

EPIZOOTIOLOGY OF FELINE LEUKEMIA VIRUS IN THE FLORIDA PANTHER

By

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by

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“The role of disease in wildlife conservation has probably been radically underestimated”
(Aldo Leopold, 1933).

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Feline leukemia virus (FeLV) has been reported only rarely in non-domestic felids and was not detected in Florida panthers (*Puma concolor coryi*) during almost 20 yr of routine surveillance. The finding of two FeLV antigen-positive panthers during the 2002-2003 capture season led to a prospective and retrospective investigation of the epizootiology of this disease in the population. Archived serum was tested for FeLV antibodies to assess history of exposure. To determine prevalence and distribution, panthers were captured throughout their range and tested for FeLV antigen by ELISA. Positive tests were confirmed by immunofluorescent antibody (IFA) test and viral culture. The outcome following exposure in panthers was inferred from ELISA antigen and antibody, IFA, and PCR results. All infected panthers were monitored by radio-telemetry and necropsied following detection of a mortality signal. Between 1990 and 2005, the prevalence of positive antibody tests increased significantly and were concentrated in the northern portion of panther range. The prevalence of antigenemia (positive ELISA antigen) among panthers and Texas pumas ≥ 1 yr of age, not previously

vaccinated for FeLV, and sampled between July 2002 and June 2005, was 7% (5 of 71). Antigenemic panthers were captured or recovered in the Okaloacoochee Slough State Forest (OKS) in the northern portion of panther range. All antigenemic panthers were positive by viral culture and three were IFA positive at capture. Clinical signs and clinical pathology at capture ($n = 4$) included lymphadenopathy, moderate to severe anemia, lymphopenia, and acute lymphoblastic leukemia. All infected panthers died during the study period; causes of deaths were septicemia ($n = 2$), intraspecific aggression ($n = 2$), and unknown ($n = 1$). Average time from diagnosis to death was 9.25 (SD ± 10.3) wk in antigenemic panthers. Following exposure, panthers developed transient, latent, or persistent infections. The high localized prevalence of antigenemic panthers in OKS (45.5%) demonstrates the potential impact of this disease on the population. Management to control the epizootic currently includes vaccination and test-removal. No new cases have been diagnosed since July 2004.

CHAPTER 1 INTRODUCTION

The Florida panther (*Puma concolor coryi*) is one of the most endangered mammals in North America, at one time numbering as few as 30 individuals. With protection and management the population has rebounded to almost 100; however, the panther is now threatened by feline leukemia virus (FeLV). Feline leukemia virus infection is a fatal infectious disease, common to domestic cats (*Felis catus*), that is rare in non-domestic felids. Routine FeLV antigen testing in panthers was negative for almost 20 yr until two positive panthers were detected during the 2002-2003 capture season. These findings resulted in a prospective and retrospective investigation into the epizootiology of this disease in the panther population. Information gained from this research will not only be used to help manage the epizootic in this critically endangered population but may also benefit managers of other non-domestic felid populations.

Background

The Florida Panther

The Florida panther is an endangered subspecies of puma whose range was once contiguous with other puma subspecies including the Texas puma (*P. concolor stanlyana*). By the early part of the 20th Century; however, habitat destruction, exploitation, and human population growth had reduced the panther to an isolated remnant population. The panther was eliminated eventually from all previous range with the exception of the relatively inaccessible and, historically, undesirable Big Cypress and Everglades ecosystems of south Florida. Protection of the panther began with state

classification as a game animal in 1950 followed by complete state protection in 1958. The panther was listed federally as an endangered species in 1967. Nevertheless, the population dwindled to an estimated 20 to 30 individuals by the early 1970's (Nowak and McBride, 1973).

Researchers noticed morphologic differences among panthers from different areas of south Florida. Subsequent genetic analyses revealed two genotypes: 1) original or canonical Florida panthers, concentrated in the Big Cypress ecosystem, and 2) Florida panther/South American puma intercrosses which primarily occupied the Everglades ecosystem (O'Brien et al., 1990). The canonical genotype traced its lineage from the original remnant population while the South American puma intercrosses likely resulted from the release of Florida panther/captive puma hybrids into the free-ranging panther population between 1957 and 1967 (Vanas, 1976). Panthers with genetic evidence of South American puma ancestry, although representing a minority, had a greater genetic diversity and fewer congenital anomalies than panthers retaining the canonical genotype (Roelke et al., 1993a). Among canonical panthers, the level of mitochondrial DNA variation, frequency of polymorphic allozyme loci, and average heterozygosity of allozyme loci was lower than any other similarly studied feline except the cheetah (*Acinonyx jubatus*) (O'Brien et al., 1990; Newman et al., 1985; Roelke et al., 1993a).

The consequences of inbreeding in panthers were believed to have included cryptorchidism (Roelke et al., 1993a; Mansfield and Land, 2002), atrial septal defects (Cunningham et al., 1999), poor seminal traits (Barone et al., 1994), and poor fecundity (Roelke et al., 1993a). Putative impaired immunocompetence was suspected to increase

susceptibility to parasites and infectious diseases including dermatophytosis (Rotstein et al., 1999). Many of these traits are still seen in canonical Florida panthers today.

Without intervention the Florida panther was predicted to become extinct within 25 to 40 yr (Seal and Lacy, 1989). However, in 1995 eight female Texas pumas were released into south Florida as part of a genetic restoration program (Seal, 1994). The resultant introgression was designed to restore the genetic diversity to levels comparable to other puma subspecies and to lower the incidence of congenital anomalies in the panther population.

As of September 2004, over half of the population had Texas puma genes (D. Land, pers. commun.). The distribution of genotypes was not uniform however, with more canonical panthers present in the northern portion of panther range. Recent microsatellite DNA analyses also provided evidence for a third and more recent introgression. Several captive pumas of unknown western ancestry escaped from the Seminole Indian Reservation (SIR) north of Big Cypress National Preserve (BCNP) between 1996 and 1999. Although most were eventually recaptured, successful breeding with free-ranging panthers apparently occurred, and evidence of this genotype was present in 6-10% of panthers sampled between 2000 and 2004 (D. Land, pers. commun.). This genotype was concentrated also in the northern portion of panther range.

The prevalence of congenital anomalies among intergrades was reduced greatly and was limited to the occasional kinked tail or cowlick. As a result of the genetic introgressions, both deliberate and unintentional, and other management measures, the panther population had rebounded to a minimum of 87 by 2003 (McBride, 2003). However, this increase in density may have resulted in an increased risk of infectious

disease transmission and expansion of the wildland-urban interface. These factors may have set the stage for the current FeLV epizootic.

Feline Leukemia Virus

Feline leukemia virus is a Gammaretrovirus in the family Retroviridae. Following penetration of the host cell by the viral RNA, reverse transcriptase transcribes viral RNA into double-stranded DNA which is then incorporated into the host genome. Incorporated viral DNA, known as provirus, codes for viral proteins and serves as a template for the production of viral RNA. There are numerous strains of FeLV and few isolates in nature are identical (Hoover and Mullins, 1991). Feline leukemia virus is classified into subgroups A, B, and C based on envelope antigens (Jarrett et al., 1973; Sarma and Log; 1973). All FeLV-infected cats carry subgroup A (Jarrett et al., 1978), which is the least pathogenic and only transmissible form. Subgroup C results from mutation of subgroup A while subgroup B arises from recombination between subgroup A and endogenous retroviral DNA (enFeLV) (reviewed by Miyazawa, 2002). EnFeLV are non-coding, non-immunogenic sequences (Mandel et al., 1979; Rigby et al., 1992) that became incorporated in the domestic cat genome early in their phylogenetic history. Most non-domestic felids, including Florida panthers, do not have enFeLV.

The domestic cat is the definitive host for FeLV and the virus has a worldwide distribution. Although several non-felid cell lines have shown in vitro susceptibility (Nakata et al., 2003) infection has not been described in non-felid species. The worldwide prevalence of FeLV in healthy domestic cat populations ranges from 1-8% (Levy, 1999) with prevalences over 30% in some closed populations (Grant et al., 1980; Gertsman, 1985). There is evidence that the overall prevalence of FeLV in domestic cats is decreasing, possibly due to vaccination and other control measures (Levy and Crawford,

2005). In Florida, the prevalence among feral cats is less than 4% (Lee et al., 2002). Infection is more prevalent among male cats, mixed breed cats, and cats between 1 and 7 yr of age (Levy, 2005). The highest infection rate occurred in cats less than 2 yr of age (Levy, 1999). In contrast, the prevalence of FeLV antibodies, indicating exposure, continues to increase with age (Rogerson et al., 1975).

Feline leukemia virus is an enveloped virus and is therefore quite fragile. The virus immediately begins losing viability outside of the host and, on dry surfaces, is completely inactivated between two and three hr (Francis et al., 1979). Therefore transmission is primarily by direct contact. The virus is shed in highest concentrations in the saliva (Francis et al., 1977), and horizontal transmission occurs primarily via the oronasal route and by bite wounds. Prolonged contact is generally necessary for effective transmission (Hardy et al., 1973). Transplacental and transmammary transmission of the virus are also important (Hardy et al., 1976).

Following exposure most domestic cats will eventually clear the virus while approximately one-third will become persistently infected and eventually succumb to FeLV related diseases. However, there is a dynamic relationship between the host and virus, and progression of disease depends on a number of factors. Outcome following exposure depends on host age (Hoover et al., 1976), genetics (Hoover and Mullins, 1991), and immunocompetence (Hoover et al., 1980), as well as route of exposure, virus burden, and strain of virus (Rojko and Kociba, 1991; Hoover and Mullins, 1991). The progression of infection can be predicted by provirus burden using quantitative polymerase chain reaction (PCR) (Hofmann-Lehmann et al., 2001). Cats clearing infection early have no or low provirus burdens, those latently infected retain moderate

levels of provirus, while those becoming persistently infected have high provirus burdens that peak at about 4 wk post-exposure (Hofmann-Lehmann et al., 2001). These researchers also demonstrated an inverse correlation between ELISA antibodies and provirus load beginning approximately 3 wk post-exposure. Cats that resisted persistent infection had a more pronounced humoral response and lower provirus burdens than cats that progressed to persistent infections. Cell-mediated immunity is important also in the early immune response to FeLV infection (Flynn et al., 2002). Regardless of the outcome, the course of infection is established usually by 8 wk post-exposure (Torres et al., 2005). Outcome following exposure in domestic cats is summarized in Fig. 1-1.

Following exposure the virus replicates in local lymphoid tissues. Approximately 40% of cats mount an effective immune response and clear the virus before further progression (Hoover and Mullins, 1991). These cats remain antigen and provirus negative throughout their lives (Torres et al., 2005). If the infection progresses, however, viral replication within a small number of circulating leukocytes will lead to infection of lymphoid organs including the thymus, spleen, and lymph nodes (Rojko et al., 1979). Cats at this stage may be transiently antigenemic and may even be briefly infectious. Clinical signs during this primary viremia may include fever, lethargy, leukopenia, anemia, and lymphadenopathy (Pedersen et al., 1990; Levy, 1999). However, approximately 50% of cats reaching this stage are still able to mount an effective immune response and clear the infection (Hoover and Mullins, 1991). Failure of viral containment will lead to infection of the bone marrow, salivary glands, and other tissues between 3 and 13 wk. Nevertheless, an adequate immune response early in this process may rescue the cat from persistent infection. These cats will retain provirus in peripheral and marrow

leukocytes for variable periods and are considered latently infected. Latently infected cats do not shed virus and are not infective to other cats. Reactivation of latent infections following stress is possible but becomes less likely ≥ 1 yr post-infection (Pedersen et al., 1984). Generally, cats recovering from transient or latent infections are immune to re-infection.

Progression to persistent infection occurs in approximately 35% of exposed cats and is characterized by infection of the bone marrow and the development of cytosuppressive and cytoproliferative diseases. Severity and type of disease in persistently infected cats depends on host age (Hoover et al., 1976), concurrent feline immunodeficiency virus (FIV) infection, and virus subgroup and strain. Following establishment of a persistent infection, a period of dormancy ensues lasting weeks to years during which few if any clinical signs are apparent. Eventually, persistent infections result in any of three clinical syndromes: immunosuppression, anemia, and/or neoplasia.

Immunosuppression is believed to result in opportunistic infections. Co-infections were the most frequent finding in FeLV infected cats examined at North American veterinary schools (Levy, 1999). Anemia, whether primary or secondary, is the next most common clinical finding in FeLV infected cats. Anemias are most commonly non-regenerative and include pure red cell aplasia, red blood cell macrocytosis, erythemic myelosis, bone marrow infiltration, and anemia of chronic disease (Hardy, 1980a). Finally, hematopoietic neoplasms may also result from FeLV infection. Lymphoma is the most common FeLV-related neoplastic disease; leukemias, myeloproliferative diseases, and fibrosarcomas are also common (Hardy, 1980a). Mortality among persistently

infected cats is approximately 5-fold that of uninfected cats and 83% die within 3.5 yr (McClelland et al., 1980).

Co-infections of FIV and FeLV are believed to work synergistically to result in more severe disease (Grindem et al., 1989; Pedersen et al., 1990; Hofmann-Lehmann et al., 1997). Beebe et al. (1994) suggested that immunosuppression caused by pre-existing FeLV infection affected disease development upon subsequent FIV infection. Feline immunodeficiency virus infected cats experimentally infected with FeLV had more severe disease with a more rapid onset than cats infected with either virus alone. Further, CD4⁺ T-lymphocytes were much more depressed in co-infected cats than cats infected with either virus alone (Hofmann-Lehmann et al., 1995). It is unknown whether the order of infection (FeLV or FIV first followed by the other) is important in the clinical outcome (Hofmann-Lehmann et al., 1997). Finally, virus/virus interactions such as the formation of FeLV/FIV pseudotypes does not appear to be a mechanism of disease potentiation (Beebe et al., 1994).

The outcome following introduction of FeLV into naïve domestic cat populations depends on population size, density, dispersal patterns, and spatial and social structure (Fromont et al., 1998a,b; Fromont et al., 2003). Based on computer models, FeLV becomes established in large natural domestic cat populations at a prevalence of between 0.8% and 12.4% depending on the parameters used (Fromont et al., 1998a,b) and reduces population size by 3% (Courchamp et al., 1997) to 7% (Fromont et al., 1997). Inclusion of FIV in Courchamp's et al. (1997) models more than doubled the population impact of FeLV. Fromont et al. (1998a) also predicted that FeLV fails to become established in

small isolated populations numbering <100 individuals although extinction of the virus may take several years.

Feline leukemia virus can be diagnosed and staged using a variety of techniques. The enzyme-linked immunosorbent assay (ELISA) antigen test is the most common screening method. The ELISA detects soluble p27 antigen in blood (Lutz et al., 1980a) usually within 3 wk post-infection (Hofmann-Lehmann et al., 2001). Positive test results may indicate transient or persistent infection and are an indicator of viremia. Confirmation of positives is accomplished by immunofluorescent assay (IFA), which detects p27 antigen within neutrophils and platelets of blood smears (Hardy et al., 1973). A positive IFA test indicates infection of the bone marrow and usually indicates persistent infection. Viral culture is highly specific and may be used to detect transient, latent, or persistent infections and to identify subgroup. Polymerase chain reaction is a highly sensitive and specific technique that has been used to detect integrated provirus or free FeLV in formalin-fixed tissues, fresh tissues, bone marrow, and blood. Most transient and persistent infections are detectable by PCR 1 wk post-infection and all are detectable by 2 wk (Hofmann-Lehmann et al., 2001). Detection of FeLV antibodies helps stage the disease, especially identifying previous transient infections, but has little importance in diagnosis. Feline leukemia virus ELISA antibodies are most frequently found in those groups clearing the infection (Lutz et al., 1980b). Finally, sequencing of virus is used to identify strain and subgroup. Expected test results during various stages of FeLV infection are summarized in Fig. 1-1.

Infection of non-domestic felids by FIV, also a retrovirus, is relatively common and usually does not result in clinical signs. Approximately 28% of Florida panthers carry the

puma lentivirus strain of FIV (Olmstead et al., 1992) and pathology has not been observed. In contrast to FIV, FeLV infections in non-domestic felids are quite rare. Feline leukemia virus infection has been documented in a handful of captive non-domestic felids including a bobcat (Lynx rufus) (Sleeman et al., 2001), puma (Meric, 1984), clouded leopard (Neofelis nebulosa) (Citino, 1986), and several cheetahs (A. jubatus) (Briggs and Ott, 1986; Marker et al., 2003). Feline leukemia virus has also been isolated from a leopard cat (F. bengalensis) cell line (Rasheed and Gardner, 1981). In all cases, the source of infection was believed to be infected domestic cats.

Despite extensive testing for FeLV in free-ranging felid populations (Rasheed and Gardner, 1981; Mochizuki et al., 1990; Roelke et al., 1993b; Paul-Murphy et al., 1994; Hofmann-Lehmann et al., 1996; Miyazawa et al., 1997; Osofsky et al., 1996; Biek et al., 2002; Munson et al., 2004; Riley et al., 2004; Ryser-Degioris et al., 2005) published reports of FeLV infection have been limited to a puma (P. concolor) in California (Jessup et al., 1993) and a sand cat (F. margarita) in Saudi Arabia (Ostrowski et al., 2003). Approximately 10 to 24% of European wildcats (F. sylvestris sylvestris) were also FeLV positive (Daniels et al., 1999; Fromont et al., 2000), although interbreeding with domestic cats occurs frequently in this subspecies (Daniels et al., 1998).

There have been reports of positive FeLV test results in free-ranging non-domestic felids that were not confirmed with additional tests. Rickard and Foreyt (1992) detected FeLV antigen in 2 of 2 free-ranging pumas found dead in Washington but virus isolation was not attempted. Schmitt et al. (2003) diagnosed FeLV infection by IFA in 11 of 16 (69%) captive and free-ranging felids from Brazil, a biologically inconsistent percentage, but did not confirm the results by ELISA antigen or viral culture.

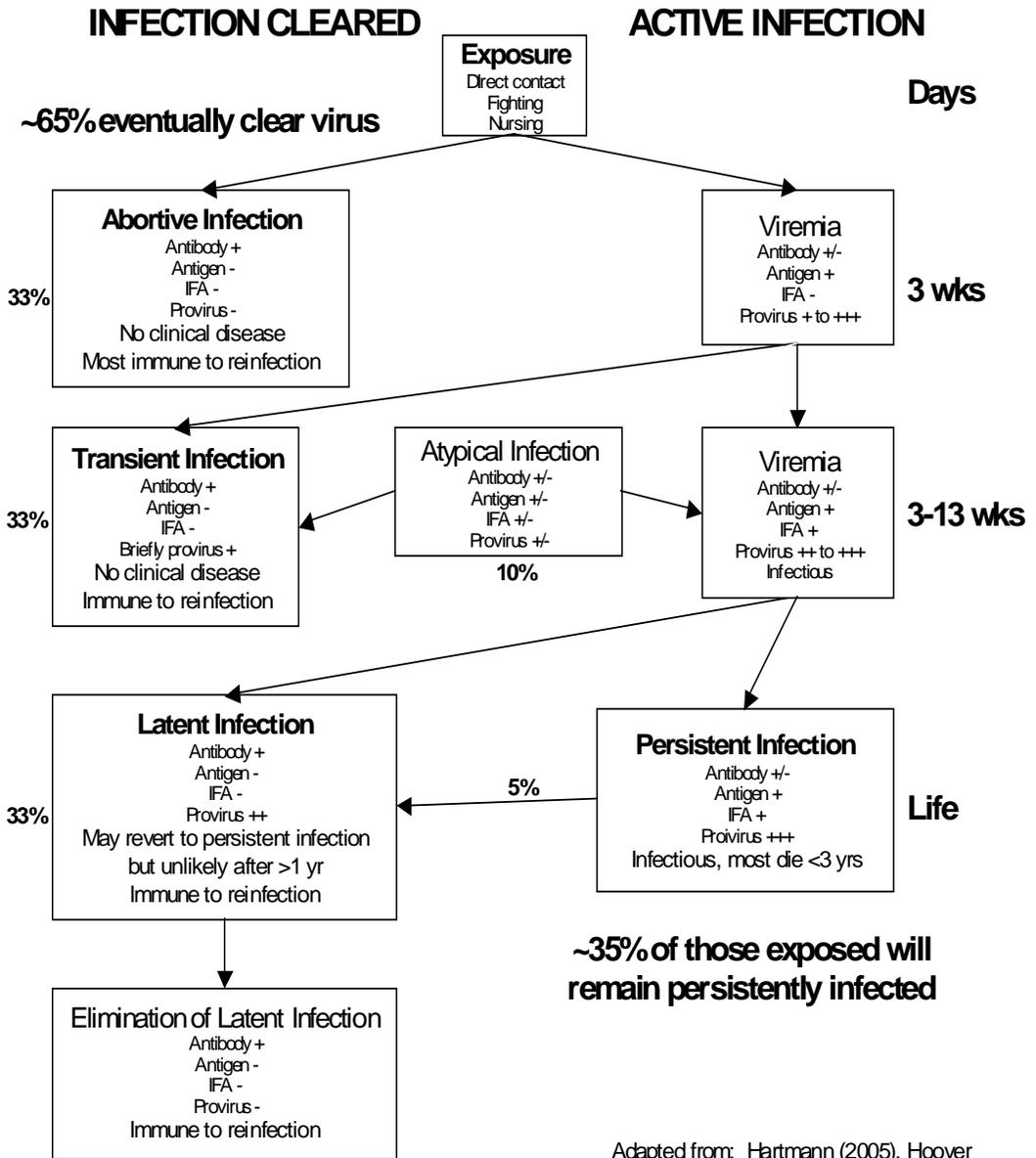
Testing for FeLV antibodies has been performed only rarely on samples collected from non-domestic felids. Feline leukemia virus antibodies were found in a transiently infected captive clouded leopard (Citino, 1986) and two captive Siberian tigers (Panthera tigris altaica) (Meric, 1984).

Most infections in non-domestic felids were self-limiting. In a survey of North American zoos, 7 of 11 (64%) non-domestic felids that originally tested FeLV-positive, were negative when retested. The remaining four were not retested and did not go on to develop clinical signs of FeLV. Clinical signs in non-domestic felids with self-limiting infections were minimal and included lethargy, peripheral lymphadenopathy, and dehydration. Terminal infections were seen in a free-ranging and captive puma, a bobcat, and a cheetah. Clinical pathology and necropsy findings included anemia, lymphopenia, other cytopenias, septicemia, lymphadenopathy, opportunistic infections, and lymphoma (Meric, 1984; Jessup et al., 1993; Sleeman et al., 2001; Marker et al., 2003).

In Florida panthers, routine FeLV ELISA antigen testing was negative since testing began in 1978 through late 2002 (Roelke et al., 1993b; Florida Fish and Wildlife Conservation Commission, unpubl. data); however, during the 2002-2003 capture season, two panthers tested antigen-positive. These findings launched the investigation detailed in this report.

Objectives

The objectives of this study were to determine for FeLV in Florida panthers 1) the history of exposure, 2) the prevalence and geographic distribution, 3) the outcome following exposure, 4) the clinical signs, clinical pathology, and pathological changes associated with infection, and 5) risk factors for infection.



Adapted from: Hartmann (2005), Hoover and Mullins (1991), and Torres et al. (2005).

Figure 1-1. Outcome following exposure to feline leukemia virus in domestic cats.

CHAPTER 2 MATERIALS AND METHODS

Study Area and Period

Florida panthers were sampled in southern peninsular Florida (south of 28° N) primarily in the Big Cypress and Everglades ecosystems. For ELISA antibody comparisons, capture/sampling locations were divided into north and south of I-75 (approximately 28.05° N) (Fig. 2-1). The prospective study period was 1 July 2002 to 5 June 2005. Archived tissues collected between 1990 and 30 June 2002 were retrospectively evaluated. For analysis of ELISA antibody prevalence, the study period was divided into before (1990-1995) and after (1996-2005) genetic restoration. Previously published and unpublished FeLV ELISA antigen test results from 1983 to 30 June 2002 are included in this report (Roelke, 1990; Roelke et al., 1993b; Dunbar, 1994; FWC, unpubl. data).

Florida Panther Capture and Immobilization

Free-ranging Florida panthers and translocated Texas cougars were captured using trained hounds. Panthers either bayed on the ground or were treed, and then were darted with a 3 ml compressed-air dart fired from a CO₂-powered rifle. Since 2002, immobilization drugs included various combinations of ketamine HCl (Congaree Veterinary Pharmacy, Cayce, South Carolina, USA), medetomidine (Domitor®, Pfizer Animal Health, Exton, Pennsylvania, USA), tiletamine HCl/zolazepam HCl (Telazol®, Fort Dodge Animal Health [FDAH], Fort Dodge, Iowa, USA), midazolam HCl (Abbott Laboratories, North Chicago, Illinois, USA), and xylazine HCl (Congaree Veterinary

Pharmacy, USA) (Shindle et al., 2003; Shindle et al., 2004). Following immobilization, treed panthers were caught with a net and, in some cases, a crash bag (McCown et al., 1990). Propofol (PropoFlo™, Abbott Laboratories, North Chicago, Illinois, USA) was administered intravenously (IV) either as a bolus or continuous drip to maintain anesthesia. Butorphanol tartrate (0.1-0.4 mg/kg, FDAH) or midazolam HCl (0.03 mg/kg) was administered intramuscularly (IM) to smooth recovery in some panthers. Panthers were left to recover in a shaded area away from water. Xylazine HCl and medetomidine HCl were reversed with yohimbine HCl (Yobine®, Lloyd, Inc., Shenandoah, Iowa, USA) and atipamezol HCl (Antisedan®, Pfizer Animal Health, Exton, Pennsylvania, USA), respectively, at ½ to ¼ their recommended dosages.

Physical Examination

Vital signs (temperature, heart rate, respiration rate, and capillary refill time) and depth of anesthesia were monitored and recorded. A sterile petrolatum ophthalmic ointment was applied to the eyes for lubrication. All animals underwent a physical examination to assess general health and physical condition. For each panther handled, the skin over the medial saphenous vein was clipped, prepped, and an IV catheter aseptically placed. Sterile isotonic fluids were administered either subcutaneously (SQ) or IV. Panthers were implanted with a SQ transponder identification chip (Trovan®, Douglas, United Kingdom), ear-tattooed, measured, and weighed.

Live-capture Sample Collection

Approximately 70-140 ml of blood (depending on body weight) were collected from the medial saphenous or cephalic veins using a butterfly catheter (19 or 21 gauge), luer adapter/hub, and Vacutainer® tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA) (approximately 50 ml in serum separator, 40 ml in EDTA, 9 ml in Na Heparin, and

9 ml in ACD tubes). From uncollared panthers, eight skin biopsies (4 mm) were collected aseptically from the medial aspect of the hindlimbs and saved in biopsy transport media. Defects were closed with surgical glue. Hair clipped from blood collection and biopsy sites and pulled hair were saved in sample collection bags; clipped hair was saved also from the ventral abdomen. Other samples such as bacterial cultures, skin scrapings, and diagnostic biopsies were taken if indicated. Between November 2002 and April 2004, blood smears were made from EDTA whole blood on glass slides approximately 6 to 24 hr after collection. Beginning May 2004, blood smears were made in the field from fresh whole blood.

Vaccination and Treatment

Panthers >4 mo old were vaccinated SQ against feline viral rhinotracheitis (FVR), feline calicivirus (FCV), feline panleukopenia virus (FPV) (Fel-O-Vax® PCT [FDAH], 1 ml, lower left leg), and rabies (Rabvac™ 3 [FDAH], 1 ml, lower right leg). Beginning June 2003, captive and free-ranging panthers were also vaccinated against feline leukemia virus (FeLV, Fel-O-Vax® Lv-K [FDAH] or Fevaxyn® FeLV, Schering-Plough Animal Health Corporation, Omaha, Nebraska, USA, 2 ml, lower left leg). Some panthers were given a FeLV booster (2 ml) IM remotely by darting 3-16 wk post initial inoculation. Captured panthers were dewormed with ivermectin (0.1 mg/kg, Ivomec®, Merial Limited, Iselin, New Jersey, USA) and praziquantel (3.75 mg/kg, CestaJect™, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri, USA) administered SQ in the lateral aspect of thigh. Penicillin G procaine/benzathine (USVet®, Hanford Pharmaceuticals, Syracuse, New York, USA) was administered IM at 22,000 to 44,000 U/kg.

Radio-instrumentation

Captured adult and juvenile panthers were fitted with a VHF or VHF/GPS radio-collar and monitored three times weekly as described by Shindle et al. (2004). If a mortality signal was detected the carcass was recovered the same day for necropsy.

Neonatal Kittens

Neonatal kittens were handled according to Land et al. (1998) and marked with a SQ transponder identification chip. Pyrantel pamoate (22 mg/kg, Anthelban V, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri, USA) was administered orally. Blood was collected from the jugular vein.

Necropsy

All FeLV-positive Florida panthers and/or those found dead due to infectious disease or unknown causes were completely necropsied by board-certified pathologists at the University of Florida (Veterinary Medical Teaching Hospital, Gainesville, Florida, USA) or Disney's Animal Kingdom (Celebration, Florida, USA). One severely autolyzed FeLV-positive panther (FP109) and all panthers dying of known trauma were necropsied by the FWC veterinarian at the Wildlife Research Laboratory (FWC, Gainesville, Florida, USA).

When carcass condition allowed, tissue samples were collected at necropsy from all major organs. Fluids collected included heart blood, venous blood, thoracic blood, aqueous humor, and urine. Blood samples were centrifuged at 2000 rpm for 10 minutes and the supernatant decanted. Representative tissues from fresh (unfrozen) and some previously frozen panthers were placed in 10% neutral buffered formalin. Fixed tissues were embedded in paraffin, sectioned at 5 to 6 μm and stained with hematoxylin and eosin.

Specimen Storage

All tissues from live-captured and necropsied panthers not immediately analyzed were archived at -20° to -70°C .

Age Determination and Genetic Status

Panther ages were either known (handled as kittens) or were estimated from tooth wear. Panthers were classified as neonates (<8 wk-old), dependents (8 wk to <1 yr), subadults (1 to <2.5 yr), adults (2.5 to <10 yr), and older adults (≥ 10 yr).

Panthers were grouped by genotype (canonical Florida panther, Texas puma, Texas puma/Florida panther intergrade, Texas puma/Everglades/Florida panther intergrade, SIR/Florida panther intergrade, and Everglades/Florida panther intergrade) (W. Johnson, unpubl. data).

Diagnostics

Enzyme-linked Immunosorbent Assay Antibody

Antibodies to FeLV were detected at Hansen Veterinary Immunology (Dixon, California, USA) according to techniques described by Lutz et al. (1980b). Optical densities (OD) of less than 0.25 were considered negative, 0.25 to <0.35 were low positive, 0.35 to <0.5 were medium positive, and those ≥ 0.500 were high positive. For statistical analysis any OD ≥ 0.25 was considered positive.

Enzyme-linked Immunosorbent Assay Antigen

Serum for ELISA antigen testing (ViraCHEK® FeLV, Synbiotics Animal Health, San Diego, California, USA) was shipped overnight to the New York State Diagnostic Laboratory (Cornell University, Ithaca, New York, USA). Adsorbing reagents were used to remove heterophile antibody. Fluids collected from live-captured and necropsied panthers were tested for FeLV antigen with a rapid immunochromatic assay (SNAP

Combo, IDEXX Laboratories, Westbrook, Maine, USA). Beginning November 2003, EDTA whole blood from captured panthers was tested in the field using the SNAP combo. The SNAP Combo was also used to test fluids collected from necropsied panthers. Fluids included blood collected from the thoracic cavity, heart chambers, vessels, and marrow cavity, and aqueous humor.

Immunofluorescent Assay and Immunohistochemistry

Panthers testing positive by ELISA antigen were also tested by IFA.

Immunofluorescent assays were performed on EDTA or fresh whole blood smears using techniques described by Hardy et al. (1973) at the National Veterinary Laboratory (Franklin Lakes, New Jersey, USA). Immunohistochemistry to identify p27 antigen was performed on formalin-fixed paraffin-embedded tissues at the Diagnostic Center for Population and Animal Health (Michigan State University, Lansing, Michigan, USA) using a labeled streptavidin-biotin peroxidase detection system on an automated stainer (Ramos-Vara et al., 2002).

Polymerase Chain Reaction, Genetic Sequencing, and Viral Culture

Polymerase chain reaction and subsequent genetic sequencing was performed at the Laboratory for Genomic Diversity (National Cancer Institute, Frederick, Maryland, USA) on tissues collected from panthers at capture and necropsy. Viral culture was performed at the Center for Retrovirus Research (The Ohio State University, Columbus, Ohio, USA). Materials and methods used, and complete results for PCR and genetic sequencing (M. Brown, unpubl. data) and viral culture (K. Hayes, unpubl. data) will be presented in separate reports.

Complete Blood Count and Serum Chemistry

Complete blood counts (CBC) and serum biochemical parameters were determined by Antech® Diagnostics (Smyrna, Georgia, USA). Blood smears were examined at the Veterinary Medical Teaching Hospital (University of Florida, College of Veterinary Medicine, Gainesville, Florida, USA) for hemoparasites, white blood cell differential counts, and red blood cell morphology.

Other Diagnostic Testing

Necropsied panthers were tested for rabies by IFA at the Jacksonville Central Laboratory (Jacksonville, Florida, USA). Viral isolation and real-time and conventional PCR for canine distemper virus (CDV), pseudorabies virus, flaviviruses, and alphaviruses were performed at the Southeastern Cooperative Wildlife Disease Study (Athens, Georgia, USA) on brain, heart, and other tissues collected from panthers dying of unknown causes.

Other serological tests included Western blot for FIV and kinetics-based enzyme-linked immunosorbent assay (KELA) for feline coronavirus antibodies (FCV) (New York State Diagnostic Laboratory). Polymerase chain reaction for Mycoplasma haemofelis and M. haemominutum was performed on EDTA whole blood from FeLV positive panthers at the University of Illinois (College of Veterinary Medicine, Urbana, Illinois, USA) and Cornell University (Ithaca, New York, USA).

Statistics

Prevalence was calculated as the percentage of panthers/pumas positive for FeLV antibodies by ELISA (OD >0.251). Raw prevalence estimates were examined for each potential categorical predictor (age class, genotype, FIV status, location, time period, and gender). Logistic regression using Egret® software (Cytel Software Corporation,

Cambridge, Massachusetts, USA) was performed to investigate ELISA antibody status as a binary response variable. Odd ratios and their 95% confidence limits were calculated for each state of the categorical predictors in comparison to an arbitrary reference state. Significance of difference from 1.0 was determined for the odd ratios by the Wald test. To account for correlation among replicate outcomes from individuals with multiple test results, panther identification was modeled as a random effect within the logistic regression model. Significance of the random effect was evaluated by a likelihood ratio test. Test results were considered significant at $P < 0.05$. The two significant predictors emerging from univariate analyses (location and time period) were included in a multiple predictor logistic regression analysis and their interactions examined.

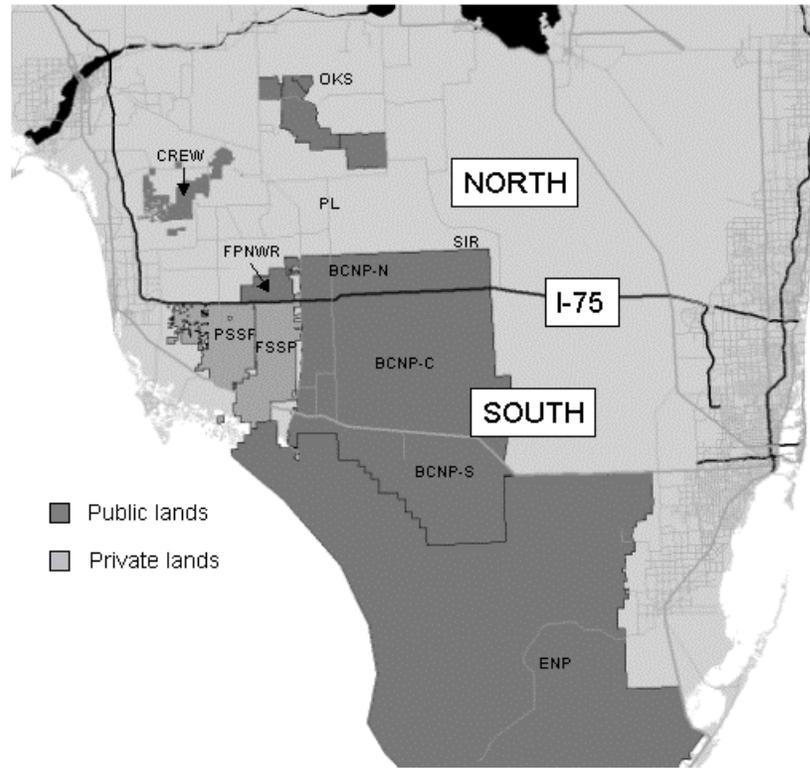


Figure 2-1. Study area in south Florida, USA.

CHAPTER 3 RESULTS

Diagnostic Tests

Enzyme-linked Immunosorbent Assay Antibody

ELISA antibody ODs were determined for samples collected from 128 Florida panthers/Texas pumas on 257 occasions between 2 January 1990 and 29 March 2005. Eighteen (7%) samples from 17 individuals were positive (1 high OD, 3 medium OD, 14 low OD).

The prevalence of positive antibody ODs was significantly greater in the period 1996-2005 compared to 1990-1995 ($P = 0.032$). The prevalence of positive antibody ODs was significantly greater among panthers sampled north of I-75 compared to south ($P = 0.014$). No positive ODs were found in the southern portion of panther range (south of US41). The odds of having a positive antibody OD were not affected by age, gender, genotype, or FIV status. Of panthers sampled on multiple occasions, six had low or medium positive ODs at their initial sampling but seroconverted to negative status when re-sampled 10 mo to 3 yr later.

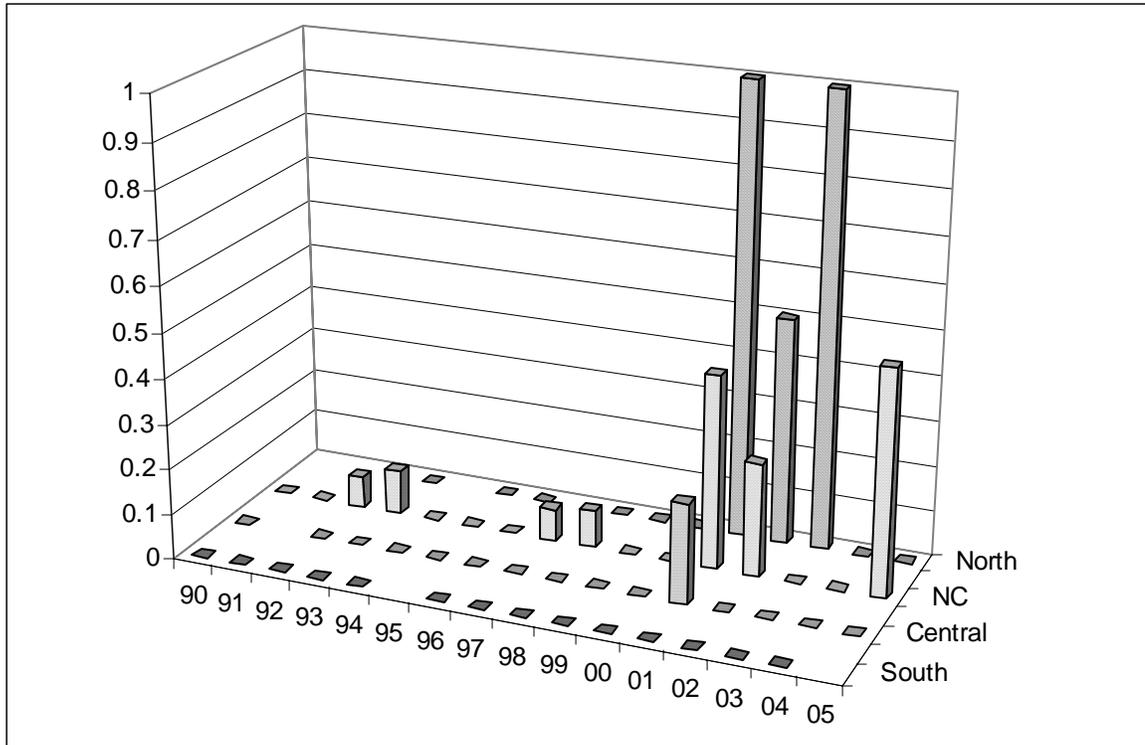


Figure 3-1. Distribution of positive feline leukemia virus positive ELISA antibody optical densities in Florida panthers/Texas pumas by region and year 1990-2005. North refers to lands north of CR846, NC refers to lands between CR846 and I-75, Central refers to lands between I-75 and US41, and South refers to lands south of US41.

Enzyme-linked Immunosorbent Assay Antigen

Prior to the study period, all ($n = 143$ sampled on 322 occasions) Florida panthers and Texas pumas sampled were negative for p27 antigen by ELISA based on review of published and unpublished data and retrospective testing. During the study period (1 July 2002 to 5 June 2005), 91 panthers/Texas pumas were tested on 113 occasions for FeLV antigen by ELISA. Fifty-five panthers or pumas were sampled on 66 occasions at capture, 40 were sampled at necropsy, 10 were sampled at both capture and necropsy, and seven were tested as neonatal kittens. Panther number, age, gender, FIV status, and results of FeLV diagnostic tests are presented in Table A-1.

The prevalence of antigenemia (positive ELISA antigen) among panthers and Texas pumas ≥ 1 yr of age, not previously vaccinated for FeLV, and sampled during the study period, was 7% (5 of 71). All antigenemic panthers were captured in Okaloacoochee Slough (OKS). The prevalence of antigenemia in OKS (NC region, Fig. 3-2) was 45.5% (5 of 11).

Antigenemia was only detected in adult panthers (3 males, 2 females). The average age of antigenemic panthers was 4.85 yr (standard deviation [SD] ± 3.5) and ranged from 2.25 to 11 yr. Genotypes included canonical Florida panthers ($n = 3$), Texas puma/Florida panther intergrade ($n = 1$), and SIR captive/Florida panther intergrade ($n = 1$). Case histories of antigenemic panthers are presented in Appendix B.

Feline leukemia virus antigen was detected by SNAP test in all fluids tested in those viremic panthers suitable for testing at necropsy. Fluids testing positive included thoracic blood (FP115, 122, 123, 132), splenic blood (FP115), venous blood (FP132), and aqueous humor (FP115, 122, 123, 132).

Immunofluorescent Assay and Immunohistochemistry

Three (FP122, 123, 132) of the 5 (60%) panthers positive for FeLV antigen by ELISA were also IFA positive. Results for two viremic panthers (FP109, 115) were inconclusive. Spleen and lymph node from 2 of 2 (100%) viremic panthers (FP115, 132) were positive for p27 antigen by IHC.

Other serology

During the study period, 37.5% of panthers/Texas pumas tested were positive for FIV antibodies by Western blot. Three of five (60%) FeLV antigen-positive panthers also tested positive for the puma lentivirus strain of FIV (J. Troyer, unpubl. data). Serology for FCV was negative for all panthers sampled during the study period ($n = 64$).

Clinical Findings

Clinical signs observed at capture in four antigenemic panthers included a peripheral lymphadenopathy ($\underline{n} = 2$, 50%) and muscle wasting ($\underline{n} = 1$, 25%).

Clinical Pathology

Complete blood counts were performed on four antigenemic panthers sampled while living. Significant findings included a mild to moderate non-regenerative anemia ($\underline{n} = 3$ [75%]), lymphopenia ($\underline{n} = 3$), low hemoglobin ($\underline{n} = 3$), monocytosis ($\underline{n} = 1$ [25%]), and elevated nucleated red blood cell count ($\underline{n} = 1$). Large immature mononuclear cells with prominent nucleoli, consistent with acute lymphoblastic leukemia, were seen in two panthers (FP122, 123, 50%). The mean hematocrit of antigenemic panthers was 29.3% (SD \pm 7.9, range 22.5-42.5%), hemoglobin 9.3 g/dL (SD \pm 2.4, range 7.2-13.2 g/dL), red blood cell count $6.2 \times 10^6/\mu\text{l}$ (SD \pm 1.78 $\times 10^6/\mu\text{L}$, range 4.2-8.75 $\times 10^6/\mu\text{l}$), and lymphocyte count 1165/ μl (SD \pm 811.9/ μl , range 490-2250/ μl). Serum biochemical values in antigenemic panthers were unremarkable. Clinical pathology of antigenemic panthers and normal values for panthers are summarized in Table B-1.

Pathology

Gross

Three antigenemic panthers (FP115, 122, 132) were suitable for complete necropsy based on carcass condition. Completely necropsied panthers had evidence of anemia (pale mucus membranes and skeletal muscle, $\underline{n} = 2$ [66.7%]), moderate to severe dehydration ($\underline{n} = 2$), lymphadenopathy ($\underline{n} = 2$), septicemia ($\underline{n} = 2$), bronchointerstitial pneumonia ($\underline{n} = 2$), abscesses ($\underline{n} = 1$, [33.3%]), and puncture wounds ($\underline{n} = 1$). Lacerations and puncture wounds associated with intraspecific aggression (ISA) were seen in the two autolyzed/decomposed carcasses (FP109, 123).

Microscopic

Microscopic examination was performed on three panthers (FP115, 122, 132). Sections of bone marrow from two (66.7%) panthers were hypercellular with approximately 90 to 100% of the marrow space occupied by hematopoietic cells. Megakaryocytes were present in normal to moderately increased numbers. No marrow evidence of acute lymphoblastic leukemia was seen. Microscopic changes consistent with septicemia were seen in most tissues in FP115 and 132. See Appendix B for complete histological results.

Opportunistic infections

Aerobic culture of multiple tissues from FP115 and 132 resulted in heavy growth of Escherichia coli and β -hemolytic Streptococcus sp., respectively. Rabies IFA and viral isolation and PCR for canine distemper virus, pseudorabies virus, flaviviruses, and alphaviruses were negative in FP122.

Two of four (50%) ELISA antigen-positive panthers (FP109, 115) were PCR positive for M. hemominutum; FP115 was also positive for M. haemofelis (J. Messick, unpubl. data). Organisms were not seen on blood smears made from EDTA whole blood. Rare Cytauxzoon felis organisms were seen on blood smears from FP109; blood smears from FP115, 122, and 123 were negative.

Mortality

Suspected causes of death for the five antigenemic panthers included septicemia ($\underline{n} = 2$), intraspecific aggression ($\underline{n} = 2$), and anemia/dehydration ($\underline{n} = 1$). Time from diagnosis to death averaged 9.25 (SD \pm 10.3, range 2-24.6) wk in panthers antigenemic at capture (FP109, 115, 122, 123). Time from diagnosis to death in the two panthers believed to have died due to FeLV-related diseases was 2 (FP122) and 24.6 wk (FP115).

Time from exposure to death for one panther (FP132) dying of FeLV-related disease was 18.4 wk.

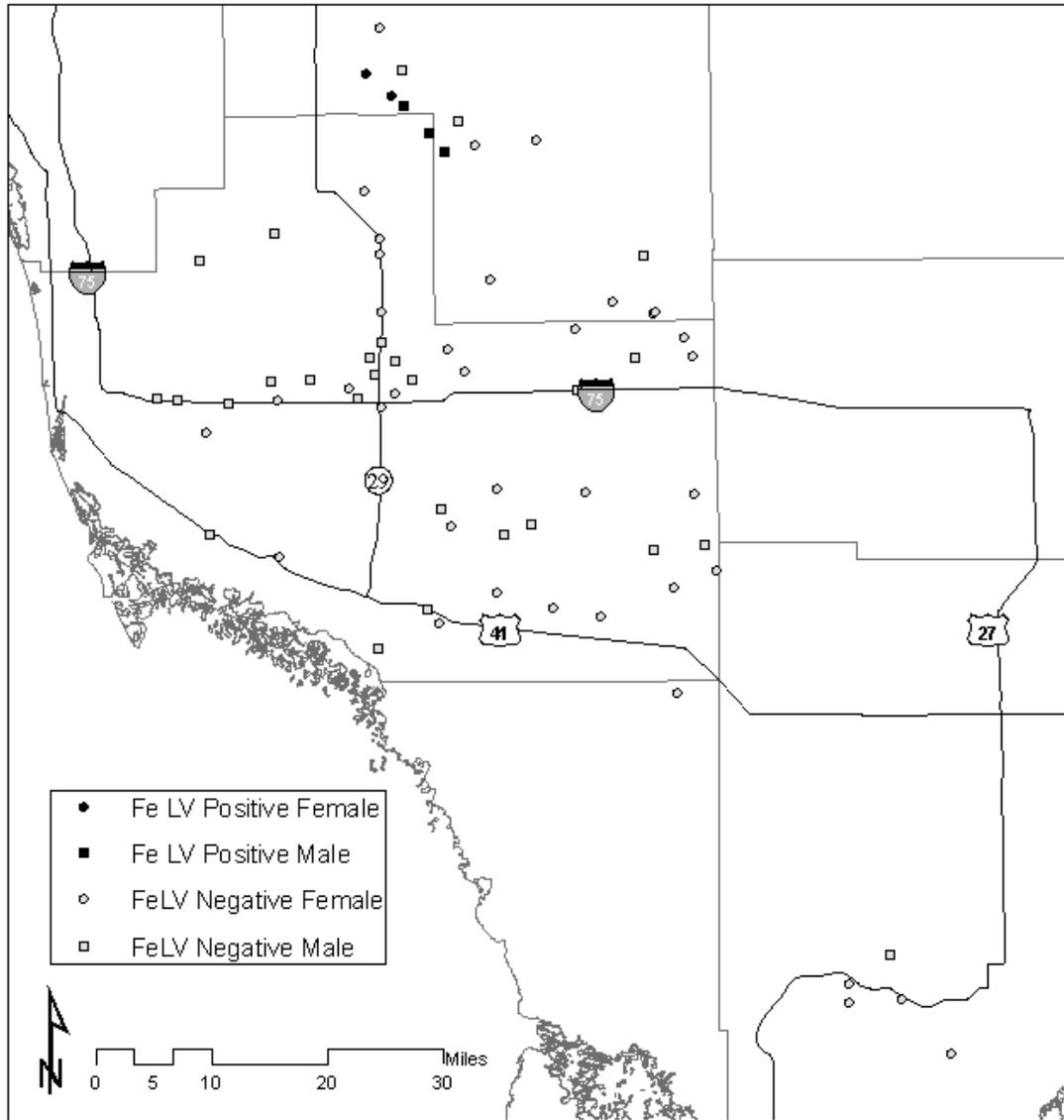


Figure 3-2. Feline leukemia virus (FeLV) ELISA antigen results for panthers ≥ 1 yr, not previously FeLV vaccinated, and sampled in South Florida (south of Caloosahatchee River) between 1 July 2002 and 5 June 2005.

CHAPTER 4 DISCUSSION

Diagnostics

Diagnostic tests validated for domestic animals but used on wildlife must be interpreted with caution (Hietala and Gardner, 1999). Nevertheless, the test results in this study were biologically consistent and appeared to be appropriate and suitable for use in panthers.

The ELISA antibody test detects exposure to FeLV and is considered more sensitive but less specific than Western blot analysis. ELISA antibody testing has only been used rarely in non-domestic felids. Ryser-Degiorgis et al. (2005) found serum from 58 of 102 (58%) Eurasian lynx (L. lynx) to be FeLV positive by ELISA antibody but negative by Western blot. The authors speculated that cross-reactions with E. coli antigen (test preparation) or antibodies to murine leukemia viruses may have been responsible for the false-positive results. Our positive ELISA antibody tests were not confirmed by Western blot. Further, antibody ODs in panther serum were tested incrementally. Incremental testing of serum for ELISA antibodies may lead to inconsistent results due to between-batch variation. Additionally, degradation of antibodies in stored serum may have resulted in the apparent increase in positive ODs in recently collected samples. However, FeLV antibodies are stable when frozen in serum (S. Hansen, pers. commun.). Additionally, ELISA antibody results in Florida panthers were consistent biologically with other test results and observations. Panthers seroconverted following vaccination (data not shown) and positive ODs were geographically and temporally clustered.

ELISA antigen tests detect the FeLV p27 protein and therefore should be suitable for use in exotic species. Nevertheless, false-positives have occurred in some tests that used murine-derived reagents in domestic and non-domestic cats that had naturally occurring anti-murine antibodies (Lopez and Jacobson, 1989). False positive results were reported in one Florida panther tested in 1987 (Lopez, 1988). In this case anti-mouse antibodies were believed to have resulted from vaccination with a rabies vaccine of mouse brain origin. Changes in test procedures and reagents effectively eliminated this problem by the early 1990s (Jacobson and Lopez, 1991). False positives may occur also due to insufficient washing of vessels in micro-well systems (Jarrett et al., 1982), a problem not encountered when using rapid immunoassay test kits.

The effectiveness of using body fluids from known infected panthers for detection of p27 antigen was evaluated. Hemolyzed thoracic, heart, and venous blood; bone marrow; and aqueous humor from infected panthers consistently tested positive by rapid immunoassay (SNAP Combo), even on severely autolyzed specimens. The p27 antigen is only 27,000 daltons and is thus small enough to cross into the aqueous humor in healthy felids (K. Gellatt, pers. commun.). Thus aqueous humor, and the other fluids described above, may be useful for FeLV monitoring not only in panthers but in other populations of pumas.

Immunofluorescent assay and IHC detect p27 antigen in platelets and neutrophils of blood smears and paraffin-embedded fixed tissues respectively. Three of 5 panthers positive by ELISA antigen were also positive by IFA; two (FP109, 115) were inconclusive. Inconclusive results in these panthers may have been due to a delay in testing and/or improper slide storage. Alternatively, if the samples were true negatives,

sampling may have occurred soon after exposure, before infection of the bone marrow. In domestic cats neutropenia or thrombocytopenia can lead to false negatives; however, these values were normal in FP109 and FP115. Spleen and lymph node from FP115 did test positive by IHC when collected 5 mo after initial positive antigen findings. However, tissues from FP109 were severely autolyzed and unsuitable for IHC when collected at necropsy approximately 1 mo after initial positive antigen findings.

Epizootiology

History of Exposure

The FeLV epizootic in free-ranging Florida panthers was foreshadowed by evidence of increasing exposure based on ELISA antibody tests. Beginning in the late 1990s the prevalence of positive ODs increased dramatically, peaking in 2001 when 9 of 26 (34.6%) were positive (Fig. 3-1). Positive ODs were also geographically clustered with 16 of 18 (88.9%) positive ODs occurring north of I-75 (Fig. 3-1). In domestic cats, antibody ODs increase with age; however, this was not seen in panthers – probably the result of small sample size.

ELISA antibody tests support the theory of multiple introductions of the virus. One introduction may have occurred on the Florida Panther National Wildlife Refuge (FPNWR) between January and November of 2001. Four of five (80%) panthers sampled during the spring of 2001 (2000-2001 capture season) were negative for ELISA antibodies. Two of these panthers (FP96, 99) were recaptured the next season (2001-2002). FP96 had seroconverted from a negative to low positive OD, and FP99 went from a low to medium positive OD. Two other panthers captured in the Fall of 2001 also had positive ODs (FP107, low; FP78, medium). Based on telemetry data, FP96 and 107

(siblings) and FP99 formed a loosely associated group between August and December 2001 (Land et al., 2002). This may have facilitated exposure among these panthers if any were shedding virus at the time. Indeed, one of these, FP96, was found to be latently infected (PCR positive, M. Brown, unpubl. data) at necropsy after being killed by another male in early 2002. Although no panthers from FPNWR tested antigen-positive when sampled, we speculate that FP96 became transiently viremic and exposed the panthers that were accompanying him. He apparently overcame the infection, perhaps aided by a relatively high antibody OD.

Prevalence and Distribution

Before the 2002-2003 capture season, routine ELISA antigen testing of captured or necropsied Florida panthers had been negative since 1978. However, between July 2002, and June 2005, 5 of 71 (8%) free-ranging panthers/pumas ≥ 1 yr of age sampled had active FeLV infections based on ELISA antigen, IFA, and/or viral culture results. All infected panthers had overlapping home ranges in the OKS ecosystem in the north-central portion of panther range (Fig. 3-2).

Outcome Following Exposure

In domestic cats, prolonged exposure is generally necessary for transmission. Indeed the percentage of adult domestic cats becoming persistently infected following a single exposure event is only 3% (Hartmann, 2005). However, FP132 became infected apparently after an aggressive encounter with an infected panther (FP123). At examination approximately 2 days after the fight, FP132 had only minor scratches and two puncture (presumably bite) wounds. Although FP132 was FeLV antigen negative at this time, he developed a persistent FeLV infection and died 4 mo later. We speculate that bite wounds are an important mode of FeLV transmission in panthers. Feline

leukemia virus is present in highest concentrations (10^6 infectious units/ml) in the saliva (Francis et al., 1977). A relatively larger dose of saliva would be expected to be transferred between fighting panthers versus domestic cats and may explain the apparent ease of transmission.

The presence of infection in females is evidence that transmission also may occur during breeding. Males and females will pair for 2 to 5 days and transmission may occur during copulation, mutual grooming, or biting. Although FeLV virus is present in the semen of domestic cats, venereal transmission is not considered important (Hoover and Mullins, 1991).

Self-limiting infections

In many respects, the outcome following exposure to the virus in panthers is similar to that in domestic cats. Following exposure, a panther can clear the virus early (abortive/transient infections) or can become latently or persistently infected.

Based on the relatively large number of panthers with positive ELISA antibody ODs but antigen and PCR negative (M. Brown, unpubl. data) test results, many panthers exposed to the virus are able to clear the infection soon after exposure. Assuming a similar pathogenesis to that occurring in domestic cats, panthers in this category would have cleared the infection within several weeks of exposure – before infection of the bone marrow. The majority of domestic cats in this category are considered refractory to re-infection (Hardy, 1980b). Based on telemetry data, at least one female (FP110) with evidence of a previous abortive/transient infection survived exposure to at least two FeLV positive males without developing persistent viremia.

It is possible that FP109 was transiently infected when captured in January 2003. At capture he was anemic, lymphopenic, and had a profound lymphadenopathy. Levy

(1999) described similar signs in transiently infected domestic cats. FP109 also had a high ELISA antibody OD. Antibodies detectable by ELISA appear shortly after infection in domestic cats (Lutz et al., 1980b), and high antibody ODs in domestic cats are a good prognostic indicator for recovery (Hofmann-Lehmann et al., 2001).

There is also evidence that some panthers can become latently infected – as evidenced by positive PCR and antibody ODs but negative ELISA antigen tests. These panthers presumably failed to control infection until later in the course of infection, and retained provirus in leukocytes sufficient to be detectable by PCR. No latently infected panther has developed a persistent infection, and at least one latently infected panther is still surviving in the wild at least 2 yr after diagnosis.

Persistent infections

In domestic cats, persistent infection is usually characterized by bone marrow infection (positive IFA), viremia persisting for ≥ 16 wk, and eventual FeLV-related clinical signs. A diagnosis of persistent infection in panthers was also based on test results, duration of infection, and clinical signs; however, premature deaths, severe autolysis, and limited ability to re-sample panthers while living precluded complete determination of disease progression. Nevertheless, persistent infections were diagnosed in four panthers (FP115, 122, 123, and 132). These diagnoses were based on viremia ≥ 16 wk (FP115), positive IFA (FP122, 123, 132), and the presence of FeLV-related diseases (FP115, 122, 123). The latter criterion is speculative; FeLV-infected domestic cats are subject to the same diseases as non-infected cats (Levy, 1999). Nevertheless, the finding of septicemia in two necropsied infected panthers and apparent acute lymphoblastic leukemia in two live-captured infected panthers appears to be unique to those infected

with FeLV. Septicemia without apparent cause or neoplasia was not observed in 73 panther necropsies performed 1978-1999 (Taylor et al., 2002).

Persistently infected panthers had relatively low antibody ODs. Although FP115 had a medium positive OD, FP122, 123, and 132 had negative ODs suggesting a muted humoral response to infection. In domestic cats, low ELISA antibody ODs are characteristic of persistent infections (Hoffmann-Lehmann, 2001).

Immunosuppression is a common feature of FeLV infection and is believed to result in increased susceptibility to infectious diseases. Co-infections were the most frequent finding in FeLV infected domestic cats examined at North American veterinary schools (Levy, 1999). Infectious and parasitic diseases seen more commonly in FeLV-infected domestic cats than non-infected cats included bacterial infections, hemobartonellosis (Mycoplasma spp.), FCV, upper respiratory infections, babesiosis, stomatitis, coccidiosis, and toxoplasmosis (Grant et al., 1980; Reinacher, 1989; Reinacher et al., 1995). Of these, bacterial infections, including β -hemolytic streptococci, were most important. Jessup et al. (1993) diagnosed septicemia and leptospirosis in a FeLV-infected puma from California.

The most significant apparent opportunistic infections in panthers were bacterial; FP115 had an E. coli septicemia while a mixture of opportunistic bacteria, predominately β -hemolytic streptococci, was cultured from FP132. Other opportunistic infections in viremic panthers may have included M. haemofelis and M. haemominutum; however, approximately 70% of FeLV-negative Florida panthers also tested positive for these mycoplasmas (J. Messick, unpubl. data). Feline infectious peritonitis has not been diagnosed in panthers regardless of FeLV status.

Anemias, primarily non-regenerative, are also a frequent finding in FeLV-infected domestic cats. Non-regenerative anemias were seen in FP109 and FP122 when live captured, and may have been the cause of death in FP122. Severe anemia in FP132 was also suspected at necropsy. Bone marrow sections from FP122 and FP132 were examined histologically. Sections were hypercellular with approximately 90% of the marrow space occupied by hematopoietic cells; however, erythroid precursors were decreased in number, and few maturing erythroid cells were present.

Finally, hematopoietic neoplasias occur frequently in domestic cats (Reinacher, 1989). Under controlled conditions opportunistic infections can be reduced and neoplasia becomes the most important cause of mortality in FeLV-infected cats (Hofmann-Lehmann et al., 1997). Given the apparently rapid progression of infection, FeLV-positive panthers may not have survived long enough to develop terminal neoplasia. Atypical lymphocytes consistent with acute lymphoblastic leukemia were seen on blood smears from FP122 and FP123; however, no evidence of leukemia was seen on histological examination of bone marrow or other organ tissue from FP122 (FP123 was unsuitable for histological examination). The role, if any, in the deaths of these panthers is unknown.

All antigenemic panthers died relatively soon after diagnosis. FP115 and FP132 died from septicemias (*E. coli* and β -hemolytic streptococci respectively). FP122 is believed to have died from severe anemia. FP109 and FP123 died from ISA; however, anemia or other FeLV-related diseases (acute lymphoblastic leukemia) may have impaired their ability to fight. For example, FP109 had a hematocrit of 24% (normal is 36.4% [Dunbar et al., 1997]) when handled 1 mo prior to death, which may have resulted

in exercise intolerance. Although FP123 was apparently healthy when handled 6 wk prior to death, he inflicted only a few minor punctures and scratches to the panther that killed him (FP132). Further, due to severe autolysis, it is unknown if these panthers may have died from secondary bacterial infections. Neither FP109 nor FP123 had obviously fatal ISA-related wounds. Of concern was the presence of healing wounds associated with ISA in FP132 at the time of death – suggesting that he may have exposed another panther before dying. Further, these bite wounds may have been the source of infection leading to septicemia in this panther.

Progression of infection appears to be quite rapid in panthers. Although 50% of viremic domestic cats die within 6 mo of exposure (Jarrett, 1983), adult cats enjoy a longer induction period and less severe disease compared to younger age groups (Hoover et al., 1976; Levy, 1999). All viremic panthers were adults and, although the time of infection is unknown in most infected panthers, the average time from diagnosis to mortality was just over 9 wk. In the one case of known exposure, FP132 died 18 wk after exposure to an infected male. Lack of supportive care and presumably increased exposure to pathogens may play a role in this apparently more rapid clinical course.

Progression to persistent infection following exposure depends on a number of host and viral factors. The most important host factor in domestic cats is age, but inbreeding (genetic variation) and FIV status also affect the outcome. Important viral factors include strain, dose, and duration of exposure. However, with the average age of viremic panthers approaching 5 yr, maturity did not appear to be protective against infection. Genetic variation also did not appear to significantly influence the outcome following exposure. While some panthers had very low genetic variation, at least two had H_e much greater

than the average for the population (M. Roelke, unpubl. data). Ancestry also did not appear to play a role. Although three viremic panthers had canonical or original genotypes, two were intergrades (Florida panther/Texas puma and Florida panther/SIR captive) (W. Johnson, unpubl. data).

Feline immunodeficiency virus and FeLV, both retroviruses, have overlapping host cell tropism. In domestic cats, co-infection with FIV results in marked synergism of immunosuppression and clinical disease induction (Pedersen et al., 1990). Three of five (60%) FeLV viremic panthers were co-infected with FIV (Shindle et al., 2003; J. Troyer, unpubl. data); however, the impact of co-infection in the panther is unknown.

Historically, approximately 28% of the free-ranging panther population was infected with the puma-lentivirus strain of FIV (Olmstead et al., 1992). However, there is a disturbing trend in FIV prevalence – 76% (13 of 17) of panthers captured during the 2004-2005 capture season were positive for FIV antibodies. The consequences of this trend are unknown but if pre-existing FIV infection affects subsequent FeLV infection, then an increasing FIV prevalence may alter the epizootiology of FeLV infection in panthers.

Viral factors may play a more important role in the apparent greater impact of FeLV on panthers. Although infection pressure is expected to be low in this reclusive solitary species, viral load may be quite high. Although the virus concentration in saliva of infected panthers is unknown, the dose transmitted during fighting or breeding would be expected to be higher as panthers may be as much as 15-fold larger than domestic cats. However, virus strain may be the most important factor in the current epizootic. Based on

preliminary viral culture results, the strain isolated from the current epizootic in panthers may be similar to virulent domestic cat strains (K. Hayes, unpubl. data).

Epizootiology

The source of infection in panthers is unknown. Texas pumas introduced in 1995 tested antigen negative during quarantine (Dunbar, 1995), and western pumas in captivity at SIR tested negative when handled in 1999 (Shindle et al., 2000). In reports of FeLV infection in non-domestic felids, authors speculated or provided direct evidence that an infected domestic cat was the source – and this is the most likely explanation for FeLV in panthers. Domestic cat remains have been found in the stomachs of necropsied pumas from California (Jessup et al., 1993) and there have been observations of panthers killing domestic cats in Florida (L. Richardson, pers. commun.). Kennedy-Stoskopf (1999) speculated that “[c]onsumption of FeLV infected domestic cats by larger nondomestic felids would...be an effective way to transmit the virus.”

Private land in panther range continues to be developed at an astounding pace. As humans encroach on panther habitat they are accompanied by their domestic animals, including cats. Indeed free-roaming domestic cats have been observed on private lands near OKS (M. Lotz, pers. commun.). Additionally, the increasing panther population undoubtedly results in increased opportunities for exposure to domestic cats. Young dispersing males move through the fringes of the resident population and often occupy marginal habitat until an established home range becomes available (Maehr, 1997). This existence on the urban/wildland interface likely increases the risk for exposure to domestic cats. Riley et al. (2004) speculated that a higher prevalence of positive feline calicivirus titers in bobcats frequenting urban areas was due to increased exposure to domestic cats.

The transmission of FeLV from a domestic cat to a panther is likely a rare event. Domestic cat remains have never been reported in panther scat (Maehr et al., 1990) or in stomach contents (M. Cunningham, unpubl. data). Further, the odds of a free-ranging domestic cat in Florida having FeLV is less than 1 in 20 (Lee et al., 2002). Should these unlikely events occur the panther would still need to become persistently infected following exposure.

Given that these events, however unlikely, probably did occur, once the species barrier was crossed the virus was likely spread panther-to-panther. The apparent transmission of FeLV from FP123 to FP132 supports this theory. Higher panther densities undoubtedly facilitate this panther-to-panther transmission. The population has tripled since the early 1990s while panther habitat has been reduced.

Feline leukemia virus infection in panthers is likely a disease of adult cats. Although mother-to-offspring transmission is probably the most important mode of transmission in domestic cats (Levy, 2005) it is unlikely to be a factor in the epizootiology of the disease in panthers. Given the apparent severity and rapid progression of the disease in panthers, infected females are unlikely to survive to raise kittens. Further, if a female were to successfully reproduce, infected kittens would be unlikely to survive to independence – the age at which an infected kitten would first be expected to encounter susceptible panthers and potentially spread the virus.

The current epizootic likely began in the OKS area in late 2001 or early 2002, possibly as a result of cross-species transmission from an infected domestic cat on a local ranch. The first positive antibody tests in OKS began to appear in February 2002. The infection was then likely spread panther-to-panther resulting in the infection of at least

five panthers. However, since July 2004, none of 30 panthers have tested FeLV antigen-positive indicating that the epizootic may be over. Several factors may have contributed to this. First, the rapid progression of disease may have limited the number of exposure events – infected panthers die before transmitting the disease. Additionally, some panthers appear to be refractory to infection thus limiting the number of susceptible individuals capable of perpetuating the disease. Small population size and geography may have also helped. Fromont et al. (1998a) demonstrated that populations less than 100 individuals were unlikely to sustain FeLV infections. Since this epizootic occurred in the northernmost portion of panther range the disease could effectively spread only to the south. Finally, vaccination may have helped end the epizootic. Vaccination of free-ranging panthers began in August 2003, and as of June 2005, 34 panthers have received at least one inoculation. Six of these have died due to non-FeLV causes, therefore, based on a population size of between 80 and 100, approximately 28 to 35% of the population has received at least one inoculation. However, because vaccination efforts were targeted at OKS and adjacent lands, the percentage vaccinated in these areas is much greater. Using computer models, Lubkin et al. (1996) estimated that 23% to 73% of a population with a FeLV prevalence of 10% must be effectively vaccinated to eliminate infection.

Conclusion

Kennedy-Stoskopf (1999) speculated “[t]he lack of antigen-positive animals and absence of clustered clinical cases with FeLV-related diseases are evidence that the virus is not maintained in [non-domestic felid] populations.” However, the finding of five antigenemic panthers over almost 2 yr is evidence that the disease had, at least temporarily, become established in the Florida panther population. Small populations are at greater risk of extinction due to infectious diseases than larger populations

(Berger, 1990). With the exception of the occasional dispersing male, all free-ranging Florida panthers are part of a single contiguous population in south Florida. As such, the population is at risk of a catastrophic disease outbreak. Also of concern is the apparent increased FeLV susceptibility of panthers compared to domestic cats. Transmission appears to be occurring despite low infection pressure (few exposure events) and host maturity, and the progression of infection appears to be more rapid compared to domestic cats. Finally, FeLV prevalence among free-ranging domestic cats in Florida is <4% (Lee et al., 2002), and in a review of FeLV in domestic cats, Levy (1999) speculated that “[t]rue ‘outbreaks’ of FeLV infection are unlikely to occur.” However, the prevalence of FeLV in panthers sampled in OKS went from 0% prior to 2002 to 45.5% between November 2002, and June 2005. If the disease spreads to the core population the impact could be devastating.

Further Research

More research is needed to further elucidate the epizootiology of FeLV in panthers. Western blot antibody tests are needed to confirm positive ELISA antibody tests. Further, quantitative PCR may be used to estimate provirus burden in panthers. This technique may be more sensitive than conventional PCR for detecting latent infections. (Hofmann-Lehmann et al., 2001). In domestic cats latent infections may eventually be cleared. Testing to determine if living PCR-positive panthers converted to negative status should be performed to determine the duration of the latent state. Finally, further research is needed to determine the source of infection. Bobcats should be tested in the OKS region. Domestic cats from this area should be tested as well, and virus recovered from infected cats should be sequenced for comparison to strains isolated from panthers.

APPENDIX A
FLORIDA PANTHER/TEXAS PUMAS SAMPLED DURING THE STUDY PERIOD

Table A-1. Florida panthers and Texas pumas tested for feline leukemia virus (FeLV) antigen by ELISA 1 July 2002, to 5 June 2005.

ID ^a	Date	Event ^b	Sex ^c	Age (yr)	Location ^d	Area ^e	FeLV			FIV
							Antibody OD ^f	Antibody result ^g	ELISA antigen ^h	Western Blot ⁱ
FP 32	9/12/2002	N	F	15	FPNWR	North	—	—	N	—
FP 48	2/18/2005	L	F	10.25	BCNP-N	North	0.227	N	N	P
FP 55	4/4/2003	L	F	10	BCNP-C	South	0.188	N	N	P
FP 59	4/20/2004	L	M	9.33	FSSP	South	0.176	N	N	E
FP 59	11/22/2004	N	M	9.42	PL-Collier Co.	North	—	—	N	—
FP 60	6/29/2004	L	M	8.75	BCNP-S	South	0.219	N	N	P
FP 67	1/15/2003	N	F	5.5	PL-Collier Co.	North	—	—	N	—
FP 69	1/3/2005	L	F	7.75	BCNP-N	North	0.252	L	N	P
FP 70	3/10/2003	L	F	5.83	BCNP-C	South	0.131	N	N	N
FP 71	2/18/2004	L	F	6.75	SIR	North	0.173	N	N	N
FP 73	2/27/2003	L	F	6	BCNP-N	North	0.17	N	N	N
FP 75	5/6/2003	L	F	5	BCNP-N	North	0.184	N	N	N
FP 78	10/16/2002	N	F	6	FPNWR	North	—	—	N	—
FP 79	3/17/2004	L	M	8.5	BCNP-C	South	0.116	N	N	N
FP 79	3/3/2005	L	M	9.5	BCNP-C	South	0.176	N	N	P
FP 82	12/6/2002	L	F	6	OKS	North	0.262	L	N	N
FP 83	3/31/2004	L	F	4.8	FSSP	South	—	—	N	—
FP 85	2/18/2003	L	M	4	ENP	South	0.165	N	N	N
FP 85	3/1/2004	N	M	5	ENP	South	—	—	N	—
FP 86	4/1/2003	L	F	3.92	BCNP-C	South	0.165	N	N	N
FP 86	11/6/2003	L	F	3.92	BCNP-C	South	0.094	N	N	N
FP 91	3/18/2003	L	F	3.75	BCNP-C	South	0.216	N	N	N
FP 91	12/12/2003	N	F	4.5	BCNP-C	South	—	—	N	—
FP 94	1/30/2003	L	F	3.5	ENP	South	0.141	N	N	N
FP 95	3/24/2003	L	F	5.17	ENP	South	0.168	N	N	N
FP 98	7/1/2002	N	M	4	SR29	North	—	—	N	—
FP 99	11/27/2002	N	M	2.8	CR846	North	—	—	N	—
FP 100	1/6/2004	L	M	7	BCNP-N	North	0.155	N	N	N

Table A-1. Continued.

ID ^a	Date	Event ^b	Sex ^c	Age (yr)	Location ^d	Area ^e	FeLV			FIV
							Antibody OD	Antibody result ^f	ELISA antigen ^g	Western Blot ^h
FP 102	3/24/2004	L	F	6	BCNP-C	South	0.121	N	N	N
FP 103	2/27/2004	L	F	3.75	BCNP-C	South	0.143	N	N	N
FP 104	12/13/2002	L	M	2.13	BCNP-N	South	0.132	N	N	P
FP 104	3/1/2005	L	M	5.4	BCNP-C	South	0.133	N	N	P
FP 106	2/20/2003	N	F	3	SR29	North	—	—	N	—
FP 107	12/6/2004	L	F	4.7	FPNWR	North	0.23	N	N	P
FP 108	11/17/2002	N	M	1.9	BCNP-C	South	—	—	N	—
FP 109	1/24/2003	L	M	11	OKS	North	0.546	H	P	N
FP 109	2/21/2003	N	M	11.08	OKS	North	—	—	N ^j	—
FP 110	11/25/2002	L	F	1.08	OKS	North	0.196	N	N	E
FP 112	9/11/2002	N	F	4	BCNP-N	North	—	—	N	—
FP 113	10/23/2002	L	F	0.54	FPNWR	North	0.183	N	N	P
FP 113 ^k	4/7/2004	L	F	2	FPNWR	North	0.237	N	N	E
FP 114	10/23/2002	L	M	0.54	FPNWR	North	0.242	N	N	P
FP 114 ^k	10/17/2003	N	M	2.5	FPNWR	North	—	—	N	—
FP 115	11/26/2002	L	F	4.5	OKS	North	0.499	M	P	P
FP 115	5/17/2003	N	F		OKS	North	—	—	P	—
FP 116	1/20/2003	L	F	0.63	PL-Hendry Co.	North	0.149	N	N	N
FP 116 ^k	3/22/2004	L	F	1.8	PL-Collier Co.	North	0.142	N	N	N
FP 117	2/25/2003	L	M	0.88	BCNP-N	North	0.17	N	N	N
FP 117	12/3/2003	L	M	1.58	SIR	North	0.157	N	N	N
FP 117 ^k	7/28/2004	N	M	2.25	OKS	North	—	—	N	—
FP 118	3/5/2003	L	F	0.92	SIR	North	0.157	N	N	N
FP 118	4/2/2003	N	F	1	SIR	North	—	—	N	—
FP 119	4/2/2003	L	M	1	BCNP-C	South	0.125	N	N	P
FP 119	11/17/2004	L	M	2.7	FPNWR	North	0.237	N	N	P

Table A-1. Continued.

ID ^a	Date	Event ^b	Sex ^c	Age (yr)	Location ^d	Area ^e	FeLV			FIV
							Antibody OD	Antibody result ^f	ELISA antigen ^g	Western Blot ^h
FP 120	4/8/2003	L	F	3	BCNP-C	South	0.193	N	N	N
FP 120	7/14/2004	L	F	4	BCNP-C	South	0.163	N	N	P
FP 121	12/2/2003	L	F	2.5	SIR	North	0.148	N	N	N
FP 122	1/30/2004	L	F	2.25	OKS	North	0.183	N	P	N
FP 122	2/13/2004	N	F	2.25	OKS	North	—	—	P	—
FP 123	2/2/2004	L	M	3.5	OKS	North	0.151	N	P	N
FP 123	3/15/2004	N	M	3.6	OKS	North	—	—	P	—
FP 124	2/13/2004	L	F	3.5	BCNP-S	South	0.155	N	N	P
FP 125	2/13/2004	L	M	0.67	BCNP-S	South	0.135	N	N	N
FP 126	2/13/2004	L	M	0.67	BCNP-S	South	0.155	N	N	N
FP 126 ^k	5/28/2004	L	M	0.96	BCNP-S	South	0.163	N	N	—
FP 127	2/16/2004	L	M	2	BCNP-C	South	0.149	N	N	P
FP 127 ^k	3/29/2005	L	M	3	BCNP-N	South	0.196	N	N	P
FP 128	2/18/2004	L	F	3.7	SIR	North	0.171	N	N	N
FP 129	2/20/2004	L	F	3	BCNP-C	South	0.157	N	N	P
FP 130	3/4/2004	L	M	0.8	OKS	North	0.181	N	N	N
FP 130 ^k	3/10/2005	L	M	1.83	PL-Highlands Co.	North	0.385	M	N	P
FP 131	3/10/2004	L	M	5	FPNWR	North	0.193	N	N	N
FP 132	3/17/2004	L	M	3	OKS	North	0.108	N	N	N
FP 132	7/22/2004	N	M	3.33	OKS	North	0.23	N	P	N
FP 133	11/18/2004	L	M	4.5	BCNP-N	North	0.214	N	N	P
FP 134	12/14/2004	L	M	2.5	BCNP-N	North	0.246	N	N	P
FP 135	12/17/2004	L	M	1.7	FPNWR	North	0.24	N	N	N
FP 136	1/13/2005	L	F	5	BCNP-C	South	0.207	N	N	N
FP 137	1/25/2005	L	M	2.5	OKS	North	0.239	N	N	N
FP 138	1/31/2005	L	M	4	BCNP-C	South	0.212	N	N	P
FP 139	3/31/2005	L	M	2.83	OKS	North	0.213	N	N	N

Table A-1. Continued.

ID ^a	Date	Event ^b	Sex ^c	Age (yr)	Location ^d	Area ^e	FeLV			FIV
							Antibody OD	Antibody result ^f	ELISA antigen ^g	Western Blot ^h
K 94	8/17/2004	N	M	3	I75	North-South	—	—	N	—
K 149	6/11/2003	L	F	0.05	OKS	North	—	—	N	—
K 150	6/11/2003	L	M	0.05	OKS	North	—	—	N	—
K 156	8/2/2004	N	M	0.5	US41	South	—	—	N	—
K 175	2/10/2005	L	M	0.04	BCNP-S	South	—	—	N	—
K 176	2/10/2005	L	M	0.04	BCNP-S	South	—	—	N	—
K 178	3/7/2005	L	M	0.05	OKS	North	—	—	N	—
K 179	3/7/2005	L	F	0.05	OKS	North	—	—	N	—
K 180	3/21/2005	L	F	0.05	FPNWR	North	—	—	N	—
K 181	3/21/2005	L	F	0.05	FPNWR	North	—	—	N	—
TX 105	1/27/2003	L	F	10.5	ENP	South	0.161	N	N	P
TX 106	1/8/2003	L	F	9.5	PSSF	South	0.129	N	N	P
TX 108	11/18/2002	L	F	10	ENP	South	0.189	N	N	N

Table A-1. Continued.

ID ^a	Date	Event ^b	Sex ^c	Age (yr)	Location ^d	Area ^e	FeLV			FIV
							Antibody OD	Antibody result ^f	ELISA antigen ^g	Western Blot ^h
UCFP 48	11/11/2002	N	F	0.7	CR846	North	—	—	N	—
UCFP 49	11/25/2002	N	F	1.58	PL	North	—	—	N	—
UCFP 50	1/25/2003	N	M	6.5	PL	North	—	—	N	—
UCFP 51	3/10/2003	N	M	2	NC	North	—	—	N	—
UCFP 54	6/3/2003	N	M	0.75	PL	North	—	—	N	—
UCFP 58	6/30/2003	N	F	0.83	PL	North	—	—	N	—
UCFP 59	11/2/2003	N	M	0.3	CR858	North	—	—	N	—
UCFP 60	12/9/2003	L	M	1.5	BCNP	South	0.144	N	N	N
UCFP 61	12/25/2003	N	F	2.5	CR833	North	—	—	N	—
UCFP 62	1/11/2004	N	F	0.63	BCNP-C-S	South	—	—	N	—
UCFP 63	2/26/2004	N	M	3.5	I75	North-South	0.166	N	N	—
UCFP 65	4/6/2004	N	M	1.5	SR29	North	—	—	N	—
UCFP 66	6/27/2004	N	M	1.75	I75	North	—	—	N	—
UCFP 67	9/2/2004	N	F	0.003	FPNWR	North	—	—	N	—
UCFP 68	9/28/2004	N	F	U	BCNP-C	South	—	—	N	—
UCFP 69	10/25/2004	N	F	2	SR29	North	—	—	N	—
UCFP 70	12/1/2004	N	F	1	SR29	North	—	—	N	—
UCFP 71	2/4/2005	N	M	2.5	US41	South	—	—	N	—
UCFP 74	6/4/2005	N	M	3	195 Flagler Co.	North	—	—	N	—

TABLE A-1 LEGEND

^aFP (Florida panther), TX (Texas puma), K (Florida panther previously handled as a neonatal kitten), UCFP (Uncollared Florida panther).

^bL (live-capture), N (necropsy)

^cF (female), M (male).

^dBCNP (Big Cypress National Preserve – C [BCNP between I-75 and US-41], N [BCNP north of I-75], S [BCNP south of US-41]), CR (County Road), CWMA (Crew Wildlife Management Area), ENP (Everglades National Park), FPNWR (Florida Panther National Wildlife Refuge), I (Interstate), OKS (Okaloacoochee Slough State Forest), PL (private lands), PSSF (Picayune Strand State Forest), SIR (Big Cypress Seminole Indian Reservation), SR (State Road), US (United States Road).

^eN (panther range north of I-75), S (panther range south of I-75), N-S (killed by vehicular collision on I-75).

^fOD (optical density).

^gN (negative), L (low positive), M (medium positive), H (high positive).

^hN (negative), P (positive).

ⁱN (negative), P (positive), E (equivocal).

^jTest results unsuitable due to severe autolysis.

^kPreviously vaccinated for feline leukemia virus.

APPENDIX B
CASE REPORTS: ANTIGENEMIC FLORIDA PANTHERS

FP115

On 26 November 2002, a 4.5 yr-old female Florida panther was captured in OKS. Capture was routine and the panther appeared healthy weighing 52.7 kg. Physical exam was unremarkable and routine biomedical samples were collected. The panther was vaccinated with Fel-O-Vax PCT®, de-wormed, radio-instrumented and released.

Complete blood count revealed a mild non-regenerative anemia (28.4%), low hemoglobin (9.2 g/dL), and lymphopenia (736/μl). Biochemical alterations were limited to an elevated BUN (57 mg/dL), glucose (183 mg/dL), and creatinine phosphokinase (609 U/L). FeLV ELISA antigen and FIV ELISA antibody (KELA and Western Blot) were positive, although IFA of blood smears were inconclusive. Feline leukemia virus was cultured from EDTA whole blood at the Ohio State University (OSU).

Radio-telemetry over the ensuing 5 mo indicated normal movements with a minimum convex polygon (MCP) home range of 104.6 km² (Shindle et al., 2003). However, between 12 and 17 May 2003 movements became increasingly restricted. On 17 May 2003, the panther was located in a palmetto thicket and died at approximately 1730 hrs.

The panther was immediately collected, placed on ice, and transported to Disney's Animal Kingdom the next day for complete necropsy. The panther had lost 20.4 kg since capture; moderate SQ and abdominal fat were present although there was mild muscle wasting. A copious red-tinged mucosy fluid drained from the nares. The right

submandibular lymph node exuded a bloody purulent yellow fluid. The tracheobronchial lymph nodes were markedly enlarged and had a nodular appearance on cut section. A frothy yellow mucoid material was observed at the tracheal bifurcation extending into the distal airways. The lungs had a diffusely mottled red/black appearance with a nodular texture which extended into the cut surface. The caudal lung lobes were most severely affected. The thymus contained multifocal hemorrhages, and the pericardial sac contained fibrin. The mesenteric lymph nodes were diffusely prominent. The stomach contained a small amount of hog hair and mucus. The spleen was slightly enlarged and had a meaty texture. The liver contained 1-3 mm multifocal tan foci. Each ovary contained two 3-5 mm diameter corpora lutea, and the uterus showed no evidence of previous pregnancy.

Histologically, the lungs contained nodular collections of alveoli containing dense colonies of gram-negative bacteria intermixed with degenerative neutrophils, fibrin, extravasated erythrocytes, and necrotic debris. There was necrosis of type I pneumocytes and scattered hyperplasia of type II pneumocytes. Adjacent alveoli contained edema fluid, macrophages, and neutrophils. Within the kidneys there was multifocal mineralization of cortical tubules associated with necrosis of tubular epithelial cells. The tubular epithelial cells contained a golden brown granular pigment. Throughout the splenic parenchyma there was a mild increase of macrophages with mild hyperplasia of white pulp. Megakaryocytes were scattered throughout the red pulp. Fibrin was seen in a few splenic sinuses. Within the submandibular lymph node there was a focally extensive area of necrosis infiltrated by large numbers of degenerate neutrophils. Other lymph nodes showed evidence of multifocal cortical hyperplasia with sinuses containing

macrophages and lymphocytes. All other tissues appeared histologically normal. Immunohistochemistry of spleen and lymph node was positive for p27 antigen.

Aerobic culture of the lung, liver, and submandibular lymph node resulted in pure growth of E. coli. Viral isolation of the lung was negative.

Although IFA was inconclusive at capture, the persistence of antigenemia for >5 mo combined with clinical signs is consistent with persistent infection.

FP109

FP109 was initially captured and radio-instrumented as a 10 yr-old male 10 February 2002, in OKS. At capture he had injuries consistent with intraspecific aggression. ELISA ag for FeLV at Cornell University was negative as was ELISA antibody (HVL). Ten days later FP109 had to be recaptured to replace a defective radio-collar and ELISA ag was again negative. The radio-collar failed several wk later.

FP109 was recaptured 24 January 2003, at 11 yr of age. At capture he appeared to be in excellent condition but had a pronounced peripheral lymphadenopathy. Benign hyperplasia was diagnosed from fine-needle aspirates of the popliteal lymph nodes. Complete blood count revealed a moderate non-regenerative anemia (23.8%), low hemoglobin (7.5 g/dL), and lymphopenia (490/ μ l). Rare C. felis organisms were seen on blood smears. Serum biochemical abnormalities were minor with only an elevated glucose (190 mg/dL) and decreased triglycerides (10 mg/dL). Feline leukemia virus ELISA antigen and antibody tests were positive although IFA of blood smears were inconclusive. Feline leukemia virus was cultured from EDTA whole blood at OSU.

Radio-telemetry indicated normal movements, but on 27 February 2003, FP109 was found dead. The panther had been dead for 2-3 days, and the carcass was severely autolyzed, decomposed, and partially scavenged. Partial necropsy revealed puncture

wounds in the skin over the nasal bones with symmetrical crushing fractures of the nasal bones. Injuries were consistent with ISA.

FP109 died before his true FeLV status could be determined. At capture in January 2003 he was likely in the early stages of infection. Given his high antibody OD, it is possible he may have eventually cleared the infection. Negative ELISA antigen findings in heart blood collected at necropsy supports this speculation; however, the sample was extremely autolyzed and should be considered unreliable.

FP122

On 30 January 2004, a 2.25 yr-old female Florida panther was captured in OKS. Capture was routine and the panther appeared in poor health weighing only 32.3 kg with minimal SQ fat. Physical exam was otherwise normal except for a peripheral lymphadenopathy. Routine biomedical samples were collected, and a SNAP test using EDTA whole blood in the field was positive. The panther was vaccinated, de-wormed, radio-instrumented, and released.

Complete blood count revealed a moderate non-regenerative anemia (22.5%), low hemoglobin (7.2 g/dL), and monocytosis (1020/ μ l). Mild polychromasia, mild to moderate anisocytosis, and 10 nucleated red blood cells/100 leukocytes were seen on peripheral blood smear. Additionally, large immature mononuclear cells that occasionally contained nucleoli were also seen; these findings were interpreted as an acute lymphoblastic leukemia. Serum biochemical abnormalities included low cholesterol (62 mg/dL) and triglycerides (9 mg/dL). Abnormalities seen on urinalysis of free-catch urine included 1+ blood, 3-10 WBC/HPF, 1-3 RBC/HPF, and 4+ bacteria/HPF. Specific gravity was 1.009. ELISA antigen and IFA were positive. Virus was cultured from EDTA blood at OSU.

Radio-telemetry over the ensuing 2 wk indicated normal movements and she remained within the OKS area. Approximately 1 wk after capture, field sign indicated the panther had killed and fed on a white-tailed deer (*Odocoileus virginianus*). However, on 13 February 2004, a mortality signal was detected, and her carcass was found in a hammock in OKS. Time of death was approximately 0500 hrs.

The panther was immediately collected, placed on ice, and transported to Disney's Animal Kingdom the next day for complete necropsy. At necropsy the panther was approximately 15% dehydrated, in poor body condition, and had lost 8.9 kg since capture. Mucus membranes and skeletal muscle were pale. Abdominal and SQ fat were negligible, and there was evidence of serous fat atrophy. Adrenal glands were diffusely enlarged. Peripheral lymph nodes were markedly enlarged.

Histologically the bone marrow was hypercellular with approximately 90% of the marrow space occupied by hematopoietic cells. There was also a moderate increase in the number of megakaryocytes. Erythroid precursor cells were decreased in number, and few maturing erythroid cells were present. Myeloid cell lines were relatively increased in number, and all stages of maturation were observed. Few lymphoid precursors and mature lymphocytes were seen, and there was no marrow evidence of an acute leukemia.

There was no evidence of cortical follicle formation in examined lymph nodes. Thymocytes were present in the thymus, but there was no evidence of cortical or medullary architecture; intermixed among the thymocytes were macrophages containing a bland golden brown pigment. Alveoli contained eosinophilic fluid and mildly increased numbers of alveolar macrophages. Within the spleen, much of the red pulp was autolyzed and there was scattered extramedullary hematopoiesis evident with few megakaryocytes

noted. Within the kidneys, scattered glomeruli were shrunken with markedly thickened Bowman's capsules, collapse of the glomerular tufts, and replacement of the glomerular tufts by fibrillar eosinophilic material. Rare cortical tubules were dilated. Scattered few perivascular infiltrates of lymphocytes and plasma cells were present in the cortical interstitium.

Rabies IFA was negative. Brain and heart were negative for CDV, pseudorabies virus, flaviviruses, and alphaviruses by real-time and conventional PCR and viral culture.

Persistent infection was diagnosed based on positive ELISA antigen and IFA results and clinical signs.

FP123

FP123, a 3.5 yr-old male, was captured 2 February 2004, in OKS. Capture was routine and the panther appeared healthy weighing approximately 64 kg. Physical exam was unremarkable and routine biomedical samples were collected. FeLV SNAP test using EDTA whole blood was positive. The panther was vaccinated, dewormed, radio-instrumented and released.

Complete blood count abnormalities were limited to a lymphopenia (884/ μ l), although a significant percentage (11%) of the differential contained large, apparently immature, mononuclear cells that occasionally contained nucleoli. These findings were interpreted as an acute lymphoblastic leukemia. Serum biochemical abnormalities were suggestive of dehydration and recent feeding (BUN 59 mg/dL, sodium 161 mEq/L, BUN/creatinine ratio 39, triglycerides 222 mg/dL, and calculated osmolality 336 mOsm/L). FeLV ELISA antigen at Cornell Diagnostic Laboratory and IFA of blood smears at the National Veterinary Laboratory were positive.

FP123 had a large home range (164.5 km²) traveling at least 10 km to the south (Shindle et al., 2004). However, within 6 wk of capture, FP123 was found dead 17 March 2004, in OKS following detection of a mortality signal. The carcass was severely autolyzed and decomposed; date of death was believed to have been 15-16 March. FP132 was captured the same day within 400 m of FP123. Acute injuries on FP132 were consistent with ISA.

FP123 was completely necropsied at Disney's Animal Kingdom. No gross abnormalities were noted although the carcass was severely autolysed.

FP132

On 17 March 2004, the carcass of FP123, a FeLV positive male, was recovered in OKS. External injuries indicated the cause of death to be intraspecific aggression, and the panther appeared to have been dead for approximately 24-48 hrs. Within 400 m of the carcass, a freshly killed white-tailed deer was discovered. The dogs were released and FP132, a 3 yr-old male, was captured. Capture was routine and the panther appeared healthy weighing 66.3 kg. Two acute puncture wounds over the right shoulder, presumably bite wounds, were seen on physical examination. Minor lacerations consistent with claw marks were also seen. Circumstantial evidence was consistent with FP132 as the cause of death for FP123. Routine biomedical samples were collected and a SNAP test using EDTA whole blood in the field was negative. The panther was vaccinated (including 2 ml Fel-O-Vax LvK), de-wormed, radio-instrumented, and released.

Complete blood count and serum chemistry were unremarkable. Repeat FeLV ELISA antigen test was negative.

Radio-telemetry over the ensuing 4 mo indicated normal movements with a home range of approximately 197.4 km² (Shindle et al., 2004). FP132 was treed and boosted with 2 ml Fel-O-Vax LvK on 12 April 2004. However, detectable movement based on radio telemetry ceased between 14 and 21 July 2004. On 20 and 21 July, biologists investigated and were able to approach to within 5 m of FP132 in thick brush before he would move ahead. He appeared alert and healthy but lethargic. On 22 July, the panther was located in a palmetto thicket and appeared to be in respiratory distress. He died at approximately 1000 hr.

Within 30 min of death, whole blood was collected by dissection of the brachial artery and aspiration with a needle and syringe. Blood was placed in serum separator and EDTA tubes, and blood smears were made from cells (EDTA). The panther was transported from the field and was on ice within 3 hr of death. Necropsy was performed at Disney's Animal Kingdom. At necropsy the panther was approximately 10% dehydrated and had lost over 13 kg since capture. There was moderate muscle wasting although moderate to heavy SQ and abdominal fat was present. A 15x15 mm pedunculated cutaneous mass was present over the left nasomaxillary region. Mucus membranes were icteric and pale, and a copious red-tinged fluid drained from the nares. Several healing puncture wounds and abrasions were noted in multiple sites.

Gross examination revealed a large abscess occupying the subcutis over the lateral aspect of the right quadriceps muscle. The abscess measured 29x17 cm and had a variable depth of 5-10 cm. The abscess contained several liters of tan cloudy fluid. Skeletal muscle was pale. Lungs were diffusely dark red and firmer than expected. On cut section numerous 1-5 mm tan foci were observed in all lung lobes although the left

cranial and medial lobes were most severely affected. Sections of lung tissue from these lobes did not float in formalin. The liver was pale and friable. Peripheral lymph nodes were not significantly enlarged, but mesenteric lymph nodes were larger than expected. Aerobic cultures were taken of the abscess and lungs resulting in heavy growth of β -hemolytic Streptococcus sp.

Histologically, the skeletal muscle beneath the abscess was covered by a thick band of mixed inflammatory cells representing the margin of the abscess. The superficial aspect was composed of large numbers of degenerate neutrophils subtended by mixed macrophages, lymphocytes, and plasma cells as well as immature fibroblasts and connective tissue markedly expanded by edema. Numerous colonies of large bacterial cocci were present on the superficial aspect of the lesion. Multifocally throughout the lung, large dense colonies of bacteria and associated inflammatory cell aggregates effaced the pulmonary architecture. Smaller bacterial colonies were also common in airways. Large numbers of degenerate neutrophils and alveolar macrophages were present in association with bacterial colonies and within the adjacent parenchyma. There was necrosis of alveolar epithelium with multifocal type II pneumocyte hyperplasia. Alveoli often contained strands of fibrin and edema fluid. Large areas of necrosis and hemorrhage were also present. Numerous small (approx 50um diameter) objects resembling trematode eggs were scattered throughout the liver. These eggs were bounded by a refractile rim with the central core composed of granular basophilic material. Numerous eggs were mineralized, degenerate, and were associated with small numbers of macrophages. Within the thymus there was a loss of architecture and replacement by abundant adipose tissue. There was also loss of cortico-medullary demarcation with

lymphocytes remaining in a loosely arranged fibrovascular stroma. Scattered cystic structures were present and were presumed to be Hassals corpuscles. Also present within the thymus were small mineralized structures which also represented calcified Hassals corpuscles. Increased numbers of large macrophages containing pale brown cytoplasmic pigment were present in the thymic parenchyma. Sections of bone marrow were hypercellular with approximately 100% of the marrow space occupied by hematopoietic cells. Megakaryocytes were present in normal to mildly increased numbers.

Serum biochemical abnormalities were consistent with hepatic failure (total bilirubin 5.8 U/L, ALT 455 U/L, and AST 728 U/L) and pre-renal azotemia and/or renal failure (BUN 63 mg/dL, creatinine 2.8 mg/dL). Other evidence of renal failure included a severe hyperkalemia (9.7 mEq/L), hyperphosphotemia (19.1 mEq/L), hypermagnesemia, and calculated osmolality (338 mOsm/L). These findings were likely a combination of post-mortem artifact (potassium released from platelets), tissue necrosis, metabolic acidosis, and dehydration. Hypoglycemia (61 mg/dL) was likely the result of septicemia.

FeLV SNAP test of serum and aqueous humor, and IFA of blood smears were positive. Immunohistochemistry of spleen and lymph node were positive for p27 antigen. ELISA antigen of serum at Antech Diagnostics was negative, but this is believed to be an erroneous result. Virus was cultured at OSU.

Table B-1. Selected hematological and serum biochemical values for Florida panthers testing positive for feline leukemia virus (FeLV) antigen by ELISA 1 July 2002 to 5 June 2005.

Panther	FP 109	FP 115	FP 122	FP 123	FP 132	Units	Normal ^b (SD) ^c
Sex	M	F	F	M	M	—	—
Date	1/24/2003	11/26/2002	1/30/2004	2/2/2004	7/22/2004	—	—
Age (yr)	11	4.5	2.25	4	3	—	—
FIV ^e western blot	N	P	N	N	N	—	—
FeLV ELISA	P	P	P	P	P	—	—
FeLV IFA ^f blood smear	N	N	P	P	—	—	—
Glucose	190	183	115	120	61	mg/dl	154.4 (51.0)
Blood urea nitrogen	22	57	24	59	63	mg/dl	37.7 (14.1)
Creatinine	2.0	2.1	2.4	1.5	2.8	mg/dl	1.84 (0.54)
Total protein	8.0	6.6	6.5	7.4	5.5	g/dl	7.35 (0.67)
Albumin	3.4	3.2	3.3	3.6	2.7	g/dl	3.70 (0.36)
Bilirubin	0.1	0.2	0.3	0.3	5.8	mg/dl	0.26 (0.61)
Alkaline phosphatase	7	12	8	7	4	U/l	35.4 (38.6)
Alanine aminotransferase	45	36	35	62	455	U/l	60.2 (35.0)
Aspartate aminotransferase	24	36	28	68	728	U/l	73.4 (77.8)
Calcium	10.4	9.3	10.3	9.8	10.2	mg/dl	9.92 (0.66)
Phosphorous	3.6	4.8	5.0	5.2	19.1	mg/dl	—
Globulin	4.6	3.4	3.2	3.8	2.8	g/dl	—
Creatine phosphokinase	223	609	244	470	223	U/l	515.6 (415.1)
Hemoglobin	7.5	9.2	7.2	13.2	—	g/dl	12.21 (1.70)
Hematocrit	23.8	28.4	22.5	42.5	—	%	—
White blood cells	4.9	9.2	8.5	5.2	—	X103/ μ l	12.19 (3.01)
Red blood cells (RBC)	4.91	6.85	4.18	8.75	—	X106/ μ l	7.635 (1.033)
Mean cell volume	48	41	54	49	—	fl	47.29 (2.89)
Mean cell hemoglobin (MCH)	15.3	13.4	17.2	15.1	—	Pg	16.07 (1.41)
MCH concentration	31.5	32.4	32.0	31.1	—	g/dl	34.08 (3.26)
Platelets	147	236	185	350	—	X103/ μ l	402.6 (131.5)
Neutrophils	4165	7360	4845	3848	—	—	8000 (2900)
Neutrophils %	85	80	57	74	—	% WBC's	64.3 (14.3)
Band	0	0	0	0	—	—	—
Band %	0	0	0	0	—	% WBC's	—
Lymphocytes	490	736	2550	884	—	—	3400 (1700)
Lymphocytes %	10	8	30	17	—	% WBC's	28.8 (14.5)
Monocytes	245	368	1020	416	—	—	390 (340)
Monocytes %	5	4	12	8	—	% WBC's	3.2 (2.6)
Eosinophils	0	736	85	52	—	—	420 (310)
Eosinophils %	0	8	1	1	—	% WBC's	3.4 (2.2)
Basophils	0	0	0	0	—	—	100 (60)
Basophils %	0	0	0	0	—	% WBC's	0.89 (0.57)
Nucleated RBC's (NRBC)	0	—	10	0	—	/100WBC's	1.5 (1.0)
T4	1.3	1.6	1.3	0.2	0.2	μ g/dl	—
Cytauxozoon	P	—	N	N	N	—	—

^a ELISA (Enzyme-linked immunosorbent assay).

^b Normal values for panthers (Dunbar et al., 1997).

^c SD (standard deviation).

^d OKS (Okaloacoochee Slough).

^e FIV (feline immunodeficiency virus).

^f IFA (immunofluorescent assay).

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BIOGRAPHICAL SKETCH

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