate) was undertaken as indicated. Following incubation at 25°C for 2 h, and then 3 washes with PBS/0.05 % Tween-20, HPRO-conjugate (rabbit anti-cat, diluted 1:2000 in PBS/0.05 % Tween-20) was added and the samples were incubated for 1 h at 25°C . After 3 more washes with PBS/0.05 % Tween-20, o-phenylene diamine/hydrogen peroxide (H_2O_2) substrate mixture was applied. The reaction was allowed to proceed for 15–30 min and was stopped by addition of H_2SO_4 . The optical density was determined at 492 nm using an EAR 400A ELISA Reader (SLT, Austria) and compared to controls.

SDS-PAGE, immunoblot assay (Western blot) and radio-immunoprecipitation assay (RIPA)

For SDS-PAGE and Western blot assays (Towbin, Staehelin & Gordon, 1979) using the FIV

A.



B.

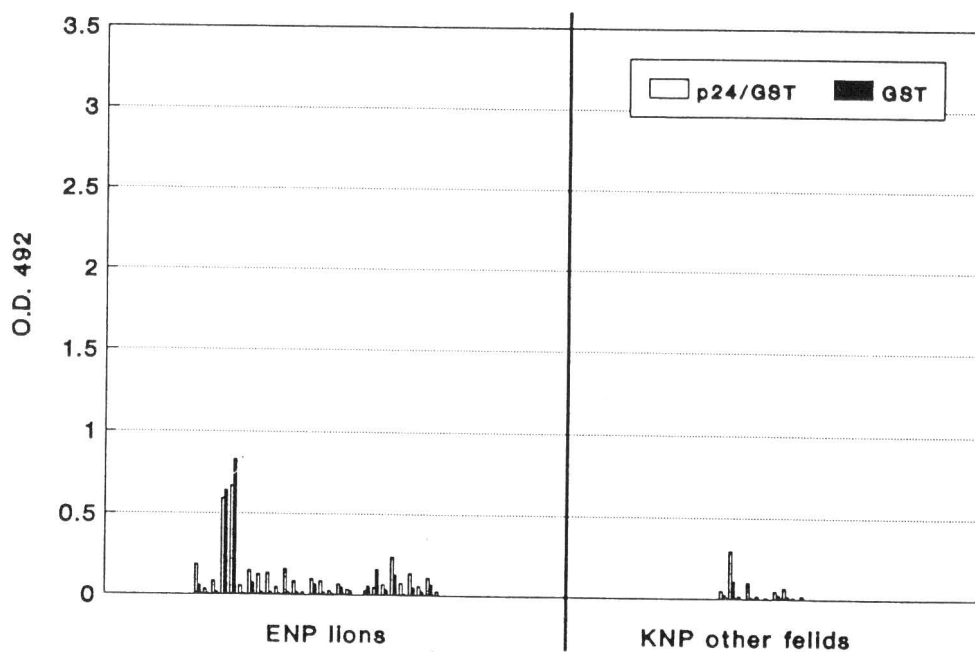


FIG. 1 ELISA analyses of felid sera collected from the KNP or ENP. In the top panel are shown the data for KNP lion sera collected during the period 1977–1991 (left to right: 1977–9 lions; 1984–7; 1985–1; 1986–4; 1987–13; 1988–22; 1989–12; 1990–2; 1991–28; total 98). For each sample the data are shown in pairs representing the results obtained with p24/GST (open columns) and control GST (filled columns), respectively. Similarly, in the bottom left panel are shown the data for ENP collected during the period 1989–1991 (left to right: 1989–9; 1990–7; 1991–12; total 28). Likewise in the bottom right panel are shown the data for 7 cheetahs, 1 leopard, and 2 genets sampled in the KNP during the period 1986–1991 (left to right: cheetahs; 1986–1; 1987–1; 1988–2; 1989–2; 1990–1; leopard: 1989–1; genets: 1991–2)

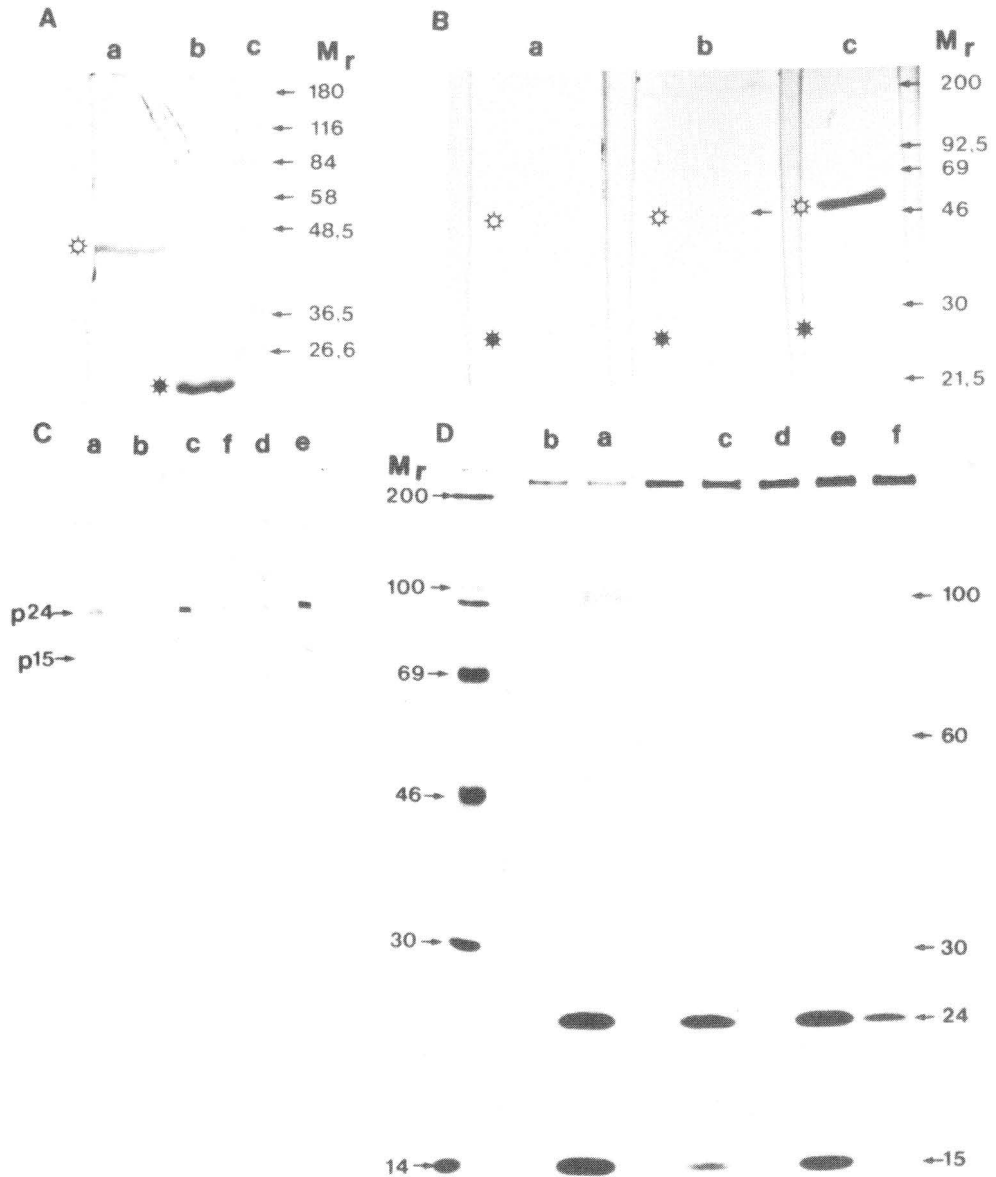


FIG. 2 SDS-PAGE of p24/GST and GST proteins and Western blot analyses of KNP lion sera. In panel A are shown the resolved kDa p24/GST (lane a) and 26 kDa GST (lane b) proteins by comparison to molecular mass markers (kDa). In panel B shown the reactions of lion sera with mixtures of p24/GST (positions indicated by open stars) and GST (filled stars). Lion sera with low (panel a), intermediate (lane b) and high (lane c) ELISA titres were employed. Reactions were evident (arrows) only the p24/GST protein and the high and intermediate titered sera. In panel C a Western blot analysis of sucrose gradient purified FIV is shown. Strips were incubated with a positive (lane a) and negative (lane b) control serum of a domestic cat and four different lion sera (lanes c, d, e and f). In panel D the electrophoretic analysis of immunoprecipitates formed between methionine labelled FIV proteins and different lion serum samples are shown. Lanes a, b, c, d, e and f represent reactions with the same sera as in panel C. The M_r of FIV-specific proteins are indicated at the right.

p24/GST fusion protein and control GST protein, proteins were resolved using a 5 % stacking gel and 15 % separating gel (Laemmli, 1970) and either stained or transferred to a nylon membrane (Hybond-N, Amersham) according to the manufacturer's instructions. The membrane was blocked at 4 °C for at least 4 h using 3 % non-fat milk powder made up in PBS. After 3 washes with PBS, the

membrane was incubated with serum (1:15 dilution in blocking solution) for 2 h at 25 °C, again washed thoroughly and incubated with HRPO-conjugated rabbit anti-cat 1:200 dilution in PBS/0.05 % Tween 20/3 % non-fat milk powder) for 1 h at 25 °C. Following a final washing, the colour was developed using a solution containing 4-chloro-1-naphtol and H_2O_2 .

For SDS-PAGE and Western blot analyses

crose gradient purified FIV, strain 113, from persistently infected CRFK cells was used. Proteins were separated by electrophoresis (15 % SDS-PAGE) and transferred to nitrocellulose sheets (pore diameter 0,45 μ m, Schleicher and Schuell) using an LKB semi-dry clotting apparatus. After transfer to nitrocellulose, the sheets were cut into strips 3 mm wide and blocked for 2 h at 37 °C in PBS containing 0,1 % Triton X-100 and 0,05 % pig skin gelatin (gelatin buffer). Sera diluted 1:50 in gelatin buffer were used for incubation of the strips (1,5 h at room temperature); they were then washed in gelatin buffer and a goat anti-cat HRPO-conjugate was added. After another hour of incubation and subsequent rinses, 4-chloro-1-naphthol was added as chromogen. The reaction was stopped by rinsing the strips in tap water.

Radio-isotope labelling of FIV infected cells and RIPA were performed essentially as described before (Egberink, Lutz & Horzinek, 1991). In short, CRFK cells persistently infected with FIV strain 113, were labelled for 16 h with 35 S-L-methionine. The supernatant was clarified at 10 000 \times g for 30 min and samples containing about 100 000 cpm 35 S-L-methionine were incubated with 5 μ l serum in Tris/EDTA/saline buffer (TESV) containing 0,5 % Triton-X100 and 2 mM phenylmethylsulfonylfluoride at 4 °C for 16 h. Alternatively, lysates of FIV-infected cells were used. The immune complexes were precipitated with formaldehyde fixed *Staphylococcus aureus* cells for 45 min at 4 °C. The precipitates were washed three times with TESP containing 0,1 % Triton X-100 and finally resuspended in Laemmli sample buffer. Proteins were analysed by SDS-PAGE and visualized by fluorography.

RESULTS

The FIV *gag* gene encodes a polyprotein of 450 amino acids (49,5 kDa, p50) which, as in other retroviruses, is processed to proteins (p15, p24 and p10

in the case of FIV) by a protease encoded by the amino-proximal part of the *pol* gene product (Olmsted, Hirsch, Purcell & Johnson, 1989). The ORF encoding the p24 protein was cloned from FIV cDNA (Morikawa, Booth & Bishop, 1991) and expressed as a fusion protein with GST using the pGEX bacterial expression system (Smith & Johnson, 1988). The purified p24/GST fusion protein was used as the antibody detecting antigen in ACP ELISAs and Western blots, with similarly purified GST protein providing the negative control.

The reactivities of large felid sera to FIV p24 antigen

A total of 98 sera from lions in the KNP, 10 other samples of KNP felid sera (7 cheetahs, 1 leopard and 2 genets) and those of 28 lions from the ENP were analysed in duplicate using the ACP ELISA. Sera were used at a 1/100 dilution. Higher dilutions gave similar results but proportionately lower signals. Lower dilutions also gave similar data but, in some cases, higher control signals. The majority of the KNP lion sera reacted with the FIV antigen (Fig. 1 top panel). A mean OD₄₉₂ of 0,10 was obtained for a panel of 50 animals, 40 lions (10 KNP, 28 ENP and 2 captive lions) and the 10 KNP non-lion felids (7 cheetahs, 1 leopard and 2 genets), that had a >3-fold higher reactivity to the p24/GST antigen over the reaction with GST alone in the p24-based ACP ELISA and also tested negative with the CITE Combo ELISA. For the purposes of this study, the positive cut off point for the p24-based ACP ELISA was therefore set at OD₄₉₂ >0,20, twice the mean OD₄₉₂ of 0,10 of the above-mentioned panel of animals. On this basis the sera of the non-lion KNP felidae and all the ENP lion sera scored negative (Fig. 1, bottom panels). The KNP lion sera yielded ELISA signals ranging from high (17 sera: >2,0 OD₄₉₂), to intermediate (52 sera: 0,5–2,0 OD₄₉₂) to low, but still positive (12 sera: 0,2–0,5 OD₄₉₂ with an

KNP LIONS: BY YEAR

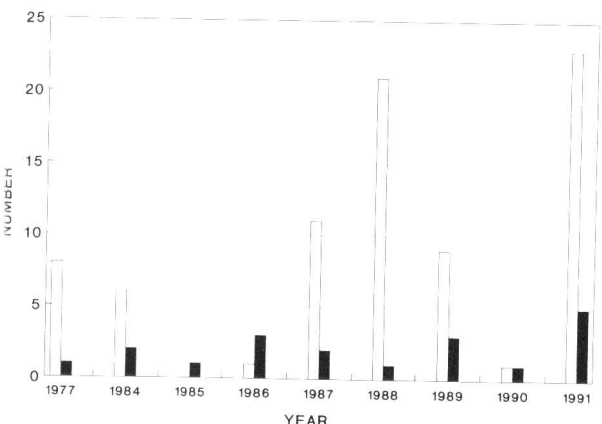


FIG. 3 Serological analyses of the KNP lions in relation to the date of serum acquisition. The numbers of sera that were scored positive (see text) using the p24/GST ELISA (open columns) and those that were scored negative (filled columns) are plotted against the date of serum acquisition

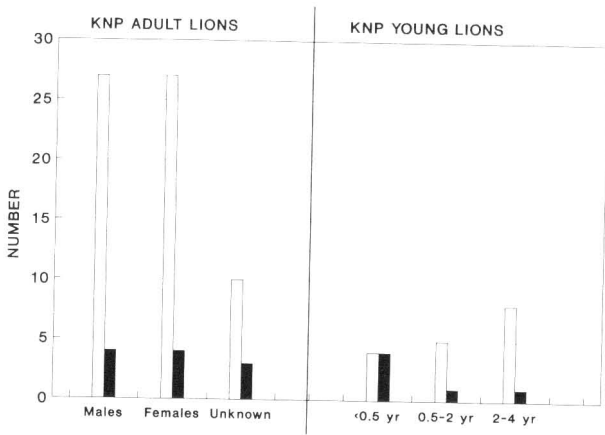


FIG. 4 Serological analyses of the KNP lions in relation to sex (where recorded) and estimated age. The numbers of lions that were scored positive using the p24/GST ELISA (open columns) and those that were scored negative (filled columns) are plotted for adult lions (left panel) against sex (where recorded), or for young lions (right panel) against estimated age (see text)

OD pos/neg ratio >3). Seventeen sera were scored negative (OD₄₉₂<0,2 and, or with an OD pos/neg ratio <3). In summary, a total of 81 of the 98 KNP lion sera were scored positive for reaction with the FIV p24 antigen (83 % seropositive). The reactivity to GST alone was generally low (OD₄₉₂ <0,2), on occasion a higher reactivity was observed (Fig. 1, blackened columns; 8/134 samples), presumably reflecting an immunity induced to the GST antigens of *Schistosoma* spp., or other sources. This was particularly evident for 2 of the ENP lions (Fig. 1, bottom left panel).

The commercial CITE Combo FeLV Ag/FIV Ab kit scored positive 38 of 74 KNP lion samples that were tested (51 %). Sera that were designated high by the p24/GST ELISA also scored positive by the CITE Combo kit. Six that scored negative in the p24-based ELISA scored positive with the kit. The tests were not repeated to verify these results. Of the 36 samples that scored negative by the kit, 20 scored intermediate and 10 scored low in the ELISA test. In summary, it appears that the commercial CITE Combo FeLV/FIV Ab ELISA kit was less sensitive than the p24-based ELISA. Also the CITE Combo kit required more serum to give a positive signal. All the sera were negative for the FeLV Ag.

To confirm the data, Western blots were undertaken on selected sera that exhibited high, intermediate, low or negative ELISA readings. Fig. 2A shows SDS-PAGE of p24/GST (open star) and GST (filled star) run in individual lanes (a, b), stained with Coomassie Brilliant Blue and by comparison with molecular mass markers (lane c). Fig. 2B (lanes a-c) shows the Western analyses of mixtures of the blotted p24/GST and GST proteins (i.e., in the same lane, respective positions indicated by open and filled stars). The reaction obtained with a lion serum with a high ELISA reading to the p24/GST fusion was strong, no reaction was obtained with GST alone (lane c). The reaction to the fusion protein by a serum of intermediate titre was evident, albeit faint (lane b), no reaction was detected with negative sera (not shown), or sera of low titre (e.g., lane a). Probing gel-resolved FIV proteins with lion sera detected the p15 and p24 *gag*-proteins (Fig. 2C lanes c, e and f). Lane d shows the reaction of a negative lion serum. Although the serum of an FIV-infected domestic cat used as a positive control (Fig. 2C lane a) disclosed only the *gag*-proteins, RIPA detected higher M_r proteins (Fig. 2D), including an *env*-protein, gp100, (Fig. 2D lane a), in addition to the *gag*-proteins. Also faint bands in the 60 kDa and 30 kDa region were recognized, probably representing *pol* gene products (reverse transcriptase and integrase, respectively). By RIPA the positive lion sera (Fig. 2D lanes c, e and f) showed no reaction with the *env*-protein of FIV, while the *gag*-proteins were again observed. The 60 kDa protein was also recognized by RIPA for one of the lion sera (Fig. 2D lane d). Using cell lysates, the 50 kDa *gag* precursor protein was recognized by the lion sera (results not shown).

Analyses of the Kruger National Park lion sera

The data obtained for the KNP lion sera were analysed in relation to the time of serum acquisition

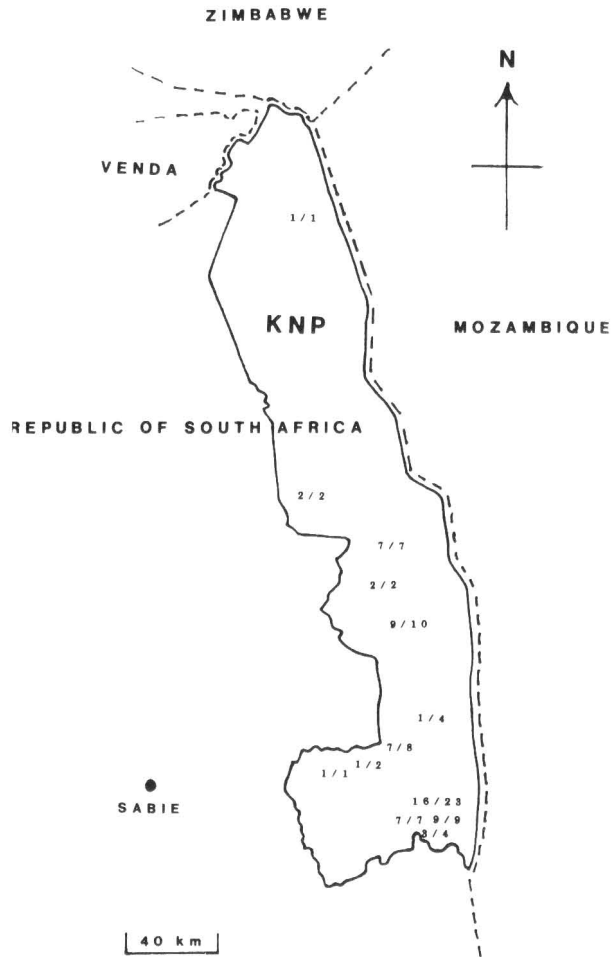


FIG. 5 Serological analyses of the KNP lions plotted against the site of acquisition of the animals. For 80 lions for which the exact site of acquisition of the sample was recorded, the number of lions scored seropositive using the p24/GST ELISA (see text) are given versus the number sampled at that site. The data are shown in relation to a map of the KNP in the Republic of South Africa

(Fig. 3), the sex and age of each animal (Fig. 4) and the distribution within the Park (Fig. 5). The sera that were analysed included samples that dated back to 1977. The collection is not comprehensive since there is a gap of 6 years from 1978 to 1984 for which no serum was available. Also for some years only a few sera were obtained (e.g., for 1985: 1 sample; 1986: 4 samples; and 1990: 2 samples). Nevertheless, the data show that antibodies reactive with FIV p24 were present in lions as far back as 1977 (Fig. 3). More than 80 % of the KNP lions tested positive for any year for which >4 sera were available. The numbers for 1986 (3/4 negative) are too small to make any specific conclusion, particularly since two of these animals were younger than 6 months (see below).

Analysis of the KNP lion results on a sex and age base was undertaken where possible (Fig. 4). Of the 98 samples, 31 came from adult males and 31 came from adult females, 13 adults had no sex indication. Both male and female adult lions were more than 80 % seropositive, indicating no sex preference.

The unknown sex group also exhibited a high infection rate. In parenthesis, the ENP lion sera collection (all FIV-seronegative) included 18 samples from male and 8 from female lions, while 2 sera had no indication of sex origin.

Of the 98 KNP sera, 23 were indicated to have been collected from young animals. These were subdivided into three groups: cubs (i.e., 8 that were younger than 6 months), those from 6–24 months old (6 animals) and those between 2–4 years of age (9 animals). The data show that by 6 months of age 50 % were seropositive. Some 83 % of the animals from 6 months to 2 years were positive which is essentially the same as the adult animals. The third group of animals (2–4 years of age) was also predominantly seropositive (89 %). Although the numbers are low, the results suggest that risk of infection may increase with age.

A plot of the seropositive lions on a map of the KNP indicated the geographical distribution of infection in the Park population (Fig. 5). The data represent a total of 80 lions for which the site of acquisition was recorded. Approximately two thirds of the samples were collected in the southern part of the KNP, and the remaining one third in the central region. Serum was obtained from only one animal that was captured in the northern-most region of the KNP. Although more sera are required for that region, the data show that infection occurs in lions throughout the KNP.

DISCUSSION

We have obtained data on lion sera which confirm and extend the results of other investigations which indicate that lions may have antibodies reactive to FIV p24 antigen. The reactivities of the lion sera to the FIV antigen were identified by both ELISA and, for high and intermediate titered sera, Western analyses. Since p24 reactivity may overestimate the prevalence of FIV-like infection, reaction to other FIV antigens may be required to verify the virus specificity of the p24 reaction. The agent that is responsible for the infections is not known. Most likely it is a lentivirus antigenically closely related to FIV. However, this requires definitive proof by isolation and characterization of the etiological agent. We have not been able to detect antibodies in lion sera to the FIV *env* gene products. The negative RIPA on FIV *env* gene products requires further verification using radio-labelled glucosamine instead of methionine to increase sensitivity. However, our result does not exclude an infection with FIV; FIV *env* gene products might be less immunogenic in lions—as they are in cats—than *gag* products. From an evolutionary point of view the putative lion lentivirus is most likely to differ from FIV in its *env* protein. In this context it is worth noting that Lutz *et al.* (in press) were not able to transmit infection using blood from an African lion seropositive for FIV to specific pathogen free cats. This is also in agreement with the concept that the putative lion lentivirus is distinct from FIV.

Interestingly, although a high percentage of KNP lions were seropositive, none of the three other felids tested from the KNP (cheetahs, a leopard and

3 genets) were seropositive. Although the sample size is too small to make a conclusion, it may reflect a species specificity of the agent to lions. In a study of captive lions and 10 other species of felids (pumas, leopards, cheetahs, jaguars, 3 species of tiger, a bobcat, servals and caracals) in the National Zoo in Pretoria and the Johannesburg Zoo, by means of the commercially available PetChek ELISA (IDEXX Corporation, Portland, Maine 04101, USA), no antibodies that reacted with FIV antigens were detected, except for the lion samples. A type-specific reagent to the lion agent is required to investigate this issue and to establish its relationship to other agents isolated from felids, including domestic and feral cats. The FIV-reactivity of the captive lions was subsequently confirmed using both the CITE Combo ELISA and the p24-based ELISA. The results of the PetCheck, CITE Combo and the p24-based ELISAs were, however, not compared quantitatively.

The 1:1 male to female distribution of FIV-reactive antibodies in lions, in contrast to domestic cats where the male to female ratio is 3:1, might be affected by behaviour. Lions, being the only truly sociable felids, feed, groom and play together in prides, often inflicting bite wounds on one another, thus exposing females and cubs more to infection than other felidae. It is of interest to note that two mothers of six lion cubs born in the Johannesburg Zoo during 1991 had FIV-reactive antibodies at the time of the births. The cubs were left with their mothers for three weeks before they were removed and hand-reared. None of them developed FIV-reactive antibodies. However, of four lion cubs born in the National Zoo in Pretoria and left with their FIV-reactive mother, three had FIV-reactive antibodies when tested at 6 months of age, and the fourth developed FIV-reactive antibodies by 18 months. These observations support the hypothesis that infection may be spread from mother to offspring through grooming and retrieval behaviour as suggested by Barr *et al.* (1989).

Surprisingly, based on the present data, none of the lion sera which came from the ENP reacted with the FIV *gag* antigen. The ENP in Namibia is essentially similar in size to the KNP. Also, animals in the ENP were sampled at various locations and over a three year period (1989: 9 lions; 1990: 7 lions; 1991: 12 lions). In a more extensive survey in which another 38 lions from the ENP were assayed with the CITE Combo kit alone, none were found to be positive (Spencer & Morkel, 1992). The lack of infection of the ENP lions may reflect their geographic isolation. In the Umfolozi Game Reserve in Natal in the Republic of South Africa, some 250 km south of the KNP, an initial survey of 17 lions has given no evidence of infection using the CITE Combo kit to detect FIV-reactive antibodies (J. A. Spencer, unpublished data). The lion population in this Reserve, now numbering 60, was originally started from 3 lions derived from the KNP in 1963/64. Provided that the animals that have been assayed from the Umfolozi Game Reserve reflect the whole population, and that the data from the CITE Combo kit are confirmed by the p24-based ELISA (since intermediate or low titered sera may score negative using the kit), it is possible that either the KNP lions

were not infected with the FIV-related agent in 1963/64, or that by chance uninfected KNP lions were selected to establish the Umfolozi Game Reserve. Further studies are required to investigate these issues.

There is no evidence to suggest that the estimated 1 500 lions in the KNP are unduly subject to disease, as might be expected if they are suffering from a FIV-like induced immunodeficiency. Further work is required to address this issue, to isolate the etiological agent and to establish its taxonomic status in relation to other viruses (retroviruses, lentiviruses, FIV, etc.), as well as to determine if infection affects the immune status of the animals.

Recently, during 1992, two leopards captured in the KNP have tested FIV seropositive with both the p24-based and the CITE Combo ELISA (unpublished observation, A. A. van Dijk and D. F. Keet).

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