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## A Serologic Survey of Wild Felids from Central West Saudi Arabia

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**ABSTRACT:** Forty-five wildcats (*Felis silvestris*), 17 sand cats (*Felis margarita*), and 17 feral domestic cats were captured in central west Saudi Arabia, between May 1998 and April 2000, with the aim to assess their exposure to feline immunodeficiency virus/puma lentivirus (FIV/PLV), feline leukaemia virus (FeLV), feline herpesvirus (FHV-1), feline calicivirus (FCV), feline coronavirus (FCoV), and feline panleukopenia virus (FPLV). Serologic prevalence in wildcats, sand cats, and feral domestic cats were respectively: 6%, 0%, 8% for FIV/PLV; 3%, 8%, 0% for FeLV; 5%, 0%, 15% for FHV-1; 25%, 0%, 39% for FCV; 10%, 0%, 0% for FCoV; and 5%, 0%, 8% for FPLV. We recorded the first case of FeLV antigenemia in a wild sand cat. Positive results to FIV/PLV in wildcats and feral cats confirmed the occurrence of a feline lentivirus in the sampled population.

**Key words:** Feline calicivirus, feline coronavirus, feline herpesvirus, feline lentivirus, feline panleukopenia virus, *Felis catus*, *Felis margarita*, *Felis silvestris*, feral cat, sand cat, wildcat.

An important threat world-wide to endangered wild felid populations are diseases transmitted by domestic carnivores (Roelke-Parker et al., 1996; Kennedy-Stoskopf, 1999). During the last decade, feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), and canine distemper virus (CDV) have been shown to infect nondomestic felids exposed to viremic domestic carnivores encroaching their habitat (McOrist et al., 1991; Jessup et al., 1993; Roelke-Parker et al., 1996).

The primary threat facing wildcats throughout their range is hybridization with domestic cats (Nowell and Jackson, 1996). In Saudi Arabia, feral domestic cats are very common in urban areas. However, during the last 30 yr they have increased in numbers outside urban areas (Harrison and Bates, 1991), encroaching in the habitat of two poorly studied and potentially threatened wild felid species; the wildcat

(*Felis silvestris*) and the sand cat (*Felis margarita*). The wildcat is widely distributed throughout the Middle East but is less frequently found close to important urban areas and in the more extensive areas of true desert (Harrison and Bates, 1991). As in Europe, hybridization between domestic cats and wildcats found in the Middle East probably takes place although its extent is not known. The sand cat is certainly the most successfully adapted felid to the environmental constraints of a hyper-arid environment and sand-dwelling existence (Kingdon, 1990). It occurs locally in sandy and stony habitats throughout the peninsula (Harrison and Bates, 1991).

Little is known about the occurrence and prevalence of potentially dangerous viral infectious agents in wild felid populations in the Middle East, an issue of concern in view of the increasing risk of disease transmission between expanding feral cats and indigenous felid populations. In Israel Mendelssohn (1989) has attributed the relative rarity of wildcats to hybridization and their susceptibility to feline panleukopenia transmitted by feral cats. The present serologic survey was initiated to provide baseline information on the occurrence and prevalence of potentially dangerous viral infectious agents in sympatric felid populations in Saudi Arabia.

The study was carried out in Mahazat as-Sayd a 2,244 km<sup>2</sup> fenced protected area in west central Saudi Arabia (28°15'N, 41°40'E) where previous studies have documented the occurrence of the domestic cat, the wildcat, and the sand cat (Olfermann, 1996; Lenain, 2000). The climate is hyper arid (<100 mm rain per year) and there is no permanent surface water in the reserve. There are two small cities respec-

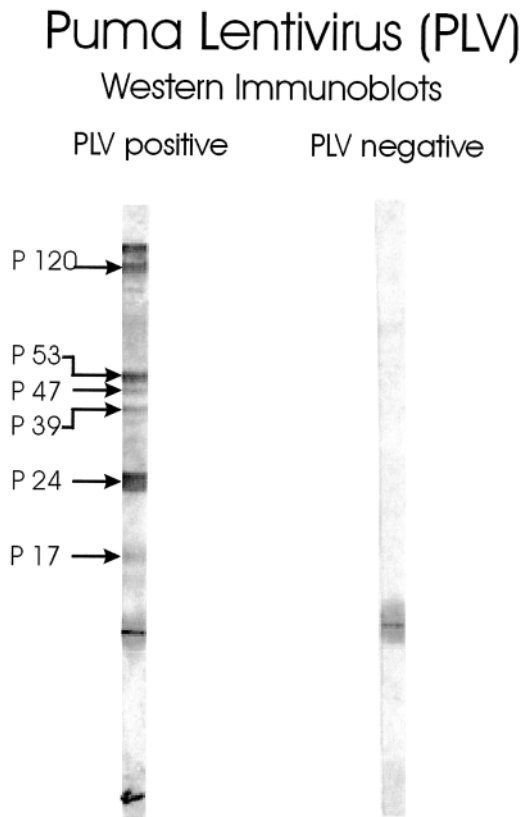


FIGURE 1. Western immunoblot of puma lentivirus (PLV). Felids presenting a p24 band and at least two other viral protein bands were determined as positive. The presence of only a single protein band was interpreted as indeterminate.

tively 15 km north and 25 km southeast of the protected area where feral domestic cats are common.

Animals were collected by two investigators during studies of carnivore population ecology conducted from May 1998 to April 2000 (Lenain, 2000). Forty collapsible double ended live traps (Tomahawk, Wisconsin, USA) baited with pieces of raw chicken were placed 1 km apart within 1×4 km trapping grid, left overnight for four consecutive nights during 24 trapping sessions and checked for capture the following morning. The phenotypes (coat color, pattern of stripes, presence of hair on the sole of the feet, and tarsal length) of wildcats and sand cats were assessed according to criteria described by Harrison

and Bates (1991). Cats that did not fully match these descriptions were categorized as feral. Although a preliminary genetic study did not find evidence of recent hybridization in the wildcat population of Mahazat as-Sayd (Essop et al., 1997) it is possible that hybrids with typical wildcat phenotypes could have been incorrectly categorized. Each cat was removed from the trap, weighed, and sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg; Merial, Lyon, France) and medetomidine chlorhydrate (80 µg/kg; Pfizer, Amboise, France). Two age classes were determined by evaluation of body mass, tooth eruption, and general appearance. Cats without fully-erupted teeth and/or small in size were considered <1 yr old and classified as juveniles. Other cats were classified as adult cats. A blood sample was collected via jugular vein puncture. Cats were released at the site of capture.

Antibodies reactive to feline coronavirus (FCoV), feline herpesvirus type I (FHV-1), feline calicivirus (FCV), and feline panleukopenia virus (FPLV) were detected by means of an indirect fluorescent antibody test (IFA) as previously described (Van Vuuren, 1990). The capture antigens used in the different IFA tests were FCoV strain WSU 79-1683(3), obtained from the American Type Culture Collection (Rockville, Maryland, USA), and field strains of FHV-1, FCV, and FPLV isolated from clinically ill domestic cats and obtained from the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa. The identity of the isolated strains was confirmed by means of electron microscopy and specific FITC-conjugated antisera (VMRD Inc., Pullman, Washington, USA). Viruses were all grown on Crandell feline kidney cells (CrFK) for preparation of the capture antigen slides. The secondary antibody used in the IFA test was sheep anti-cat IgG (H + L) (The Binding Site, Birmingham, UK).

Antibodies against FIV were detected

TABLE 1. Seroprevalence by sex and age for feline herpesvirus (FHV-1), feline calicivirus (FCV), feline coronavirus (FCoV), feline panleukopenia virus (FPLV), feline lentiviruses (FIV/PLV) and feline leukemia virus (FeLV) in wildcats, sand cats, and feral domestic cats, from Mahazat as-Sayd protected area in central west Saudi Arabia.

Species/category	FHV-1	FCV	FCoV	FPLV	FIV/PLV	FeLV
Wildcat	2/40 (5) <sup>a</sup> (0.6–17) <sup>b</sup>	10/40 (25) (12.7–41.2)	4/40 (10) (2.8–23.7)	2/40 (5) (0.6–17)	2/33 (6) <sup>c</sup> (0.8–20.8)	1/33 (3) (0.1–15)
Adult males	0/14 (0)	4/14 (29)	0/14 (0)	0/14 (0)	2/10 (20)	0/10 (0)
Adult females	2/16 (13)	6/16 (38)	2/16 (13)	0/16 (0)	0/14 (0)	0/14 (0)
Juvenile males	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	1/3 (33)
Juvenile females	0/7 (0)	0/7 (0)	2/7 (29)	2/7 (29)	0/6 (0)	0/6 (0)
Sand cat	0/14 (0)	0/14 (0)	0/14 (0)	0/14 (0)	0/14 (0)	1/13 (8) <sup>d</sup> (0.2–38) <sup>b</sup>
Adult males	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	1/9 (11)
Adult females	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Juvenile males	—	—	—	—	—	—
Juvenile females	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Domestic cat	2/13 (15) (1.9–45.4) <sup>b</sup>	5/13 (39) (13.9–68.4)	0/13 (0)	1/13 (8) (0.2–38.5)	1/13 (8) <sup>e</sup> (0.2–38.5)	0/12 (0)
Adult males	1/6 (17)	3/6 (50)	0/6 (0)	1/6 (17)	1/6 (17)	0/5 (0)
Adult females	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)
Juvenile males	1/3 (33)	2/3 (67)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Juvenile females	—	—	—	—	—	—

<sup>a</sup> Number positive/number tested (% positive).

<sup>b</sup> CI<sub>95</sub> = Confidence interval 95%.

<sup>c</sup> One wildcat was positive to FIV ELISA but negative to PLV ELISA and one wildcat was positive to PLV ELISA and PLV WB but negative to FIV ELISA.

<sup>d</sup> The FeLV positive sand cat was re-tested positive 20 mo later.

<sup>e</sup> The domestic cat was negative to FIV ELISA, borderline to PLV ELISA and positive to PLV WB.

according to a previously described method (Kania et al., 1997), with an indirect enzyme linked immunosorbent assay (ELISA) employing a puma-lentivirus-derived (PLV) synthetic peptide as coating antigen (Dr. S. Kania, University of Tennessee, Knoxville, Tennessee, USA). Sera were also tested for FIV antibodies against FIVgag and env(gp40) proteins using a commercial ELISA test licensed for domestic cat (Snap Combo Plus, Idexx, Schiphol-Rijk, The Netherlands). Sera that yielded positive or borderline results with the PLV-ELISA test were re-tested with a PLV-based western blot (PLV-WB) as previously described (Osofsky et al., 1996). The proteins used in the PLV-WB were derived from cell lysates obtained from PLV-infected cells and provided by Dr. W. D. Hardy (The Bronx-Lebanon Hospital Center, Bronx, New York, USA). Finally FeLV p27 antigen was detected with a

commercial ELISA kit (Snap Combo Plus, Idexx). Results were interpreted according to the manufacturer's specifications. All tests included positive and negative controls. Negative control sera were obtained from domestic cats that yielded negative results during previous tests for the respective viruses.

Prevalence was calculated and 95% confidence intervals were determined using exact tables (Thrushfield, 1995). The influence of sex and age on the probability of infection was assessed using Fisher's exact tests. The probability threshold for significance was 0.05.

A total of 79 different felids were captured during 3,840 trap-nights. Forty-five were wildcats (57%), 17 were sand cats (22%), and 17 were feral domestic cats (22%). Results of the serologic tests are indicated in Table 1.

Comparisons of antibody prevalence ac-

ording to sex and age categories were not significantly different ( $P>0.09$ ) apart for a higher prevalence of FCV in adult versus juvenile wildcats ( $P=0.03$ ). When comparing the serologic prevalence of the six infectious agents tested between species, feral domestic cats and wildcats presented a significantly higher risk of infection for FCV than sand cats ( $P=0.01$  and  $P=0.03$  respectively). For other viruses, the risk of infection was not significantly different between species ( $P>0.22$ ).

Do viruses occurring in wildcat and sand cat populations originate from contact with immigrant domestic cats or are the infections self-sustained in indigenous felid populations? In continental Europe, several authors have suggested that domestic cats might pose a threat to wildcats through transmission of infectious agents (Artois and Redmond, 1994; Fromont et al., 2000). Although adult wildcats are strongly territorial and intolerant of other cats and sand cats survive in extreme habitats rarely used by domestic cats (MacDonald et al., 1991; Olfermann, 1996), it is probable that sub-adult domestic cats searching for a home range may cross home ranges of wild cats, increasing the risk of disease transmission through direct contact or environmental contamination (Fromont et al., 2000). Because of the small sample sizes and low antibody prevalences recorded in this study, no conclusions concerning the risk of interspecies horizontal transmission could be drawn. More sampling and description of interspecies patterns of contact are required to address the risk level.

We confirmed the presence of antibodies to the six viruses screened in Mahazat as-Sayd felid population. With the exception of FCV antibody prevalence estimates were low, either lower (FPLV, FIV, FeLV) compared to that found in wildcats in Europe (McOrist et al., 1991; Artois and Redmond, 1994; Daniels et al., 1999; Fromont et al., 2000) or comparable (FCV, FHV-1, FCoV) (Artois and Redmond, 1994; Daniels et al., 1999).

Low antibody prevalences compared to other published studies in European wildcats or in other wild felids (Roelke et al., 1993; Paul-Murphy et al., 1994; Osofsky et al., 1996) may be difficult to interpret due to potential specificity problems. The IFA test may cross-react with parvoviruses other than FPLV (e.g., canine parvovirus) and coronaviruses other than FCoV (Roelke et al., 1993). Low antibody prevalences could also be ascribed to a relative absence of the studied viruses, limited exposure/transmission rate (FIV/PLV, FeLV, FHV-1, FCoV) likely to occur in low density desert populations, or a short-lived immunity (FHV-1) (Horzinek, 1999).

Relatively high ( $>20\%$ ) antibody prevalence to FCV in wildcats and domestic cats supports the hypothesis that this virus actively circulates in the wildcat and domestic cat populations, presumably transmitted via direct contacts. Similarly to that documented by Fromont et al. (1996) in feral domestic cats, we observed a higher prevalence of FCV in wildcats among adults than in young cats. Asymptomatic carriage of FCV could explain this trait as well as long lasting immunity likely to favour a high prevalence in older cats.

The two 20-mo-apart positive tests for p27 antigen also suggest that the sand cat is susceptible to infection with FeLV. To our knowledge this is the first report of a case of FeLV antigenemia in a wild sand cat. We also confirmed the occurrence of FIV/PLV in the sampled population. Using an ELISA licensed for domestic cat, Fromont et al. (2000) recently documented the presence of FIV antibodies in the European wildcat. In our study, the wildcat and domestic cat found seropositive to PLV ELISA and PLV WB did not cross-react in the domestic cat FIV ELISA and conversely the wildcat found positive with the domestic cat FIV ELISA was negative to PLV ELISA and PLV WB suggesting that the particular epitope being recognized was not conserved between domestic and wildcat strains. Because FIV ELISA tests may suffer low sensitivity and

low specificity (Barr et al., 1991; Osofsky et al., 1996; Kennedy-Stoskopf, 1999) the concomitant use of antigens such as puma lentiviral antigens when testing wild felids is recommended (Osofsky et al., 1996).

Because of the high ambient temperature, intense solar radiation, and desiccating conditions prevailing in the study site, it is unlikely that environmental contamination through excretions or dead animals would be of major importance in disease transmission within felid populations in Mahazat as-Sayd. However encroachment of feral domestic cats in the wildcat habitats increases the risk of direct contact and interspecies disease transmission. In view of the fact that sampled feral domestic cats had antibodies against potentially emerging diseases and did encroach in wildcat and sand cat habitats, they represent a potential epidemiologic risk to wild felids in central west Saudi Arabia.

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