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Feline Immunodeficiency Virus and Puma Lentivirus in Florida panthers (*Puma concolor coryi*): Epidemiology and Diagnostic Issues

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ABSTRACT

This study documents the seroprevalence of feline immunodeficiency virus (FIV) and puma lentivirus (PLV) in free-ranging and captive Florida panthers (*Puma concolor coryi*) (n = 51) and translocated Texas cougars (*P. concolor stanleyana*) (n = 10) from 1985 to 1998. The sera were tested for anti-FIV antibodies by enzyme-linked immunosorbent assay (ELISA) and Western blot tests. The ELISAs were read kinetically (KELA) and the sera were retrospectively examined by PLV peptide ELISA. Eleven panthers and one cougar were positive by KELA; 4 panthers and 4 cougars were equivocal; 35 panthers and 5 cougars were negative; and 1 panther had no data. Seven of the 11 KELA-positive panthers were also positive by Western blot tests and all but one were positive by PLV peptide ELISA. Ten KELA-negative and Western blot-negative cats, were positive by PLV peptide ELISA. KELA results varied within cats from one sample period to the next, but PLV peptide ELISA results were consistent. Territorial sympatry and mating behaviour, noted from radiotelemetry location data on the cats, may have contributed to viral transmission between seropositive animals. These findings suggest that Florida panthers and the introduced Texas cougars have been exposed to FIV and/or PLV.

Keywords: cougar, feline immunodeficiency virus, FIV, Florida panther, KELA, puma lentivirus, PLV, peptide ELISA

Abbreviations: CBC, complete blood count; ELISA, enzyme-linked immunosorbent assay; FIV, feline immunodeficiency virus; FP, Florida panther; KELA, kinetic ELISA; PLV, puma lentivirus; RBC, red blood cell count; TX, Texas cougar; WBC, white blood cell count

INTRODUCTION

The Florida panther (*Puma concolor coryi*) was listed as an endangered species in 1967 and, since that time, research efforts to learn about this species and the potential threats it faces have broadened and intensified (Belden, 1986). Currently, only 30–50 individuals are thought to remain in the wild and, in an attempt to increase genetic diversity, eight female free-ranging cougars (*P. c. staneleyana*) from Texas were translocated into several areas of southern Florida in 1995 (Seal, 1994; Dunbar *et al.*, 1997).

Infectious diseases are a concern because individuals from genetically inbred populations have the potential to be immunosuppressed (Brown *et al.*, 1993). *Feline immunodeficiency virus* (FIV) and *Puma lentivirus* (PLV) are single-stranded RNA retroviruses of the genus *Lentivirus* and the family *Retroviridae* (Olmsted *et al.*, 1992). They are closely related, but enough sequence variation exists to suggest limited interspecies transmission among the felidae (Carpenter *et al.*, 1996; Olmsted *et al.*, 1992). FIV-like lentiviruses have been reported to infect both domestic cats (*Felis cati*) and non-domestic felids including lion (*Panthera leo*), cheetah (*Acinonyx jubatus*), puma (P. *concolor*), bobcat (*Lynx rufus*) and flat-headed cat (*Ictailurus planiceps*); while PLV has been demonstrated to infect pumas (Barr *et al.*, 1989; Carpenter *et al.*, 1996; Olmsted *et al.*, 1992; Kania *et al.*, 1997). There is great genetic diversity in the FIV infecting pumas and multiple viral subtypes are thought to exist among Florida panthers, with the possibility of co-infectivity with multiple subtypes (Carpenter *et al.*, 1996).

In domestic felids, the clinical signs of FIV infection are classified into an acute primary phase (transient fever, neutropenia and generalized lymphadenopathy), an asymptomatic latent phase (no signs and variable in duration), and a chronic terminal phase (progressive weight loss, recurrent bacterial infections, fever, generalized lymphadenopathy, persistent/recurrent anemia or leukopenia, neoplasia, opportunistic infections and encephalopathy) (Sherding, 1994). Abnormal physical findings in the oral cavity or head region are the most common manifestations of clinical disease caused by FIV (Sherding, 1994). Feline immunodeficiency virus has been shown to be shed in saliva, and transmission can occur via bite wounds; however, vertical transmission can occur during the prenatal period (*in utero* during acute maternal infection) or the postnatal period (via colostrum or milk-borne cell-associated virus) (Brown *et al.*, 1993).

The kinetic ELISA (KELA) diagnostic method has been applied to felid serum analysis since 1983 and is currently used by the New York State Diagnostic Laboratory at Cornell University (Ithaca, NY, USA) for FIV serological analysis in domestic and non-domestic felids. The KELA method is based on the principle that the amount of analyte (antibody) detected is directly proportional to the slope of the reaction rate between enzyme and substrate, which is visibly detected by the production of a chromogenic coloured product (Barlough *et al.*, 1983). The KELA reading is calibrated to reduce both within-run and between-run variability (Barlough *et al.*, 1987), as well as operator error or incorrect interpretation of the in-house ELISA (Barr *et al.*, 1991). Barr and colleagues (1991) found that, in domestic cats, the sensitivity of

FIV ELISA was 0.93 and its specificity was 0.98, but the sensitivity and specificity were reduced to 0.79 and 0.66, respectively, for KELA equivocals, thus emphasizing the need for this classification (equivocal) to reduce the number of false reports.

The peptide ELISA is similar to Western blot testing in sensitivity and specificity, but with the added capability of discriminating between antibodies to FIV and antibodies to PLV (Kania *et al.*, 1997). This test uses a synthetic peptide identical to a region of the PLV envelope protein for detecting PLV-specific antibodies in serum samples (Langley *et al.*, 1994; Kania *et al.*, 1997). The peptide was designed to correspond with the conserved envelope region present on retroviruses, thus allowing for differentiation between the retroviruses (Kania *et al.*, 1997). A Florida panther sequence was included in the sequences used by Kania and colleagues (1997) to develop the PLV peptide. The Florida panther sequence used was that from Florida panther 21 (FP21) (M. Roelke, unpublished data).

The purpose of this retrospective study was to determine the prevalence of FIV/PLV seroreactors in free-ranging and captive Florida panthers and the Texas cougars introduced into southern Florida.

METHODS

Animals

The study population included 48 (22 male; 26 female) free-ranging and 3 (male) captive Florida panthers ranging from 6 months old to 16 years old and 10 free-ranging Texas cougars (6- to 8-year-old females) translocated from Texas to southern Florida in 1995. It is presumed that, similarly to domestic felines (Hoover *et al.*, 1977; Spencer and Burroughs, 1992; Ueland and Nesse, 1992), at 6 months of age maternal antibodies have declined to undetectable levels and therefore would not affect testing. Serum samples were collected from 1985 to 1998, from southern Florida (south of 27° 00′N). Free-ranging panthers and Texas cougars were captured at 2- to 3-year intervals and samples were collected as previously described (Taylor, 1997).

Sample collection

Blood samples were collected from the jugular vein with a 21-gauge butterfly catheter using a 7 or 10 ml clot tube and a 1.5 ml EDTA-coated tube. The serum was separated by centrifugation and aliquoted into batches for immediate testing or archived as aliquots to be frozen at –20°C. Aliquots of 0.5–1.0 ml of each serum sample and the whole EDTA specimen were shipped overnight on ice to the New York State Diagnostic Laboratory at Cornell University for serological testing and complete blood cell counts (CBC) and differential blood cell counts. Occasionally, samples were shipped to the New York State Diagnostic Laboratory via the Cornell University Feline Health Center. During 2002–2003, 0.5–1.0 ml portions of the banked frozen serum samples were shipped overnight on ice to the University of Georgia Tifton

Veterinary Diagnostic and Investigational Laboratory (Tifton, GA, USA) for retrospective PLV testing.

KELA and Western blot

Anti-FIV antibodies were detected using a commercial ELISA test kit (PetChek, IDEXX, Portland, ME, USA) and laboratory-based Western blotting. The ELISAs for FIV were performed with the commercial kit, read kinetically (3 reads, 2 min apart), and interpreted manually using the kinetic slope and the standard/positive ratio recommended by the manufacturer. The samples were then scored as negative, positive, or equivocal on the basis of chromatogenicity observations. Western blot analysis, using domestic cat and/or non-domestic Pallas cat FIV proteins as antigens, was performed as described by Barr and colleagues (1989) only to confirm or clarify test results when ELISA values were equivocal and rarely as opportunistically feasible. Only domestic cat FIV proteins were available for use in Western blot analysis prior to 1990 but both domestic cat and Pallas cat FIV proteins were available for use after 1990 (Barr *et al.*, 1995, 1997). A positive Western blot was defined as having reactivity with two or more protein bands, whereas an equivocal result had reactivity with only one band, usually p26 or p17. For this study's data analysis, animals were documented as positive if they had a positive test at any one sampling.

PLV peptide ELISA

PLV analysis was conducted by peptide ELISA following the protocol used by Kania and colleagues (1997) with some modifications. Briefly, a PLV envelope peptide, CPFKDICQL, corresponding to amino acids 610–618 of the PLV Env, was obtained from a commercial laboratory (Bio-Synthesis, Inc., Lewisville, TX, USA). Immulon 2 plates were coated with 100 μl of the peptide overnight at 4°C. The plates were then washed four times with PBS containing 0.05% Tween-20 (PBSTW). After the last wash, 100 μl of blocking solution (PBS with 1% milk and 0.01% Tween) was added and the plates were incubated for 1 h at 37°C. The plate was then washed four times in PBSTW before adding 100 μl of goat anti-cat (KPL) antibody diluted 1:300 in PBSTW followed by incubation for 1 h at 37°C. After four washes, 100 μl of ABTS substrate was added and, after approximately 1 min, the reaction was stopped by adding 100 μl of stop solution. The plate was read at 405 nm.

Statistical analysis

An age- and sex-matched comparison was conducted to determine potential virus-associated haematologicsl changes in the following parameters: white blood cell count (WBC) (cells/μl), red blood cells (RBC) (number of red cells), and absolute neutrophil and lymphocyte counts (cells/μl). Values were obtained from a retrospective analysis of

medical records. Student's *t*-test was done to compare the parameters using the In-Stat program (GraphPad InStat version 3.00, GraphPad Software, San Diego, CA, USA).

RESULTS

Based on KELA results, 34.4% (20/61) of the animals (Florida panthers and Texas cougars) were seroreactors (positive or equivocal) for FIV antibodies (Table I). KELA test results for panthers and cougars, respectively, were 11 (21.6%) and 1 (10.0%) positive; 4 (7.8%) and 4 (40.0%) equivocal; 35 (68.6%) and 5 (50.0%) negative; and 1 (2.0%) and 0 (0.0%) had no data available. Western blot test results for panthers and cougars, respectively, were 7 (13.7%) and 3 (30.0%) positive; 5 (9.8%) and 2 (20.0%) equivocal; 18 (35.3%) and 3 (30.0%) negative; and 21 (41.2%) and 2 (20.0%) had no data available. PLV peptide ELISA test results for panthers and cougars, respectively, were 20 (39.2%) and 4 (40.0%) positive; 0 (0.0%) and 0 (0.0%) equivocal; 27 (53.0%) and 3 (30.0%) negative; and 4 (7.8%) and 3 (30.0%) not tested.

The KELA and peptide ELISA test results were similar except for 17 Florida panthers (FP21, FP26, FP28, FP34, FP36, FP38, FP40, FP42, FP45, FP48, FP50, FP52, FP56, FP57, FP58, FP65, FP205) and 4 Texas cougars (TX02, TX104, TX106, TX107) (Table I). FP21, TX104, TX106 and TX107 had equivocal results by KELA but consistently positive results by peptide ELISA. FP26, FP36 and FP40 had variable (sometimes positive and sometimes equivocal) results by KELA but consistently positive results by peptide ELISA. FP28 had positive results by KELA (confirmed by Western blot) but consistently negative results by peptide ELISA. FP34, FP38, FP48, FP50, FP52, FP56, FP57, FP58, FP65 and FP205 had negative results by KELA and/or Western blot but positive results by peptide ELISA. FP42 and FP45 had KELA positive results and then negative results in later years, but the peptide ELISA results were consistently positive and negative for FP42 and FP45, respectively. FP62, FP68 and TX02 had equivocal results by KELA but negative results by peptide ELISA.

There were no significant differences between seropositive and seronegative Florida panthers and Texas cougars with regard to white and red blood cell counts or absolute numbers of lymphocytes and neutrophils. The average (with standard deviation) white blood cell count (WBC) for seropositive panthers was $11\,260 \pm 3768$ cells/µl and for those seronegative was 9414 ± 3434 cells/µl. The mean ± standard deviation (SD) red blood cell count (RBC) for seropositive panthers was $7.98 \times 106 \pm 1.22$ cells/µl and for those seronegative was 7.74 ± 1.72 cells/µl. For the absolute neutrophil and lymphocyte counts, seropositive panthers had a mean ± SD of 7629 ± 2859/µl neutrophils and 2550 ± 1914/µl lymphocytes. Feline immunodeficiency virus-negative panthers had average (±SD) counts of 5998 ± 2454/µl neutrophils and 2556 ± 1288/µl lymphocytes.

TABLE I
FIV seroreactors test results[a] in free-ranging Florida Panthers (*Puma concolor coryi*) and Texas Cougars (*P. c. stanleyana*) from southern Florida from 1985 to 1998

Cat ID[b]	Sex	Year of birth	Sample dates	Western blot	KELA	PLV peptide ELISA
FP8	Female	1974–1976	1985	ND	N	N
			1986	E	P	P
			1987, 88	P	P	P
FP14	Female	1981–1982	1986	E	P	P
			1988	P	P	P
FP16	Male	1987	1987, 88, 92	P	P	P
			1990, 91	ND	P	P
			1994, 96, 97	E	P	P
FP18	Female	1977–1979	1987, 89	P	P	P
			1997	ND	N	ND
FP21	Female	1986	1987	E	E	P
			1988, 89	E	N	P
			1994	ND	N	P
			1995	E	E	ND
			1996, 97	N	E	P
FP25	Male	1984–85	1988	E	P	ND
FP26	Male	1983	1988, 94	E	E	P
			1990	ND	P	P
			1992	P	P	P
FP28	Male	1986	1991	P	P	N
			1992	ND	P	N
FP32	Female	1987	1989, 91	ND	N	N
			1995	N	N	N
			1997	N	E	P
FP34	Male	1989	1990	N	ND	N
			1991	ND	N	ND
			1993	N	ND	P
FP36	Female	1986	1990, 92	N	P	P
			1994	E	E	P
			1997	ND	N	P
FP38	Female	1986	1990, 92, 93	N	ND	P
FP40	Female	1988	1992	P	P	P
			1994, 96	E	E	P
			1997	E	E	ND
FP42	Male	1989	1990	ND	N	N
			1991	ND	P	P
			1993	ND	N	P
FP45	Male	1990	1991, 92	N	P	N
			1995, 97	N	N	N
			1998	N	N	ND

TABLE I (continued)

Cat ID[b]	Sex	Year of birth	Sample dates	Western blot	KELA	PLV peptide ELISA
FP48	Female	1992	1992	ND	N	N
			1993, 98	ND	N	P
			1995	N	N	ND
			1997	ND	N	ND
FP50	Male	1990	1992	ND	N	P
FP52	Female	1991	1992	ND	N	N
			1995	N	N	P
FP56	Female	1992	1994	N	N	P
			1997, 98	ND	N	ND
FP57	Female	1992	1995, 98	N	N	P
			1997	ND	N	ND
FP58	Male	1994	1995	N	N	N
			1996	ND	N	P
FP62	Male	1996	1997	E	E	N
			1998	ND	N	N
FP65	Male	1996	1997	ND	N	P
FP68	Male	1994	1998	N	E	N
FP204	Female	1990	1991, 94, 95	ND	N	N
			1996	E	N	N
			1997	N	N	N
FP205	Female	1990	1991, 92	ND	N	P
TX02	Female	1983	1997	P	E	N
TX104	Female	1991	1995	ND	N	N
			1997	E	E	P
TX105	Female	1991	1995	N	N	N
			1997	ND	N	P
			1998	P	P	ND
TX106	Female	1991	1995	ND	E	N
			1997	P	E	P
TX107	Female	1992	1997	E	E	P

[a]P, positive; E, equivocal; N, negative; ND, no data

[b]FP, Florida panther; TX, Texas cougar

DISCUSSION

The peptide ELISA test was performed retrospectively on all available sera from Florida panthers sampled in the period covered by this study (1985–1998). Unfortunately, in some cases, sera were no longer available for testing. In general, cats testing positive or equivocal by KELA, at any one of multiple sampling times, were positive by peptide ELISA, except for 5 cats that consistently tested negative by

peptide ELISA (Table I). This may reflect the specificity of the test for PLV versus FIV from domestic or other non-domestic cats. Kania and colleagues (1997) found that lentivirus-infected non-domestic felid species (cougar/Florida panther and Pallas cat) had strong reactivity with the PLV peptide but Panthera species (tiger, Siberian tiger and lion) did not. Further, Kania and colleagues (1997) found that sera from FIV-positive cats were not reactive with the PLV peptide. Therefore, cats with FIV may be negative on PLV peptide ELISA. Additionally, the Florida panther sequence used in the selection of the conserved peptide sequence was that of FP21. The only other Florida panther retrovirus sequenced is that of FP14 (mother of FP21), and the envelope sequences are identical (M. Roelke, unpublished data). Therefore high specificity of this test is not surprising. Presumptive cross-species transmission of domestic cat FIV to a puma has been documented by sequence analysis in at least one instance (Carpenter *et al.*, 1996), and there are opportunities for the Florida panthers to contact domestic cats and free-ranging bobcats in their habitats. Another possibility is genetic divergence of the infecting virus in the peptide region. The PLV peptide was identified from an envelope region that corresponds to a highly conserved FIV envelope region; however, the degree of conservation in this region in PLV isolates is not known. Diversity among PLV pol sequences is greater than the diversity seen in domestic cat isolates (Carpenter *et al.*, 1996), suggesting that the same may hold true for env sequences.

Several factors probably contribute to the high number of equivocal KELA and Western blot results seen in this study. The antigens used in both assays are from domestic cat FIV isolates (KELA and some Western blots) or from a non-domestic cat isolate (FIV-Oma) that differs somewhat from PLV. Neither source is the ideal antigenic substrate for testing for antibodies to PLV. Thus decreased antigenic cross-reaction might contribute to decreased reactivity in both assays. In addition, the Florida panthers tend to have much lower levels of antibodies produced in response to FIV infection, as compared to the response of domestic cats, and antibody levels appear to decline over time in some animals. This muted immune response to the virus contributes to the difficulty of obtaining consistently seropositive results.

Possible routes of transmission include external sources, mating, and territory sympatry. Historical records indicate that release of privately owned captive panthers occurred in one area. It is not possible to determine the role that release of such an animal (uncollared) that was also seropositive could have regarding spread of the virus. Additionally, free-ranging bobcats and feral domestic cats should be considered as potential sources of FIV infection.

Mating records provide a possible route for spread of the virus within one subset of seroreacting panthers. FP16 (a male Florida panther) had the longest duration of seropositivity and could have been a potential source for several animals. FP16 was the only positive panther that had recorded test results from <1 year of age. FP16 and its sibling FP21 were both positive by peptide ELISA at initial sample collection performed at <1 year and 1 year of age, respectively (Table I). The source of virus exposure in these siblings is uncertain but vertical exposure is possible given that the mother (FP14) was KELA and PLV positive at the time of the siblings births. Further, FP16 had an overlapping territory and mated (M. Roelke, unpublished data) with

TX105, a female Texas cougar, and may have served as the source of that cougar's positive test results. However, TX108, another mate of FP16, was negative by all tests, as was the offspring of TX108 and FP16 (FP61).

Territory sympatry was noted in two groups of seropositive cats: group 1 (FP26, FP28 and FP45 (all male Florida panthers) and FP40 (a female Florida panther)) and group 2 (TX104 (a female Texas cougar) and TX106 (a female Texas cougar) and Florida Panther FP32 (a female Florida panther)). Although territory was shared among group 1, test results do not suggest any pattern of transmission. Similarly, for group 2 the pattern is uncertain but it is interesting to note that all became seropositive around 1997, which may suggest a common source of exposure (Table I).

The potential population effects of FIV/PLV on the Florida panther remain unknown; however, one Florida panther captured in 2002 had concurrent FIV and feline leukaemia virus infections and died of an *E. coli* septicaemia (M. Cunningham, unpublished data). To date only two panthers have been documented as dying of infectious diseases, one a case of rabies and the second a case of pseudorabies (Glass *et al.*, 1994; Taylor, 1997; Taylor *et al.*, 2002). These had no observed FIV/PLV-related pathological lesions. Clinical changes consistent with FIV/PLV were not observed in any other seropositive cats and, of those cats, selected haematological parameters did not appear to differ between seropositive and seronegative panthers. In fact, the parameters for seropositive panthers were within established normal limits (Dunbar *et al.*, 1997). However, gross changes in haematological parameters are not commonly seen in FIV-infected cats, except in acute infection or in the final stages of disease (Hofmann-Lehmann *et al.*, 1997; Kohmoto *et al.*, 1998). Further investigation into lymphocyte ratios (CD4:CD8) as well as lymphocyte function could provide a more sensitive means of determining viral effects.

Although the IDEXX PetCHEK kit provides a quick, easy and inexpensive means for screening serum samples, the peptide ELISA appears to improve the detection of PLV antibodies. The limiting factors to the peptide ELISA are that the test is not commercially available, and the targeted peptide may not be conserved in all PLV serotypes, possibly leading to false-negative results. However, an equivocal reading on KELA was often associated with a positive PLV assay and thus warrants further investigation. Additionally, the Western blot assay may clarify results when KELA test results are equivocal. We suggest that testing with two or more assays and at multiple time points might give the most reliable test results in panthers.

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