

Exposure to FIV and FIPV in Wild and Captive Cheetahs

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Two RNA-containing viruses, feline infectious peritonitis virus (FIPV) and feline immunodeficiency virus (FIV), have been observed to infect cheetahs. Although both viruses cause lethal immunogenetic pathology in domestic cats, only FIPV has documented pathogenesis in cheetahs. We summarize and update here a worldwide survey of serum and plasma from cheetah and other nondomestic felids for antibodies to FIV and FIPV, based on Western blot and immunofluorescence assays. FIPV exposure shows an acute pattern with recognizable outbreaks in several zoological facilities, but is virtually nonexistent in sampled free-ranging populations of cheetahs. FIV is more endemic in certain natural cheetah populations, but infrequent in zoological collections. FIV exposure was also seen in lions, bobcats, leopards, snow leopards, and jaguars. FIV causes T-cell lymphocyte depletion and associated diseases in domestic cats, but there is little direct evidence for FIV pathology in exotic cats to date. Because of the parallels with a high incidence of simian immunodeficiency virus in free-ranging African primates without disease, the cat model may also reflect historic infections that have approached an evolutionary balance between the pathogen and immune defenses of their feline host species. Published 1993 Wiley-Liss, Inc.

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INTRODUCTION

The African cheetah has been studied in some detail over recent decades as a principal object of biological conservation. The Species Survival Plan (SSP) of the

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AAZPA and the Felid Taxon Advisory Group (TAG) have agreed to cooperate in designating captive North American cheetahs as an experimental population amenable to genetic, physiologic, and behavioral studies. Such research would contribute to the maintenance of a self-sustaining captive population, as well as stabilizing the free-ranging population [Felid Taxon Advisory Group, 1991]. Preliminary results in each of these areas (reproduction, pathology, behavior, and nutrition) form the basis for the accompanying papers in this volume. In this report, we present an update of the seroprevalence of antibodies to two pathological viruses, feline immunodeficiency virus (FIV) and feline infectious peritonitis virus (FIPV), in cheetahs. The results extend previous reports for both of these viruses [Barr et al., 1989; Olmsted et al., 1992; Letcher and O'Connor, 1991; Heeney et al., 1990; Evermann et al., 1988, 1989], and offer interpretation of their occurrence in the context of comparable data for other felid species.

FIV is a recently isolated T-lymphocyte tropic lentivirus that causes a depletion in the CD4-bearing subset of T-lymphocytes in domestic cats [Pederson, 1987; Pederson et al., 1987, 1991; Yamamoto et al., 1988, 1989]. FIV is genetically homologous and functionally analogous to the human immunodeficiency virus (HIV), the etiologic agent for AIDS. Domestic cats infected with FIV show gradual loss of immune function that in some cases results in AIDS-like opportunistic infections and death [Pederson, 1987; Pederson et al., 1987, 1991; Yamamoto et al., 1988, 1989; Ackley et al., 1990]. Transmission of FIV between individuals can occur by saliva in bites, but other routes have not been adequately demonstrated [Yamamoto et al., 1988, 1989]. Exposure to FIV in nondomestic felid species has been reported [Barr et al., 1989; Letcher and O'Connor, 1991; Olmsted et al., 1992].

Feline infectious peritonitis (FIP) is a fatal disease in domestic cats caused by an RNA-containing immunogenic coronavirus (FIPV). Documented outbreaks of FIP in cheetah colonies show an extremely high mortality (circa 60%), much greater than had been reported for domestic cat epizootics [Evermann et al., 1988, 1989; Heeney et al., 1990]. The correlation of markedly reduced genetic variability in cheetahs with increased mortality with FIPV prompted the hypothesis that genetic homogenization of this species by historic inbreeding contributed to the sensitivity of the species to pathological virus outbreaks [O'Brien et al., 1985, 1986, 1987].

MATERIALS AND METHODS

Serum Samples

Serum and plasma samples from 1,300 felids including 300 cheetahs were collected from U.S. and foreign zoos, wildlife reserves, and free-ranging populations in eastern and southern Africa, from 1991 to the present. These materials were used to screen for antibodies to FIPV, using an immunofluorescence assay plus Western blots as previously described [Evermann et al., 1988, 1989; Heeney et al., 1990].

Virus and Cell Culture

The Petaluma strain of FIV was propagated in a Crandall feline kidney cell line cultured in Dulbecco's modified essential medium, with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were split twice weekly 1:3 in a T-150 tissue culture flask. To harvest virus, 48- to 72-hour culture fluids were collected twice weekly and purified by low speed centrifugation at 2,000 rpm for 15 minutes, fol-

lowed by ultrafiltration through a 0.45 μm membrane to remove any cellular debris. To concentrate virus, the infectious viral supernatant was passed through 5 ml of a 20% sucrose gradient during ultracentrifugation at 27,000 rpm for 3 hours. The viral pellets were air-dried and resuspended in 100 μl of PBS (with Mg^{++}) and stored at -70°C . To monitor viral production during this period, Mg^{++} dependent reverse transcriptase assays were performed biweekly on the infectious culture fluids.

Gel Electrophoresis

Concentrated viral suspensions were separated by SDS-polyacrylamide gel electrophoresis (PAGE). Fifty microliters of viral stock were added to 150 μl of RIPA buffer (1 M Tris HCl, pH 7.5, 5 M NaCl, 0.5 M EDTA, 0.5% deoxycholate, 1% NP-40) and 200 μl of $2\times$ Laemmli sample buffer (0.2 M Tris, pH 6.8, 10% β -mercaptoethanol, 20% glycerol, 4% SDS, and 0.4% bromophenol blue). The mixture was boiled for 5 minutes then chilled on ice for 5 minutes. The mixture was loaded into a preparatory well in a stacked 10–20% SDS-polyacrylamide gel. The viral proteins were resolved at 35 mA of current, and a protein size standard (Amersham, Arlington Height, IL) was included on each gel to determine molecular weights of each protein.

Western Blot Analysis for FIV

The Western blot (immunoblot) procedure used was a modification of that initially described by Towbin et al. [1979]. The viral proteins were transferred from the SDS-PAGE gel onto an Immobilon-p nylon membrane (Millipore, Bedford, MA) using an electroblotting apparatus (BIO-RAD, Hercules, CA). The membranes were then blocked in 3% BSA (in PBS) for 2 hours and dried on 3MM filter paper. After being cut into individual strips, the membranes were incubated in a 1:100 dilution of the serum samples prepared in a 1% BSA, 0.2% Tween-20 diluent. Strips were rinsed thoroughly in 0.2% Tween-20 (in PBS) wash solution and reincubated with a biotin-conjugated goat anti-cat ligand (Kirkegaard & Perry, Gaithersburg, MD) for another hour at 37°C . After repeating the wash cycle, the strips were incubated with an alkaline phosphatase/streptavidin complex which tightly adheres to any bound biotin. The reaction was then developed by a BCIP/NBT substrate (Kirkegaard & Perry), and the membranes were examined for reaction with serum antibodies.

RESULTS

A recent study from our laboratory showed widespread occurrence of antibodies to FIV in captive and free-ranging populations of nondomestic felids [Olmsted et al., 1992]. We extend these findings here to include the Western blot (Fig. 1) analysis of 297 cheetah samples. Sixty samples were collected from the Serengeti ecosystem in East Africa (including Serengeti National Park and the Ngorongoro Crater, Tanzania); Kruger Park, Republic of South Africa; and Etosha Pan, Namibia (Table 1). We also screened 181 serum samples from zoos in North America, Europe, and Australia, plus 56 samples collected at DeWildt Cheetah Research and Breeding Center in South Africa [O'Brien et al., 1985]. These results are compared (Table 1) to exposure levels seen in lion populations from the same locales [Olmsted et al., 1992].

We observed that 20% of the wild cheetah specimens were positive for antibodies to FIV. Twenty-six percent of the cheetahs from the Serengeti were infected, but the sample from Namibia was negative. Similarly, lions from the Serengeti revealed high levels of exposure (84%), while Namibian lions were negative. The

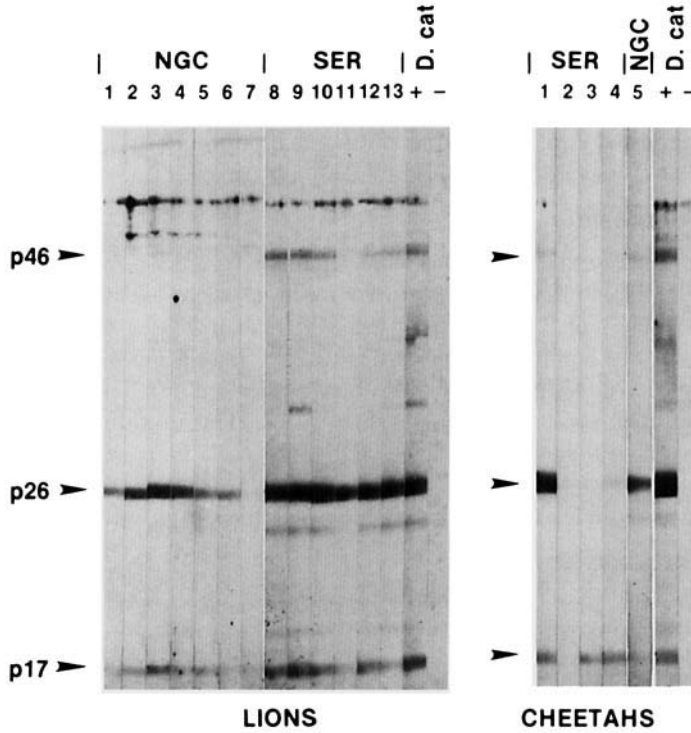


Fig. 1. Western blot analysis of some serum/plasma samples taken from free-ranging East African cheetahs and lions. Lanes 1 thru 7 are lions from the Ngorongoro Crater, and lanes 8 thru 13 include lions from the Serengeti National Reserve, Tanzania. Lanes + and - are positive, FIV-infected sera and negative, control domestic cat sera, respectively. Lanes 1 thru 4 of the cheetah samples were taken from the Serengeti National Park, while the cheetah sample in lane 5 came from the Ngorongoro Crater.

Kruger Park lions had high levels of exposure (91%), but the cheetah sample from Kruger was too limited to draw any conclusions. The captive populations of 237 cheetahs were very low in FIV prevalence, with only 3 individuals (1.3%) displaying antibodies.

FIV exposure was also observed in other feline species including bobcats, leopards, snow leopards, and jaguars (Table 2). To date, there has been no documented evidence for FIV-associated pathology in these species. It is important to note, however, that attention to FIV-associated disease has only recently been directed toward these species.

The occurrence of antibodies to FIPV was monitored by immunofluorescence assays in 87 serum/plasma samples collected in Africa, and in 181 samples collected from 21 North American and English zoological facilities holding cheetahs between 1986 and 1987 (Table 3). Five North American facilities showed increased frequency of FIPV-positive individuals over time, a pattern indicative of an ongoing epizootic [Heeney et al., 1990]. Four other facilities consistently displayed cheetahs with no or very low (≤ 25) antibody titers that did not change over time. We have interpreted these low titers in the absence of pathology or increasing incidence as reflecting cross-reaction to coronavirus not associated with FIP disease [Heeney et al., 1990].

TABLE 1. Seroprevalence of feline lentivirus exposure in cheetahs and lions*

	Cheetahs			Lions	
	No. positive	No. tested	% Positive	No. tested	% Positive
<u>Free-ranging</u>					
East Africa					
-Tanzania					
Serengeti National Park	10	39	26	250	83.6
-Ngorongoro Crater	2	2	100	50	70
-Kenya	0	9	0	—	—
South Africa					
-Kruger Park	0	1	0	44	91
Namibia	0	9	0	44	0.0
TOTAL	12	60	20	388	73
<u>Captive</u>					
DeWildt Cheetah Research and Breeding Center					
-South Africa	1	56	1.8	—	—
Zoological facilities	2	181	1.1	139	22.3
TOTAL	3	237	1.3	139	22.3

*All serum samples (1:100 dilution) were tested by Western blot assay.

TABLE 2. Seroprevalence of feline lentivirus exposure in felid species

	No. positive	No. tested	% Positive
<u>Free-ranging</u>			
Cheetah (<i>Acinonyx jubatus</i>)	12	60	20
Lion (<i>Panthera leo</i>)	284	388	73.2
Bobcat (<i>Lynx rufus</i>)	2	23	9
Tiger (<i>Panthera tigris</i>)	0	5	0.0
Leopard (<i>Panthera pardus</i>)	1	17	5.9
<u>Captive</u>			
Cheetah	3	237	1.3
Lion	31	139	22.3
Tiger	0	97	0.0
Snow leopard (<i>P. uncia</i>)	2	63	3
Jaguar (<i>P. onca</i>)	1	16	6
Leopard	1	122	0.8
Serval (<i>Leptailurus serval</i>)	0	8	0.0
Flat-headed cat (<i>Ictailurus planiceps</i>)	1	3	33
Domestic cat (<i>F. catus</i>)	1	43	2.3

The remaining 12 facilities had some positive titers, but could not be adequately interpreted, because only single time samples were obtained.

Samples from three locations in Africa were assayed as well (Table 3). Most sera were from captive animals, although 25 samples from the Serengeti were free-ranging animals collected in 1985. FIPV antibody titers in Africa were negligible or very low. Although we did not have continuous samples of these individuals, their

TABLE 3. Worldwide prevalence of cheetah coronavirus exposure

Location	Date serum collected	No. of cheetahs with coronavirus titers ^a of				
		≤25	25	100–125	400–625	≤1,600
Wildlife Safari, Oregon	Before June 1982	25	0	0	0	0
	June 1983	0	0	2	18	15
	June 1985	0	1	5	7	3
North American zoos FIPV ⁺ (4 zoos)	1986–1987	43	14	13	19	18
North American zoos FIPV ⁻ (4 zoos)	1986–1987	41	0	0	0	0
North American zoos (11 zoos)	1986–1987	17	6	0	3	7
Whipsnade, U.K. ^b	1985	11	1	0	0	0
Africa						
DeWildt, S. Africa ^c	1982	49	3	0	0	0
Serengeti, Natl. Park	1985	17	7	1	0	0
Kenya, East Africa	1985	8	3	1	0	0

^aIFA titers are expressed as the reciprocal of the highest dilution of serum resulting in positive immunofluorescence. These results are presented in greater detail in Heeney et al. [1990].

^bWhipsnade Park, a preserve operated by the Zoological Society of London.

^cDeWildt Cheetah Research and Breeding Center, Pretoria, Republic of South Africa.

pattern is most similar to FIPV-negative facilities in North America, and likely represents cross-reaction to non-pathological coronavirus.

DISCUSSION

The pattern of FIPV and FIV exposure in cheetahs provides some important parallels as well as some striking differences. Both viruses are pathogenic in domestic cats and threaten the immune system, although by different methods. FIPV stimulates an acute immune reaction to particles that complicate organ function by accumulation of immune complexes in the peritoneum. There are three known forms of FIP infection in cats. The effusive or wet FIP, which causes pleuritis, is usually fatal. The dry form of FIP causes fibrinous peritoneal deposition and is also fatal, while the third form, a subclinical enteritis, is usually not pathologic to domestic cats [Heeney et al., 1990; Pederson, 1987]. FIPV causes a high morbidity and mortality in cheetahs [O'Brien et al., 1985; Heeney et al., 1990; Pederson, 1987], much higher than the same disease in domestic cats.

FIV acts by infection and gradual destruction of the CD4-bearing T-lymphocytes, making the host susceptible to opportunistic infections normally dispatched by immunosurveillance [Ackley et al., 1990; Pederson et al., 1991]. So far there has been no documented immunological or pathological impairment associated with FIV infection in nondomestic felid species. Because T-lymphocyte depletion is observed in FIV-infected domestic cats [Ackley et al., 1990], it seems important to monitor feline T-cell subsets in these species. Until these data are forthcoming, however, it is

not possible to conclude whether FIV has a pathological outcome in nondomestic cat species.

The pattern of exposure to these viruses is very different. FIPV is primarily observed in domestic cats and cheetahs, but only rarely seen in other felid species. By contrast, FIV and its feline lentivirus relatives have been observed in several species in captivity and in the wild, including lions, pumas, bobcats, snow leopards, and cheetahs. A phylogenetic analysis of the *pol* gene sequence of puma lentivirus (PLV) revealed that in PLV, genetic variation was restricted to puma isolates and did not overlap with FIV variation from domestic cats [Olmsted et al., 1992]. These results were interpreted to suggest that the species-specific lentiviruses were evolving independently within their host species and that cross-species transfer of lentivirus is very rare.

An interesting result is the discordance between the incidence of FIV infection in cheetahs (20%) compared to sympatric lions (73%). It may be that the highly social behavior of lions in prides facilitates virus spread in that species while the more isolated social organization of cheetahs makes spread less likely. Proof of this hypothesis, however, would require epidemiological data on the time and manner of FIV spread between individuals in these species.

The absence of serum antibodies to FIV in cheetahs (and lions) in Namibia in the face of widespread exposure in eastern (Serengeti) and southeastern (Kruger Park) Africa suggests that virus spread is restricted geographically, perhaps by the Kalahari desert, an effective faunal barrier in southern Africa. Similarly, Asiatic lions from the Gir Forest Sanctuary are also negative for FIV antibodies [Letcher and O'Connor, 1991; Olmsted et al., 1992]. These geographic partitions, which are also evident from population genetic subdivision of the cat species, suggest that lentivirus infection may have occurred subsequent to their geographic separation. The combined virological, genetic, and phylogenetic analyses of viral occurrence offers an opportunity to discuss a more complete picture of the natural history of the viruses and their infected host species.

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