

## FELINE IMMUNODEFICIENCY VIRUS INFECTION IN NONDOMESTIC FELIDS

Margaret C. Barr, D.V.M., Paul P. Calle, V.M.D., Melody E. Roelke, D.V.M., and  
Fred W. Scott, D.V.M., Ph.D.

**Abstract:** Feline immunodeficiency virus (FIV) infection has been detected by serologic methods in several species of nondomestic felids, both in captive and in free-ranging populations. Antibody to FIV was detected using an enzyme-linked immunosorbent assay (ELISA) test; positive tests were confirmed by indirect immunofluorescence assay (IFA) and immunoblot procedures. Infected animals included two snow leopards (*Panthera uncia*), one lion (*P. leo*), and one jaguar (*P. onca*) from the Cheyenne Mountain Zoo, and one white tiger (*P. tigris*) and one lion from a separate facility. Six free-ranging Florida panthers (*Felis concolor coryi*) and one bobcat (*F. rufus floridanus*) from the Everglades National Park, Big Cypress National Preserve and nearby areas in southern Florida were also found to be infected. A tentative correlation between FIV seropositivity and clinical disease could be made in some of these animals; the presence of FIV infection thus may complicate the already difficult management situation of several endangered cat species.

**Key words:** Feline immunodeficiency virus, FIV, nondomestic felids, lentivirus, retrovirus.

### INTRODUCTION

Feline immunodeficiency virus (FIV) is a pathogen of domestic cats (*Felis catus*) that causes an immunodeficiency-like syndrome,<sup>9,14,18,19</sup> usually after months to years of clinically inapparent infection. The virus has been classified as a retrovirus belonging to the lentivirus subfamily based on its magnesium-dependent reverse transcriptase activity, the characteristic morphology of virions on electron micrographs, and the lengthy period of clinical latency following initial infection.<sup>14</sup> Recent serologic surveys have identified FIV antibodies in 10.2%,<sup>16</sup> 14%,<sup>18</sup> and 43.9%<sup>10</sup> of clinically ill domestic cats and in 1.4%,<sup>16</sup> 1.2%,<sup>18</sup> and 12.4%<sup>10</sup> of healthy domestic cats.

Several viruses of domestic cats are known to infect the nondomestic felids. Infection with feline infectious peritonitis virus (a coronavirus) has had devastating effects on a

captive cheetah (*Acinonyx jubatus*) population since the early 1980's,<sup>6</sup> and has been shown to infect some other species of exotic cats.<sup>8</sup> Feline panleukopenia virus (a parvovirus), feline viral rhinotracheitis virus (a herpesvirus), and feline calicivirus are all considered pathogens of most exotic felids.<sup>8</sup> Transient infections with feline leukemia virus (FeLV), a retrovirus belonging to the oncovirus subfamily, have been reported in a captive cheetah,<sup>1</sup> a clouded leopard (*Panthera nebulosa*),<sup>4</sup> and a western cougar (*Felis concolor*),<sup>12</sup> but persistent viremia has not been documented in the exotic cats. A positive enzyme-linked immunosorbent assay (ELISA) for FeLV in a Florida panther (*F. concolor coryi*) has been demonstrated to be a false positive caused by vaccine-induced anti-mouse activity,<sup>11</sup> and some of the other FeLV-positive tests may have been caused by the same type of reaction. Feline syncytium-forming virus, a retrovirus belonging to the spumavirus subfamily, apparently infects some of the nondomestic *Felis* members (Roelke, Gaskin, and Evermann, unpubl.); no information is available for the *Panthera* species.

This paper presents serologic evidence of FIV infection in selected populations of both captive and free-ranging nondomestic felids.

From the Department of Veterinary Microbiology, Immunology, and Parasitology, and the Cornell Feline Health Center, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA (Barr, Scott); the Cheyenne Mountain Zoo, 4250 Cheyenne Mountain Zoo Road, Colorado Springs, Colorado 80906, USA (Calle); and the Florida Game and Fresh Water Fish Commission, Wildlife Research Laboratory, 4005 South Main Street, Gainesville, Florida 32601, USA (Roelke).

## MATERIALS AND METHODS

### Serum samples

Samples from the Cheyenne Mountain Zoo (CMZ) were banked sera that had been stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  for various lengths of time ranging from  $<1$  wk to 5 yr. Most of the samples had been subjected to one freeze-thaw cycle, and some of the older samples had undergone two accidental freeze-thaw cycles. Serum samples in the Cornell Feline Health Center's bank had been stored at  $-20^{\circ}\text{C}$  for up to 8 yr. Sera from the Florida panthers and bobcats were collected during 1986-1988 and were kept frozen at  $-20^{\circ}\text{C}$ . Most of these samples were subjected to at least two freeze-thaw cycles. Several animals had multiple sampling dates but only one sample per animal was included in Tables 1 and 2.

### Enzyme-linked immunosorbent assay (ELISA)

The ELISA (PetChek FTLV Antibody Test, IDEXX, 100 Fore Street, Portland, Maine 04101, USA)<sup>13</sup> was run and interpreted according to the manufacturer's specifications. The sample:positive ratio was calculated as follows: the corrected sample optical density (O.D.) (sample O.D. minus negative control O.D.) was divided by the corrected positive O.D. (positive control O.D. minus negative control O.D.). A sample/positive value  $\geq 0.5$  was considered positive for FIV antibody. Sample/positive values between 0.3 and 0.5 were scored as equivocal.

### Indirect immunofluorescence assay (IFA)

The IFA was performed according to standard procedures<sup>3</sup> using methanol- and acetone-fixed FIV-infected (FIV-Petaluma strain,<sup>14</sup> provided by Dr. N. C. Pedersen, University of California, Davis, California 95616, USA) feline fetal cord blood cells (FCBC's,  $1 \times 10^4$  cells/well) as the substrate. Serum samples, diluted 1:50 or 1:25 in phosphate-buffered saline (PBS), were incubated with the cells for 1 hr at  $37^{\circ}\text{C}$ . After

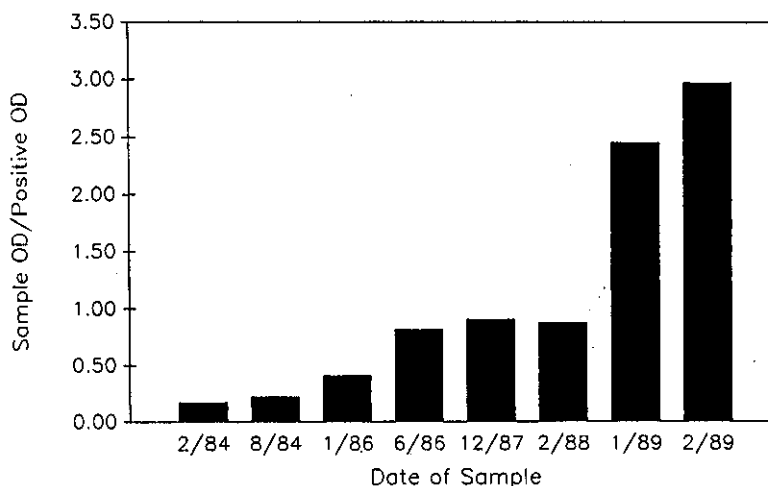
washing the slides with PBS containing 0.1% bovine serum albumin, fluorescein isothiocyanate-labeled goat anti-cat immunoglobulin G was added. The slides were incubated for 30 min at  $37^{\circ}\text{C}$ , washed, and negatively stained with dilute Evan's blue solution. Each serum was run against uninfected FCBC's as a control for nonspecific reactivity. Negative control serum from an uninfected specific pathogen free (SPF) cat and positive control serum from an SPF cat experimentally infected with FIV were also run.

### Immunoblot (Western blot) assay

The immunoblot assay was performed using described techniques.<sup>17</sup> Supernatants from FIV-Petaluma-infected FCBC's were harvested when reverse transcriptase activity reached 40,000-50,000 counts/min/ $10 \mu\text{l}$  aliquot, clarified at 3,000 g for 1 hr, pelleted through a 20% (w/v) sucrose cushion at 130,000 g for 4 hr, and resuspended in 1/100 volume PBS. After electrophoresis on a sodium dodecyl sulfate (SDS)/10% polyacrylamide gel, the proteins were transferred to a nitrocellulose membrane, incubated with serum (1:50 dilution) for a minimum of 1 hr, washed thoroughly, and incubated with horseradish peroxidase-conjugated rabbit anti-cat immunoglobulin G (1:2,000 dilution) for 2 hr. The blot was developed using a solution containing 4-chloro-1-naphthol and hydrogen peroxide. The presence of two or more virus-specific bands was scored as a positive test. Development of a single band was interpreted as equivocal, with a high probability that the sample was positive.

## RESULTS

Serologic evidence of FIV infection in cats of the genus *Panthera* was first detected in an 8-yr-old male snow leopard (*P. uncia*) at the CMZ. This male was bred to two females and sired two litters of cubs in 1988. The two cubs in the second litter were healthy, but the two cubs in the first litter developed clinical signs of paresis progress-



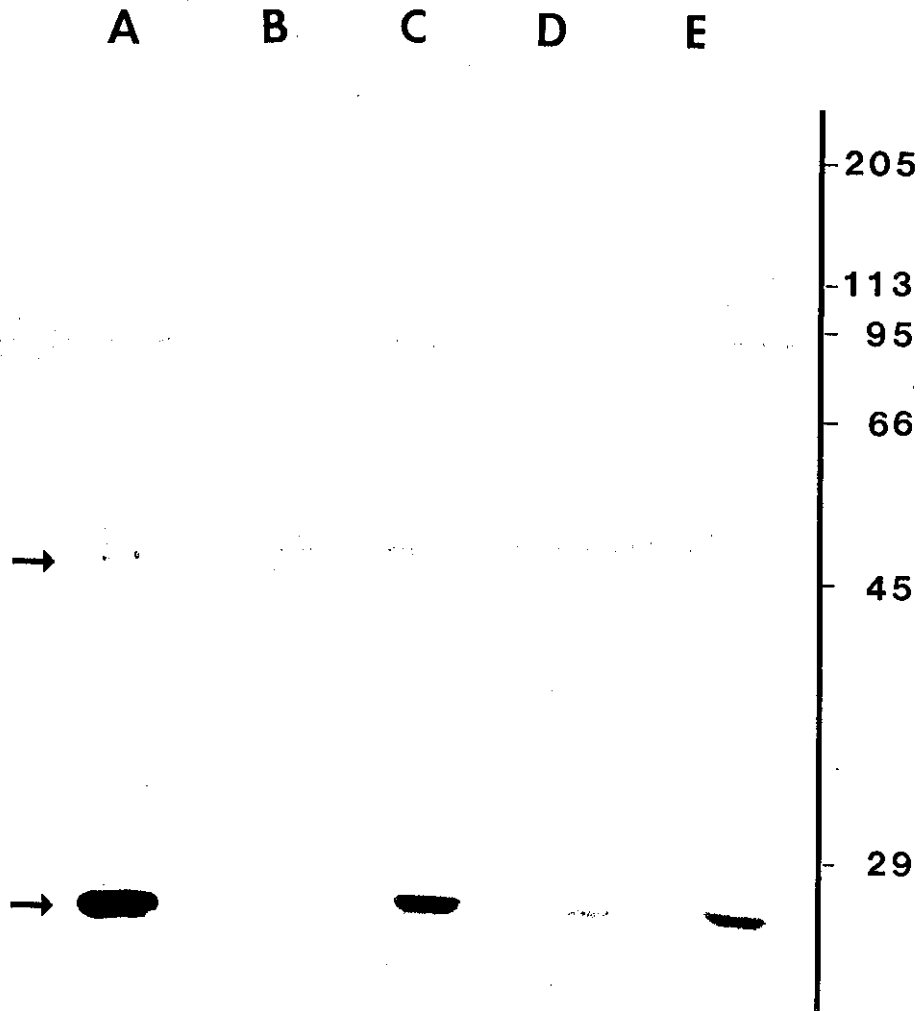
**Figure 1.** Enzyme-linked immunosorbent assay (ELISA) detection of feline immunodeficiency virus antibody in serum samples from an 8-yr-old male snow leopard (*Panthera uncia*). The samples were obtained at random time points over a period of 5 yr. A sample O.D./positive O.D. value of  $\geq 0.5$  is considered to be positive for FIV.

ing to paralysis at 5 and 6 mo of age. The cause of illness was determined to be a fungal infection of the spinal canal and epaxial muscles,<sup>2</sup> which was unresponsive to treatment. Because of the unusual nature of the illness, an underlying immunodeficiency was suspected but was not confirmed. Serum samples collected in 1988 from both parents of these cubs were tested for FeLV and FIV early this year in preparation for the new breeding season. The female leopard had no serologic evidence of infection with either of these viruses. Serum from the male tested positive for FIV antibody using an ELISA test (CITE FTLV Antibody Test, IDEXX, 100 Fore Street, Portland, Maine 04101, USA), and this serum was submitted to the New York State Diagnostic Laboratory for confirmation. The serum was weakly positive by the ELISA test (PetChek FTLV Antibody Test) but was negative by IFA and had only one very faint virus-specific band on immunoblot. Serum drawn in January 1989 was then submitted for testing, as were several other samples of stored sera from the same cat (Fig. 1). The 1989 sample was positive for FIV antibody by ELISA and IFA, and two FIV-specific bands were rec-

ognized by the immunoblot procedure (Fig. 2).

Sera from the CMZ serum bank, representing an additional 49 exotic cats, were submitted for FIV testing (Table 1). Samples from the second mate of the positive snow leopard and her cubs were negative on ELISA. Another male snow leopard, a male lion (*P. leo*), and a female jaguar (*P. onca*) were identified as positive for FIV antibody by ELISA. The IFA on the snow leopard and lion sera was positive, as was immunoblot detection of FIV-specific bands (Fig. 2). The IFA on the jaguar's serum was negative at the 1:50 dilution but appeared very weakly positive at the 1:25 dilution. One faint band was detected on immunoblot analysis.

Once evidence of antibody to FIV was found in the CMZ population, a survey was performed on sera banked at the Cornell Feline Health Center from five additional zoo populations. No positive samples were found in sera drawn in 1982–1983 from 18 sand cats (*F. margarita*), three lynx (*F. lynx lynx*), and one fishing cat (*F. viverrinus*) from four of the populations. However, sera drawn in 1987 and 1988 from a lion and a



**Figure 2.** Immunoblot detection of feline immunodeficiency virus (FIV) antibody in serum samples from exotic felids: A) experimentally infected specific pathogen free (SPF) domestic cat (*Felis catus*), B) snow leopard (*Panthera uncia*), C) lion (*P. leo*), D) Florida panther (*F. concolor coryi*), and E) bobcat (*F. rufus floridanus*). Arrows (→) indicate position of FIV-specific bands. No specific bands were detectable with serum from an uninfected SPF cat (data not shown). Positions of molecular weight markers are indicated by the numbers on the right.

young white tiger (*P. tigris*) from the fifth captive population were found to be positive for FIV antibody.

Infection by FIV of free-ranging non-domestic felids was also demonstrated. Sera

from 20 Florida panthers captured during 1986–1988 were tested (Table 2). Three of these samples were positive for FIV antibody by ELISA, IFA, and immunoblot (Fig. 2). An additional three animals were either

**Table 1.** Feline immunodeficiency virus (FIV) infection in captive exotic felids at the Cheyenne Mountain Zoo (1984–1989 sampling dates).

Species	No. animals tested	FIV positive samples <sup>a</sup>		
		ELISA	IFA	Immunoblot
Clouded leopard ( <i>Panthera nebulosa</i> )	7	0	0	0
Ceylon leopard ( <i>P. pardus fusca</i> )	1	0	0	0
African leopard ( <i>P. pardus</i> )	2	0	0	0
Snow leopard ( <i>P. uncia</i> )	17	2	2	2
Jaguar ( <i>P. onca</i> )	2	1	1	1 <sup>b</sup>
Lion ( <i>P. leo</i> )	6	1	1	1
Siberian tiger ( <i>P. tigris altaica</i> )	5	0	0	0
Sumatran tiger ( <i>P. tigris sumatrae</i> )	5	0	0	0
Mountain lion ( <i>Felis concolor hippolestes</i> )	4	0	0	0
Bobcat ( <i>F. rufus pallescens</i> )	2	0	0	0
Total	51	4	4	4

<sup>a</sup> ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence assay.

<sup>b</sup> The jaguar sample was weakly positive by ELISA (1:100 dilution) and IFA (1:25 dilution). The sample was interpreted as equivocal on immunoblot, with one faint virus-specific band visible.

positive or equivocal for FIV by ELISA, were positive by IFA at the 1:25 dilution, and were interpreted as equivocal (one band only) on immunoblot analysis. Sera from 11 bobcats (*F. rufus floridanus*) trapped in the same general area of Florida as the panthers were also tested (Table 2). One bobcat was strongly positive on ELISA and IFA, and reacted with two FIV proteins on immunoblot (Fig. 2).

#### DISCUSSION AND CONCLUSIONS

Detection of FIV antibody in domestic cats is highly correlated with FIV infection.<sup>18</sup> The presence of antibody to FIV in captive exotic cats raises the question of how these animals became infected. Feline immunodeficiency virus appears to be difficult to spread by casual contact among domestic

cats; bite wounds are implicated as the most efficient means of transmission.<sup>18</sup> In the CMZ population, none of the infected animals had contact with any of the other infected cats or with free-roaming domestic cats. Medical supplies such as syringes and needles were sterile products. The 1984 serum samples from the snow leopards and the jaguar were negative for FIV; the virus may have been introduced into this population by the acquisition of the positive lion from another institution in 1985, but no method of spread has been identified.

The CMZ exotic cats were routinely vaccinated for feline viral rhinotracheitis, feline calicivirus, and feline panleukopenia virus with an inactivated vaccine product (Fel-O-Vax, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501, USA). It is possible

**Table 2.** Feline immunodeficiency virus (FIV) in free-ranging exotic felids (1986–1988 sample dates).

Species	No. animals tested	FIV positive samples <sup>a</sup>		
		ELISA	IFA	Immunoblot
Florida panther ( <i>Felis concolor coryi</i> )	20	6	6	6 <sup>b</sup>
Bobcat ( <i>F. rufus floridanus</i> )	11	1	1	1
Total	31	7	7	7

<sup>a</sup> ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence assay.

<sup>b</sup> Three of the six samples were positive by all three tests. The remaining three had equivocal (one band only) immunoblots, with either positive or equivocal ELISA tests, and positive IFA results at the 1:25 dilution.

that contamination of such a vaccine with FIV proteins could lead to seroconversion without true infection. Antigenic analysis of the vaccine or isolation of FIV from seropositive exotic cats would be required to rule out this possibility; however, a higher number of seropositive animals would be expected if temporary reactivity to FIV were vaccine induced.

The presence of antibody to FIV in wild unvaccinated felids indicates that the virus can infect some nondomestic cats. Although two of the Florida panthers had been captured, vaccinated, and released prior to the sampling dates for their sera, four of the panthers tested positive for FIV on their initial capture date. The FIV-positive bobcat also was unvaccinated and had no previous human contact.

Spread of FIV among wild felids probably is very similar to spread among domestic cats. The high prevalence (30%) of FIV in the sampled Florida panther population may be attributed to one or more factors. The extremely low number (estimated to be <50) of animals in this population may actually facilitate more efficient spread of the virus during the breeding season. One infected female identified in the study is the dam of two other positive cats. This finding suggests that FIV may be spread efficiently from mother to offspring, possibly through accidental bite wounds or grooming; transmission of FIV through colostrum or in utero has not been documented in the domestic cat,<sup>18</sup> but studies have not been extensive enough to be conclusive. Finally, the panther may be genetically predisposed to FIV infection, or the strain of FIV infecting these animals may be more easily transmitted than most domestic cat strains.

The nonspecific and varied clinical signs associated with FIV infection in the domestic cat make it difficult to substantiate a correlation between infection and clinical disease in exotic felids. Gingivitis, dermatitis, and anemia are frequently reported in FIV-infected domestic cats,<sup>10,14,18</sup> but these signs are also seen in the absence of FIV

infection. One of the FIV-positive snow leopards has had chronic dental disease and ingrown nails, while the jaguar has been chronically anemic and has suffered from recurrent foot pad dermatitis with plasmacytic infiltrates. It should be noted that dental disease has been documented in several snow leopards from the CMZ,<sup>5</sup> and sera from most of these animals have been included in the survey summarized in Table 2. The male snow leopard represented in Figure 1 has been relatively healthy during the 3 yr since he seroconverted to FIV. His two cubs with spinal abscesses may have had an underlying immunodeficiency, but FIV infection could not be documented by serology and tissues were not available for virus isolation. In addition, the dam of the litter is seronegative for FIV and has had no significant health problems. The clinical status of the two lions and the white tiger is unknown.

One Florida panther was chronically thin and anemic but responded well to therapy during a period of captivity. Despite periodic associations with males, assumed to be normal breeding activity, she was nonreproductive during 4 yr of observation in the wild. Her death in mid-1988 was attributed to renal failure and adenomatous hyperthyroidism. A second female panther has been similarly nonreproductive for 2 yr although she appears to be healthy otherwise. Because reproductive failures have been noted in some FIV-positive domestic cats,<sup>10,14</sup> the nonreproductive status of these two females may be related to virus infection. However, a third positive female has maintained a relatively normal reproductive pattern. Hookworm infection,<sup>7</sup> chronic anemia and weight loss,<sup>15</sup> and gingivitis have been observed in some panthers, but direct correlation of these problems with FIV infection has not been demonstrated.

Most species of exotic felids are classified as threatened or endangered. With the increased emphasis on cooperative captive breeding programs for these animals, the possibility of FIV introduction and spread

among institutions must be considered. The methods of transmission of FIV within captive populations must be identified before an effective control program can be instituted. Until more information is available, screening for FIV should be included in the tests run before an animal is transferred to a different institution. Dissemination of information in the scientific literature and open communication among participating institutions regarding potentially infectious disease agents, such as FIV, are needed for the continued successful management of captive endangered felids.

The impact of FIV infection in the Florida panther population is difficult to assess. Environmental pressures have been responsible for much of the decrease in the numbers of panthers in southern Florida. Infectious diseases, however, may play an escalating role in the endangerment of these animals as the environmental concerns are addressed.

*Acknowledgments:* We thank Margaret Pough, New York State Diagnostic Laboratory, for assisting with the ELISA tests and James Williams, New York State College of Veterinary Medicine, for technical assistance. Partial funding for this project was provided by a U.S. Fish and Wildlife Service endangered species grant.

#### LITERATURE CITED

1. Briggs, M. B., and R. L. Ott. 1986. Feline leukemia virus infection in a captive cheetah and the clinical and antibody response of six captive cheetahs to vaccination with a subunit feline leukemia virus vaccine. *J. Am. Vet. Med. Assoc.* 189: 1197-1199.
2. Calle, P. P., S. B. Colter, R. A. Taylor, and A. M. Wright. 1989. Extramedullary thoracolumbar fungal (*Scopulariopsis brumptii*) abscesses in two snow leopard (*Panthera uncia*) littermates. *J. Zoo Wildl. Med.* 20(3): 346-353.
3. Carlson, J. R., J. Yee, S. H. Hinrichs, M. L. Bryant, M. B. Gardner, and N. C. Pedersen. 1987. Comparison of indirect immunofluorescence and Western blot for detection of anti-human immunodeficiency virus antibodies. *J. Clin. Microbiol.* 25: 494-497.
4. Citino, S. B. 1986. Transient FeLV viremia in a clouded leopard. *J. Zoo Anim. Med.* 17: 5-7.
5. Cook, R. A., and N. H. Stoller. 1986. Periodontal status in snow leopards. *J. Am. Vet. Med. Assoc.* 189: 1082-1083.
6. Evermann, J. F., J. L. Heaney, M. E. Roelke, A. J. McKeirnan, and S. J. O'Brien. 1988. Biological and pathological consequences of feline infectious peritonitis virus infection in the cheetah. *Arch. Virol.* 102: 155-171.
7. Forrester, D. J., J. A. Conti, and R. C. Belden. 1985. Parasites of the Florida panther (*Felis concolor coryi*). *Proc. Helminthol. Soc. Wash.* 52: 95-97.
8. Fowler, M. E. 1986. Carnivora: Felidae. In: Fowler, M. E. (ed.). *Zoo and Wild Animal Medicine*, 2nd ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 831-841.
9. Ishida, T., T. Washizu, K. Toriyabe, and S. Motoyoshi. 1988. Detection of feline T-lymphotropic lentivirus (FTLV) infection in Japanese domestic cats. *Jpn. J. Vet. Sci.* 50: 39-44.
10. Ishida, T., T. Washizu, K. Toriyabe, S. Motoyoshi, I. Tomoda, and N. C. Pedersen. 1989. Feline immunodeficiency virus infection in cats of Japan. *J. Am. Vet. Med. Assoc.* 194: 221-225.
11. Lopez, N. A., and R. H. Jacobson. 1989. False positive reactions associated with anti-mouse activity in serotests for feline leukemia virus antigen. *J. Am. Vet. Med. Assoc.* In press.
12. Meric, S. M. 1984. Suspected feline leukemia virus infection and pancytopenia in a western cougar. *J. Am. Vet. Med. Assoc.* 185: 1390-1391.
13. O'Connor, T. P., S. Tanguay, R. Steinman, R. Smith, M. C. Barr, J. K. Yamamoto, N. C. Pedersen, P. R. Andersen, and Q. J. Tonelli. 1989. Development and evaluation of immunoassay for detection of antibodies to the feline T-lymphotropic lentivirus (feline immunodeficiency virus). *J. Clin. Microbiol.* 27: 474-479.
14. Pedersen, N. C., E. W. Ho, M. L. Brown, and J. K. Yamamoto. 1988. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235: 790-793.
15. Roelke, M. E., E. R. Jacobson, G. Z. Kollias, and D. J. Forrester. 1985. Medical management and biomedical findings on the Florida panther (*Felis concolor coryi*). *Annu. Perf. Rep.* 1984/85. Florida Game and Fresh Water Fish Commission, Tallahassee, Florida.
16. Shelton, G. H., R. M. Waltier, S. C. Connor, and C. K. Grant. 1989. Prevalence of feline immunodeficiency virus and feline leukemia virus infections in pet cats. *J. Am. Anim. Hosp. Assoc.* 25: 7-12.
17. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76: 4350-4354.
18. Yamamoto, J. K., H. Hansen, E. W. Ho, T. Y. Morishita, O. Takiko, T. R. Sawa, R. M. Nakamura, and N. C. Pedersen. 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada

and possible mode of transmission. *J. Am. Vet. Med. Assoc.* 194: 213-220.

19. Yamamoto, J. K., E. Sparger, E. W. Ho, P. R. Andersen, T. P. O'Connor, C. P. Mandell, L. Lowenstein, R. Münn, and N. C. Pedersen. 1988. Patho-

genesis of experimentally induced feline immunodeficiency virus infection in cats. *Am. J. Vet. Res.* 49: 1246-1258.

*Received for publication 30 March 1989.*