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SEROPREVALENCE OF INFECTIOUS DISEASE AGENTS IN FREE-RANGING FLORIDA PANTHERS (*FELIS CONCOLOR CORYI*)

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ABSTRACT: Serum samples obtained from 38 free-ranging Florida panthers (*Felis concolor coryi*) in southern Florida, March 1978 through February 1991, were tested for antibodies against eight bacterial, parasitic, and viral disease agents. Sera were positive for antibodies against feline panleukopenia virus (FPV) (78%), feline calicivirus (56%), feline immunodeficiency virus/puma lentivirus (37%), feline enteric coronavirus/feline infectious peritonitis virus (19%), and *Toxoplasma gondii* (9%). All samples were seronegative for *Brucella* spp., feline rhinotracheitis virus, and pseudorabies virus. In addition, all the animals tested were negative for feline leukemia virus p27 antigen as determined by enzyme-linked immunosorbent assay. Feline panleukopenia virus was considered to be a potentially significant disease agent; FPV antibodies occurred in the highest prevalences in older age classes ($P = 0.027$) and in panthers living in the dense mixed hardwood swamps in the western portion of their range compared to the open cypress and sawgrass prairies to the east ($P = 0.096$). Because <50 animals remain in this relict population and the probable resultant depression of genetic diversity and lowered disease resistance, FPV or other disease agents could contribute to the extinction of this endangered subspecies.

Key words: Florida panther, cougar, *Felis concolor coryi*, free-ranging, endangered species, serosurvey, *Toxoplasma gondii*, feline calicivirus, feline enteric coronavirus/feline infectious peritonitis virus, feline immunodeficiency virus/puma lentivirus, feline panleukopenia virus.

INTRODUCTION

The endangered Florida panther, *Felis concolor coryi*, is estimated to number <50 individuals (Hines et al., 1987) and currently is protected under both state and federal endangered species statutes. This relict population of a subspecies that once occupied the entire southeastern United States is now isolated in the remote cypress swamps and hardwood hammocks of southern Florida, primarily in the Big Cypress Swamp and Everglades ecosystems (Belden, 1986). Human population growth in southern Florida and the concomitant loss of suitable panther habitat are the major threats to the continued survival of this rare mammal. A detailed review of the life history and status of the Florida panther is presented in the United States Fish and Wildlife Service (USFWS) recovery plan (U.S. Fish and Wildlife Service, 1987).

The critically small population size of the Florida panther makes the subspecies particularly vulnerable to catastrophes which could threaten its survival. Chance events such as periodic road-kills, poaching, or loss of prey species can be devastating to very small populations. In addition, disease epizootics are a constant threat to small populations, especially when those populations become relatively inbred (O'Brien and Evermann, 1988). Decreased

genetic diversity, reduced vitality, and lowered disease resistance often accompany such inbreeding in mammals (O'Brien et al., 1983, 1985). Disease outbreaks in the Florida panther could lead to extinction in the wild, such as occurred with the black-footed ferret (*Mustela nigripes*) in Wyoming (Williams et al., 1988). There is limited published information concerning disease agents of the Florida panther (Forrester et al., 1985; Barr et al., 1989; Greiner et al., 1989; Roelke et al., 1991a).

In 1978, research on the diseases and parasites of wild panthers was initiated by the Florida Game and Fresh Water Fish Commission in cooperation with the College of Veterinary Medicine at the University of Florida, Gainesville, Florida (USA). In 1983 the program was expanded to include a study of the health, reproductive, and genetic status of free-ranging Florida panthers. As part of that study, the seroprevalences of several etiologic agents infectious to Felidae and those endemic to other species of wildlife in Florida were determined.

MATERIALS AND METHODS

From March 1978 through February 1991, 74 blood samples were collected from 20 male and 18 female wild Florida panthers which either were live-captured for radio-instrumenting on one or more occasions (n = 59 samples obtained from 32 panthers) or at necropsy (n = 15). The samples were collected predominantly between January 1986 and January 1989. The age distribution of the animals ranged from 6 mo to >12 yr, with the majority being 4 to 8 yr of age. Two of the panthers were removed from the wild for rehabilitation and were sampled in captivity (only feline immunodeficiency virus/puma lentivirus data are presented from the captive dates). Necropsied animals included those killed by vehicles (n = 8), other panthers (n = 3), illegal shooting (n = 2), immobilization (n = 1), or a disease process (n = 1). Some individuals were sampled both as live animals and again at necropsy (n = 7).

All panthers were collected in southern peninsular Florida from the vicinity of Lake Okeechobee and southward (25°15′ to 27°10′N, 80°30′ to 81°30′W). Twenty-two individuals were collected from the Fakahatchee Strand/Big Cypress Swamp (FS/BCS) (Collier and Hendry Counties), representing approximately 80% of this sub-population, and seven from the Everglades National Park (ENP) (Dade County), representing 100% of this sub-population. These two areas constitute the core breeding populations. Three other individuals were collected outside this range in Glades (n = 2) and Palm Beach Counties (n = 1) and may represent transients from these areas (Fig. 1) (U.S. Fish and Wildlife Service, 1987).

Age was estimated by tooth wear (Ashman et al., 1983) and an evaluation of facial, body, and pelt features. For purposes of statistical analysis, panthers were grouped into six age classes: kitten (0 to 6 mo), juvenile (7 to 18 mo), subadult (19 to 23 mo), young adult (2 to 4 yr), mature adult (5 to 8 yr), and older adult (>8 yr).

Whole blood was obtained from each live-captured panther by venipuncture of cephalic, saphenous (medial and lateral), or jugular veins. The samples were collected in serum separator tubes and allowed to clot. Hemolyzed blood in varying states of autolysis was collected from dead panthers either by vena cava/cardiac puncture or directly from the thoracic or abdominal cavity. All whole blood samples were centrifuged at 2,000 rpm for 10 to 15 min, the serum was removed and frozen at ≤ −10 C, and held until analyzed.

Serum samples were tested for antibodies to the following disease agents: *Brucella* spp., *Toxoplasma gondii*, feline calicivirus (FCV), feline enteric coronavirus/feline infectious peritonitis virus (FECV/FIPV), feline immunodeficiency virus/puma lentivirus (FIV/PLV), feline parvovirus (FPV), feline viral rhinotracheitis virus (FVRV), and pseudorabies virus (PRV). Exposure to feline leukemia virus (FeLV) was determined by the enzyme-linked immunosorbent assay (ELISA) for antigen detection. Feline calicivirus, FPV, and FVRV values were reported for samples obtained prior to vaccination with a killed preparation of these three viruses.

The serologic test methods, source and type of antigens used, number of samples tested, positive antibody thresholds, and references for standard test procedures are summarized in Table 1 with the following modifications. Indirect immunofluorescence assay (IFA) tests for FECV/FIPV were performed as described by Evermann et al. (1986). The IFA is a group-specific test that detects cross reacting antibody to FIPV, FECV, canine coronavirus, and transmissible gastroenteritis virus of swine. The lowest serum dilution used was 1:5. Sera that consistently reacted at 1:5 or greater on repeat evaluations were considered positive. All samples tested for FECV/FIPV by IFA also were tested by KELA.

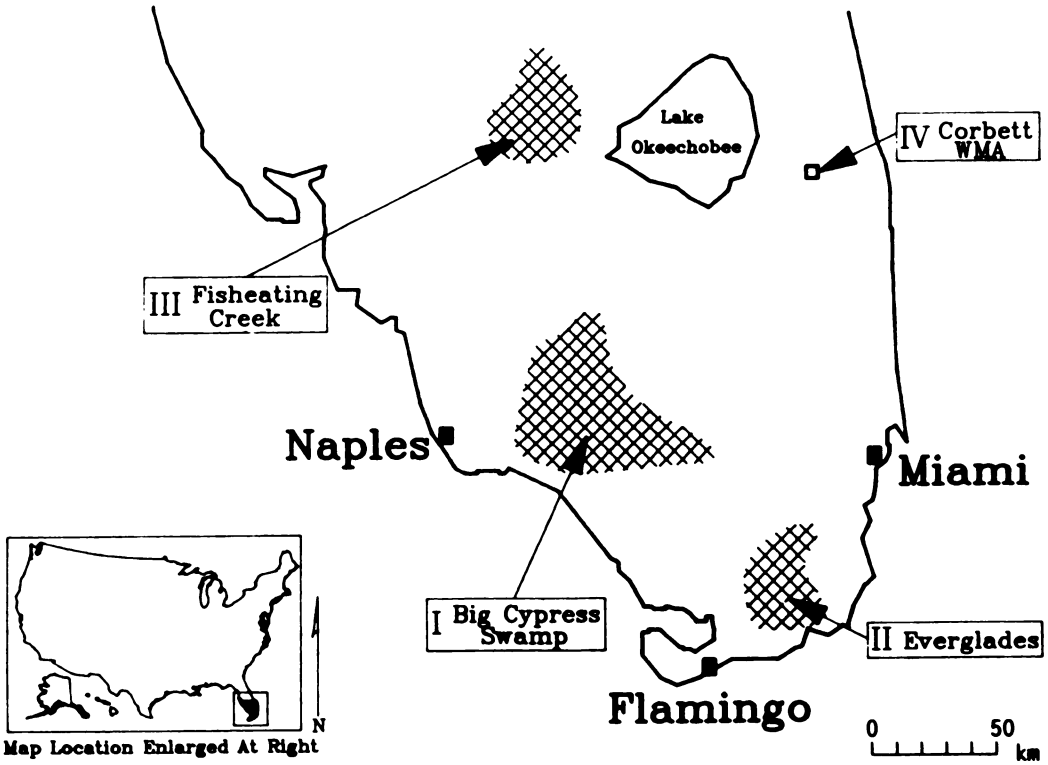

FIGURE 1. Map of the southern tip of Florida indicating locations where Florida panthers were sampled. Major populations: I, Big Cypress National Preserve, Fakahatchee Strand State Preserve, Florida Panther National Wildlife Refuge and surrounding ecosystem including privately owned land, Collier and Hendry Counties; II, Everglades National Park and East Everglades, Dade County; III, Fisheating Creek, Glades and Highlands Counties; IV, Corbett Wildlife Management Area, Palm Beach County.

The presence of FIV/PLV antibodies was detected by ELISA, IFA, and immunoblot (Barr et al., 1989). The immunoblot (Western blot) assay was modified as follows: electrophoresis was performed using the PhastSystem (Pharmacia LKB Biotechnology Inc., Piscataway, New Jersey, USA) on commercially-prepared sodium dodecyl sulfate/8 to 25% polyacrylamide gels, and the proteins were transferred passively at 45 C to a nitrocellulose membrane. Nitrocellulose strips were incubated in a 1:25 dilution of serum for a minimum of 1 hr, washed thoroughly, and incubated with horseradish peroxidase-conjugated rabbit anti-cat immunoglobin G (1:1,000 dilution) for 1 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.

The genotype of each panther was determined by molecular genetic analysis using mitochondrial DNA and nuclear markers at the Laboratory of Viral Carcinogenesis, National Cancer Institute (Frederick, Maryland, USA) according to the techniques described by O'Brien et al. (1990).

Chi-square tests (SAS Institute, 1988) on the prevalence of antibodies to parvovirus and calicivirus were applied to determine the relationships to sex, age, and location and to compare the prevalence of FIV/PLV virus with respect to capture location (FS/BCS and ENP) and genotype.

RESULTS

Panthers were seropositive for *Toxoplasma gondii*, FCV, FECV/FIPV, FIV/PLV, and FPV (Table 2). All samples were negative for antibodies against *Brucella* spp., FVRV, and PRV. All the animals tested were negative for FeLV p27 antigen as determined by ELISA.

The most significant documented infectious disease agent to which the panthers

TABLE 1. Serologic tests, antigens, and references for techniques used to detect antibodies against selected pathogenic agents in free-ranging Florida panthers, 1978 to 1991.

Agent (antigen)	No. of samples	Test	Positive threshold	Reference
Bacteria				
Brucella spp. (*Brucella abortus*)	25	Plate agglutination	≥1:25	U.S. Department of Agriculture (no date)
Protozoa				
Toxoplasma gondii	56	KELA[a]	≥1:48	Jacobson et al. (1982)
Viruses				
Feline calicivirus (FP 255)	41	Virus neutralization	>1:4	Scott (1977)
Feline enteric coronavirus/feline	56	KELA[b]	≥1:9	Barlough et al. (1983, 1987)
infectious peritonitis virus	27	IFA[c]	≥1:5	Evermann et al. (1986)
Feline immunodeficiency virus/ puma lentivirus	62	IFA, ELISA[d], & western blot	Qual.[e]	Barr et al. (1989), Olmsted et al. (1992)
Feline leukemia virus	56	ELISA[f]	Qual.	Mia et al. (1981)
Feline parvovirus	41	Virus neutralization	≥1:10	Scott et al. (1970a)
Feline viral rhinotracheitis virus (FVR-Goldstein)	41	Virus neutralization	≥1:2	Scott (1977)
Pseudorabies virus	25	Latex agglutination[g]	>1:4	Pirtle et al. (1986)

[a] KELA, kinetics enzyme-linked immunosorbent assay.
[b] These KELA results are considered to be "IFA-equivalent" titers.
[c] IFA, indirect immunofluorescence assay.
[d] ELISA-Petchek FTLV Antibody test, IDEXX, Portland, Maine, USA.
[e] Qualitative tests, no titers determined.
[f] ELISA: enzyme-linked immunosorbent assay for antigen detection; kits utilized were VIRACHEK™-FeLV by Symbiotics Corporation, San Diego, California, USA; and LEUKASSAY® F II- Feline Leukemia Virus test kit by Pitman-Moore, Mundelin, Illinois, USA.
[g] Pseudorabies virus antibody test kit-latex agglutination from Viral Antigens, Inc., Memphis, Tennessee, USA.

were exposed was FPV or a closely related parvovirus. Virus neutralizing antibodies to a parvovirus antigenically related to feline panleukopenia were detected in the sera of 25 (78%) of 32 panthers (Table 2).

Significantly ($P = 0.006$) more panthers in the Fakahatchee Strand and Big Cypress Swamp (FS/BCS) physiographic area (20 of 22) had evidence of prior infection with FPV than did panthers in Everglades National Park (ENP) (3 of 7) (Table 3). Also, seroprevalence was not related to sex ($P = 0.14$) of the panther, but was influenced by age ($P = 0.032$). There was a 3.4 times greater probability of panthers being seropositive with each successively older age class.

Within the FS/BCS ecosystem, seven of 11 of the panthers that resided primarily in the Fakahatchee Strand had high FPV antibody titers (≥1:1,000) whereas only two of eight of those that resided primarily in the adjacent Bear Island Unit of the Big Cypress National Preserve and the adjoining private lands to the north had high FPV titers. All seropositive panthers in the ENP had titers of ≤100.

Florida panthers were exposed to feline calicivirus, a potentially pathogenic virus. Eighteen (56%) of 32 panthers had VN antibodies to this virus. Based on a chi-square test there were no significant differences in the prevalence of FCV antibody titers between FS/BCS and ENP, age classes, or sexes of the panthers.

Serologic evidence of FIV/PLV infection was detected in 14 (37%) of 38 individual panthers examined between 1983 and 1991. Prevalence of FIV/PLV was significantly ($P = 0.03$) less in panthers with the historic *F. c. coryi* genotype than in those with the Everglades genotype (26% and 64%, respectively), but was not significantly different ($P = 0.18$) with respect

TABLE 2. Prevalences and reciprocal titers for antibodies to selected pathogens in wild Florida panthers, 1978 to 1991.

Agent	Number of panthers			Reciprocal titer	
	Examined	Positive	Percent positive	Minimum	Maximum
Bacteria					
Brucella spp.	24	0	0	<25	
Protozoa					
Toxoplasma gondii	32[a]	3	9	<48	308
Viruses					
Feline calicivirus	32	18[b]	56	<2 to <20[c]	>256
Feline enteric coronavirus/feline infectious	32[d]	0	0	<9	
peritonitis (coronavirus)	21[e]	4[f]	19	<5	125
Feline immunodeficiency virus/puma lentivirus	38[g]	14[h]	37	Qual.[i]	
Feline leukemia virus	32	0	0	Qual.	
Feline parvovirus (panleukopenia virus)	32	25[j]	78	<5	>10,000
Feline rhinotracheitis virus (herpesvirus)	32	0	0	<2 to <8[c]	
Pseudorabies virus	23	0	0	0	

[a] Data on five of these serum samples were presented in two earlier papers (four in Forrester et al., 1985 and one in Burridge et al., 1979). In this current study all were negative by KELA; however, one was reported by Forrester et al. (1985) to be positive by indirect hemagglutination test (1:256).

[b] Two of the positive animals were negative when first sampled but had sero-converted by a subsequent capture (after a 12 and 22 mo interval, respectively); each was included once in the tally as a positive animal.

[c] Hemolyzed sera from necropsied animals resulted in higher negative thresholds than non-hemolyzed sera and could interfere with detection of low positive titers.

[d] KELA results.

[e] IFA results.

[f] Three of the four were indeterminant and gave weak positive results when retested (1:25 to 1:5).

[g] Data from 20 of these animals have been presented by Barr et al. (1989).

[h] Three animals sero-converted, negative to positive, after a 12 to 24-mo resampling interval, and each was included once in the tally as a positive animal.

[i] Qualitative assays, no titers determined.

[j] One animal sero-converted negative to positive after a 12-mo resampling interval, and was included once in the tally as a positive animal.

to capture locations FS/BCS (9 of 29) and ENP (5 of 9). Antibody sero-conversion was detected in three individuals: a mature female, which converted from negative to positive on the third year of sampling, a young adult male, and a juvenile male. A sub-adult female (#21), the offspring and sibling of two seropositive panthers, was originally positive by IFA but had equivocal ELISA and immunoblot test results; it continued to show low and fluctuating titers on subsequent immunoblot examinations, but was consistently negative by ELISA (Table 4). With the exception of this one female, antibody positive panthers sustained their seropositive status on subsequent evaluations for at least the 6 mo to 4.0 yr that they were followed.

Four of 21 (19%) individuals were positive for coronavirus antibodies by IFA while 0 of 32 were positive by KELA. Three panthers were seropositive for *T. gondii* and appeared to maintain their relatively low titers over several sampling years—1:49 to 1:188 (2-yr interval), 1:308 to 1:150 to 1:47 (3-yr time span), and 1:17 to 1:73 to 1:66 (2-yr time span).

DISCUSSION

Feline parvovirus (FPV)

Feline panleukopenia (FPL), also known as viral enteritis or feline "distemper," is caused by FPV. Feline panleukopenia is a highly contagious, viral disease causing high mortality chiefly in young kittens, especially with stress or co-infection with

TABLE 3. Distribution of serum neutralization antibody titers against feline parvovirus in Florida panthers from different locations, 1978 to 1991.

Location of panthers	Number of panthers examined	Number (%) of individuals at each reciprocal titer						
		<10	50	100	500	1,000	5,000	>5,000
Big Cypress Swamp	22	2 (9)	0	2 (9)	7 (32)	2 (9)	3 (14)	6 (27)
Everglades National Park	7	4 (57)	1 (14)	2 (29)	0	0	0	0
Fisheating Creek	2	1 (50)	0	0	0	0	0	1 (50)
Corbett Wildlife Management Area	1	0	0	0	0	1 (100)	0	0
Totals	32	7 (22)	1 (3)	4 (13)	7 (22)	3 (9)	4 (13)	6 (19)

other agents or parasites (Scott, 1990). All members of the family Felidae and many members of the families Procyonidae and Mustelidae are susceptible to FPV (Goss, 1948; Scott, 1990). Feline panleukopenia virus has been isolated from a number of captive exotic felids including African lions (*Panthera leo*), snow leopards (*P. uncia*), and tigers (*P. tigris*) and from raccoons (*Procyon lotor*) and coatimundis (*Nasua nasua*) (F. W. Scott, unpubl.). This disease has been reported in captive cougars (*F. concolor*) (Torres, 1941; Hyslop, 1955; Bittle, 1981; Wallach and Boever, 1983) and free-ranging bobcats (*Felis rufus*) in Florida (Wassmer et al., 1988), and antibodies against the virus have been found in bobcats in New York (Fox, 1983). To our knowledge there have been no published reports of the occurrence of this virus in free-ranging populations of cougars or panthers. Parvovirus may have contributed to the deaths of two free-ranging cougars with necrotizing enteritis in 1987; FPV was found in intestinal tissue by viral isolation from an animal in southwestern Colorado (M. E. Roelke and F. W. Scott, unpubl.) and by electron microscopy from a Wyoming cougar (E. T. Thorne and E. S. Williams, pers. comm.).

Infection is thought to occur by ingestion of the virus in feces, vomitus, or urine from infected individuals (Cotter, 1980). Other potentially susceptible wild carnivores which occupy the same habitat as the Florida panther and could serve as reservoir hosts for parvovirus are the bobcat, mink (*Mustela vison*), feral domestic cat (*Felis catus*), grey fox (*Urocyon cinereoargenteus*), raccoon, red fox (*Vulpes vulpes*), and river otter (*Lutra canadensis*) (Layne, 1974; Barker et al., 1985; Roelke et al., 1985). The virus is extremely hardy and can survive for more than a year at room temperature (Poole, 1972; Cotter, 1980). Therefore, in addition to direct contact with a virus-shedding carnivore, habitat contamination may be a source of the virus.

The higher prevalence of antibodies to FPV in the FS/BCS compared to the ENP is difficult to explain, but may have resulted from differences in the two habitats. The heavily vegetated, mixed hardwood swamp of the Fakahatchee Strand and the presence of abandoned logging roads above the water level might encourage use of common travel routes by various carnivores. This would increase animal-to-animal and fecal-to-animal contact. In addition, the dense cover may protect the virus deposited in feces from inactivation by sunlight. This is in sharp contrast to the roadless, open sawgrass and cypress prairies of the Everglades habitat. Alternatively, the non-uniform distribution of panthers with FPV antibodies may be due to differences in numbers of individuals and viral-shedding capability of other susceptible host species that inhabit the area.

The high (1: ≥ 1,000) VN titers to FPV documented in the panthers of the FS/

TABLE 4. Seroconversion, persistence, and familial association of feline immunodeficiency virus/puma lentivirus antibodies in free-ranging Florida panthers, 1983 to 1991.

ID#-Location[a]	Date	Age (yr)	ELISA	IFA	Immunoblot
Individuals					
#07 M-I	27 Mar 1983	6 to 8	+	+	+/− (1 Band)
	23 Mar 1985[b]	8 to 10	+	+	ID[c]
#08 F-I	21 Feb 1985	9 to 10	−	−	−
	13 Jan 1986	10 to 11	+	ND[d]	+/− (1 Band)
	13 Apr 1987[e]	11 to 13	+	ND	+ (2 Bands)
	18 Aug 1987	11 to 13	+	+	+ (2 Bands)
	01 Jun 1988	12 to 14	+	+	+ (3 Bands)
	20 Aug 1988[f]	12 to 14	+	+	+ (3 Bands)
#18 F-I	22 Jan 1987	8 to 10	+	+	+ (3 Bands)
	23 Jan 1989[g]	10 to 12	+	ND	+ (2 Bands)
#25 M-I	16 Feb 1988	3 to 4	+	+	+/− (1 Band)
	26 Aug 1988[h]	3.5 to 4.5	+	ND	+/− (1 Band)
#26 M-I	01 Mar 1988	4 to 6	+/−	ND	+/− (1 Band)
	10 Feb 1990[i]	6 to 8	+	ND	ND
#28 M-I	30 Dec 1988	1.5 to 2	−	ND	ND
	07 Jan 1991[j]	3.5 to 4	+	ND	ND
Family members					
#14 F-II	04 Dec 1986[j]	4 to 6	+	+	+/− (1 Band)
(mother)	11 Apr 1988	6 to 8	+	ND	+ (2 Bands)
	22 Feb 1990[k]	8 to 10	+	ND	ND
#16 M-II	12 Jan 1987[l]	1 to 1.5	+	+[l]	+ (2 Bands)
(offspring 1st litter)	02 Feb 1988	2 to 2.5	+	ND	+ (2 Bands)
	21 Feb 1990	3 to 3.5	+	ND	ND
	04 Feb 1991[j]	4 to 4.5	+	ND	ND
#21 F-II	16 Mar 1987[l]	1 to 1.5	+/−	+	+/− (1 Band)
(offspring 1st litter)	23 Jul 1988[m]	2 to 2.5	−	−	+/− (1 Band)
	19 Oct 1988	2.5 to 3	−	−	−
	28 Jan 1989	3 to 3.5	−	ID	−
	24 Feb 1989	3 to 3.5	−	+[l]	+/− (1 Band)
	23 May 1989	3 to 3.5	−	+[l]	+ (2 Bands)
	12 Jun 1989[n]	3 to 3.5	−	ID	+/− (1 Band)
#42 M-II	06 Mar 1990	0.8	−	ND	ND
(offspring 2nd litter)	05 Feb 1991[j]	1.8	+	ND	ND

[a] See Fig. 1 (I—Big Cypress Swamp, II—Everglades National Park).
[b] Died October 1985, hit by car, otherwise apparently healthy.
[c] ID = Indeterminate results.
[d] ND = Not determined.
[e] Removed from wild due to declining health: anemia, weight loss, and lack of reproductive success. All subsequent dates were in captivity.
[f] Post mortem sample; died in captivity of renal and hepatic failure and adenomatous hyperthyroidism.
[g] Died of intraspecific aggression 10 October 1990.
[h] Post mortem sample; died of septicemia following intraspecific aggression.
[i] Alive as of 31 December 1991.
[j] Trio of panthers captured which functioned as a family, traveling and eating together. Subadults dispersed from their dam approximately 1 April 1987.
[k] Died 21 June 1991 of renal disease plus mercury contamination.
[l] Weak IFA result.
[m] Panther hit by car and removed from wild for rehabilitation. All subsequent dates were in captivity.
[n] Alive and still in captivity as of 31 December 1991. This panther was discovered by Butt et al. (1991) to be concurrently infected with *Cytauxzoon felis* and had periodic problems with weight loss, low grade anemia, and slow bone healing.

BCS and maintained over subsequent years in serially sampled individuals (Roelke, 1990) were consistent with findings in domestic cats where infection with virulent virus results in life-long immunity with high VN antibody titers (Scott et al., 1970b). However, these persistent high titers also may suggest that the virus is ubiquitous in that environment and that those animals are continually encountering the virus, resulting in higher antibody titers, similar to that seen with repeated vaccination (Scott, 1971). Alternatively, there may be differences in the virulence and antigenicity of the strain of FPV present in the respective locations, resulting in differences in antibody response to infection.

Virus neutralizing antibody titers $\geq$1:10 are considered protective in domestic cats (Fastier, 1968; Scott et al., 1970b). Since the majority of adult panthers and bobcats (Roelke, unpubl.) living in the FS/BCS were seropositive and probably had antibody levels that were protective, the probability of a catastrophic epizootic event occurring due to FPV would be low. However, in areas where the prevalence and intensity of antibody response to FPV is low or non-existent there is greater potential for the disease to cause high morbidity and mortality. This latter scenario may have allowed the epizootic of FPL in the wild bobcat population at Archbold biological station (near Lake Placid, Highlands County, Florida) that killed 11 of 18 radio-collared animals over a 3-mo-period in 1979–80 (Wassmer et al., 1988).

To date, no Florida panther deaths have been attributed to parvoviruses. Most panthers sampled have been sub-adults and adults; however, kittens and older juveniles are of most concern. The least is known about these latter two age classes, but mortality from birth to 6 mo of age is estimated to be approximately 50 to 70% (Roelke, unpubl.). The role of FPV in this early mortality is unknown, but should be seriously examined. Van Rensburg et al. (1987) describes a significant negative impact of FPV on an island population of domestic cats. The primary effects following the introduction of FPV were lowered fecundity, a dramatic decrease in the population density, and adverse changes in the age structure. Despite an 82% decline in the population over a 5-yr-period (from 3,409 down to 615) no clinically affected animals were documented. Further, the authors believed that there was an annual epizootic of FPV among the susceptible young kittens at the time the maternal antibody waned. Perhaps kittens in the FS/BCS likewise are succumbing to FPV.

Feline calicivirus

Feline calicivirus is a primary cause of feline viral respiratory disease in domestic cats and many species of non-domestic felids (Ford, 1989; Fowler, 1986). Although mortality generally is low, virulence can vary with the strain of virus and the age of the host, with most mortality occurring in young kittens $<$10 wk of age (Ford, 1989). This virus does not survive for long periods outside the host and transmission is by direct animal-to-animal contact through saliva and aerosolized respiratory fluids (Ford, 1989). Most recovered domestic cats become chronic carriers, and many shed the virus from their oropharynx for $\geq$2 yr (Ford, 1989).

Since there was no significant difference in the prevalence of FCV by age, sex, or location, the virus may be present throughout the panther population, with transmission similar to that in the domestic cat—usually a persistently infected mother-to-offspring pathway. Calicivirus was isolated from a 6-mo-old kitten with oral and labial ulcerative lesions and from its asymptomatic, seropositive mother (M. E. Roelke and F. W. Scott, unpubl.). Social interactions between adults or between non-maternal adults and kittens also may facilitate transmission; one adult male panther seroconverted during a 2-yr sampling interval during which he consorted at regular intervals with a sero-positive female. The

significance of this virus to Florida panthers is unknown at this time, but it could play a role in neonatal morbidity and mortality.

Feline immunodeficiency virus/puma lentivirus

Feline immunodeficiency virus is a recently recognized pathogen of domestic cats which causes an immunodeficiency-like syndrome (Pedersen et al., 1987; Yamamoto et al., 1989), usually after months or years of clinically inapparent infection. To date we have not noted immunodeficiency related illness or mortality in the free-ranging panthers associated with FIV/PLV infection. However, many general signs of disease such as anemia and weight loss, that we observed in some of these animals, were consistent with those documented in domestic cats infected with FIV. Other seropositive panthers followed for a number of years appeared to remain clinically normal, even though an FIV-like puma lentivirus was isolated recently from a few of these persistently infected animals (Olmsted et al., 1992). Further, diagnosis of clinical FIV in domestic cats is generally made ante-mortem and it is probable that hematopoietic anomalies or bone marrow suppression would be missed on the post mortem exam of the typically autolyzed dead Florida panther.

From our limited data, at least three possible routes of FIV/PLV transmission are suggested. First, a familial route is probable. This is based on serology, virus isolation, and DNA sequence analysis demonstrating that a virtually identical virus infected an adult ENP female panther and her kittens from two sequential litters (serologic data are presented in Table 4) (Olmsted et al., 1992). Whether the transmission of the virus might have occurred by transuterine, transmammary, or by direct contact is unknown. A second possible route is through fight wounds or copulation. A seropositive panther male (#07) moved into the territory of a seronegative panther female (#08). Based on radio-telemetry data over the next year, he had repeated close contact with this female at regular intervals, suggesting breeding activity (J. C. Roboski, pers. comm.). On subsequent recapture, she had fresh fight wounds, perhaps inflicted by the male and had a positive antibody titer. Over the ensuing 2.5 yr until her death, her antibody titer continued to rise. A third possible route is through the food chain, such as by consuming bobcats or domestic cats, both of which have been documented to carry FIV (Barr et al., 1989).

Within the free-ranging Florida panther population there are two major genotypes: animals descending from historic *F. c. coryi*, and animals also carrying nuclear and mitochondrial genes common to Central or South America (O'Brien et al., 1990). Because the prevalence of FIV/PLV in animals carrying non-North American genes (primarily the ENP population) was significantly higher than in animals of pure *F. c. coryi* descent, the virus may have originated in Latin America and was imported later. Other alternatives exist. The virus appears to occur within families (mothers and offspring) and it has been determined that the ENP population can be traced back to a limited number of females (perhaps one) (O'Brien et al., 1990); thus, the higher prevalence of FIV/PLV within the ENP population may merely reflect the familial/maternal connection between those panthers. A less likely hypothesis is that environmental and ecological conditions in the ENP are conducive to survival of FIV/PLV.

Feline enteric coronavirus/feline infectious peritonitis virus

The IFA test can cross-react to other coronaviruses of cats, canine coronavirus, and transmissible gastroenteritis virus; thus, the presence of low antibody titers (1:5 to 1:25) may indicate exposure to a virus other than a feline coronavirus. Further studies would be important to elucidate the potential pathogenicity of these infections in the panther. Lack of detectable response by the KELA indicates that this assay may

be very specific for FIPV, and not detect other coronavirus antibodies at lower dilutions. Further comparative studies between the IFA and KELA in the panther and domestic cat are recommended.

Pseudorabies virus

Pseudorabies can cause 100% mortality in experimentally infected domestic cats (Horvath and Papp, 1967). The clinical course of the disease in domestic cats is <48 hr; this peracute death does not allow time for a serologic response to occur (Hand, 1989). If Florida panthers succumb as rapidly as domestic cats, detecting the clinical phase or retrieving a carcass fresh enough to make an accurate histologic and virologic diagnosis would be very difficult. The fact that all sampled panthers were seronegative could indicate that they had never been exposed to PRV rather than its absence in the environment. In addition to the 23 animals tested in the present study, 19 other Florida panthers and 33 bobcats from southern Florida also were seronegative for PRV (Roelke, 1990).

Nettles and Erickson (1984) and E. P. Gibbs (pers. comm.) have reported prevalences of PRV antibodies of 30 to 80%, in feral hogs (*Sus scrofa*) from panther habitats. Asymptomatic, experimentally infected wild hogs shed PRV in saliva and nasal discharge with subsequent infection occurring in contact animals (Tozzini et al., 1982). In areas where hogs are abundant, they are primary prey of Florida panthers (Belden, 1986; Roelke et al., 1986; Maehr et al., 1990); therefore, it would be possible for panthers to contract the virus by consuming flesh from infected hogs (Gustafson, 1986). This could explain the absence or low numbers of panthers in areas of high hog density and high PRV seroprevalence (Sarrasota and Glades Counties with 80% and 65% of the hogs infected with PRV, respectively) (Belden, 1978; Roof and Maehr, 1988; R. C. Belden, pers. comm.; E. P. Gibbs, pers. comm.). In these areas the virus could be the limiting factor in the northern extension of panther distribution in southern Florida (Roelke, 1988).

Feline viral rhinotracheitis virus

To date, no unvaccinated panthers have demonstrated positive antibody titers to FVRV, however, several have had detectable titers 1 to 2 yr following vaccination. Since wild bobcats from the same ecosystem were seropositive for FVRV (Roelke, 1988), we cannot be sure whether the positive titers in the panthers were the result of natural exposure or vaccinal response.

Toxoplasma gondii

Burridge et al. (1979) reported that 18% of the raccoons and armadillos (*Dasypus novemcinctus*) in southern Florida were positive for *Toxoplasma gondii* antibodies by the hemagglutination-inhibition test (titers ≥ 1:64 = positive). These two prey items constitute a considerable proportion of the panther's food base in the Fakahatchee Strand State Preserve (Roelke et al., 1986) where two of the three antibody-positive panthers lived. The low seroprevalence found in the panthers is curious considering their carnivorous diet. It is possible that the KELA *T. gondii* assay did not accurately detect antibody in panther sera; a test for specific IgG antibody may be more appropriate (Lappin et al., 1991).

Infectious disease interaction

While no illnesses attributed to these various infectious agents were seen, many viral and parasitic agents found in wild Florida panthers could interact to influence the survival of this endangered animal. For example, the hookworm (*Ancylostoma pluridentatum*) (Forrester et al., 1985) may exacerbate the pathogenicity of FPV by enhancing mitotic activity of intestinal epithelial tissue (Carlson et al., 1977; Carlson and Scott, 1977). Similarly, immunosuppression due to FIV/PLV could allow otherwise sequestered agents, such as FCV or *Toxoplasma gondii*, to recrudesce in carrier panthers, resulting in possible mortality. This type of interaction

has been documented with human immunodeficiency virus/acquired immunodeficiency syndrome and *T. gondii* in humans (Frenkel, 1990). Innate or acquired immunity, as well as an individual's heterozygosity, can dramatically affect the outcome of exposure to infectious disease agents. Feline enteric coronavirus/feline infectious peritonitis virus has devastating effects on cheetahs (*Acinonyx jubatus*), which lack the breadth of genetic diversity seen in other species of cats (Evermann et al., 1986; O'Brien et al., 1985). With its limited population size, the Florida panther also might be compromised in its genetic diversity and disease resistance compared to other sub-populations of cougars, and be more susceptible to viral agents such as FPV or FECV/FIPV.

MANAGEMENT IMPLICATIONS

Quality panther habitat is dwindling rapidly under constant pressure from human activity, land development, and agricultural interests. The nutritional and social stress caused by habitat degradation, along with environmental contaminants such as methyl mercury (Roelke et al., 1991b) may further compromise the animals' ability to resist disease. In addition to a waning habitat, the utility of the remaining land may be decreased for panthers by the presence of infectious diseases. This could be the case with pseudorabies virus in feral hogs, which may have the potential to limit the range of Florida panthers much as the tsetse fly prevented the movement of domestic cattle into tropical Africa (Rogers and Randolf, 1988). All of these factors render this endangered subspecies exceedingly vulnerable to sporadic or catastrophic mortality, and eventual extinction.

Given the small number of panthers remaining in the wild, it is imperative that measures be taken to protect all individuals from potential pathogens wherever possible. There is no direct evidence that panthers have died from the diseases mentioned in this paper, but their presence has been documented and they should be taken into consideration in managment protocols. Measures should include judicious sanitation and sterilization of equipment between panther captures, and the administration of appropriate anthelmintics and killed viral vaccines to protect against FPV, FCV, and FVRV each time a panther is handled. Also, as animals are removed from the wild for rehabilitation or captive breeding, care must be taken to prevent transmission of disease agents from these individuals to the captive felid population or from captive animals to those which will be released and could potentially contaminate the free-ranging population (Roelke et al., 1991a).

It is possible that our initiation of the above vaccination and anthelmintic treatment program in 1985 may have prevented subsequent disease outbreaks from occurring in our radio-collared population, thus making it difficult if not impossible to document the true impact of these diseases on the Florida panther population (Roelke et al., 1991a).

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