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Research paper

Feline immunodeficiency virus (FIV) in wild Pallas' cats

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ABSTRACT

Feline immunodeficiency virus (FIV), a feline lentivirus related to HIV, causes immune dysfunction in domestic and wild cats. The Pallas' cat is the only species from Asia known to harbor a species-specific strain of FIV designated FIV_{Oma} in natural populations. Here, a 25% seroprevalence of FIV is reported from 28 wild Mongolian Pallas' cats sampled from 2000 to 2008. Phylogenetic analysis of proviral *RT-Pol* from eight FIV_{Oma} isolates from Mongolia, Russia, China and Kazakhstan reveals a unique monophyletic lineage of the virus within the Pallas' cat population, most closely related to the African cheetah and leopard FIV strains. Histopathological examination of lymph node and spleen from infected and uninfected Pallas' cats suggests that FIV_{Oma} causes immune depletion in its' native host.

1. Introduction

Pallas' cat (*Otocolobus manul*) is a rare but widely distributed small Felidae species resident in arid, rocky shrub steppe habitats in Central Asia. It is classified as Near Threatened (Convention on International Trade in Threatened Species, 2006) primarily because of habitat loss, over-hunting and prey base depletion through poisoning (Ross, 2009). Pallas' cats in captivity have a unique and marked susceptibility to infectious agents, especially *Toxoplasma gondii*, in comparison to other captive non-domestic cat species (Brown et al., 2005). These and other cases of opportunistic infections have been associated with suspected (Ketz-Riley et al., 2003) and confirmed (Barr et al., 1995) cases of immunodeficiency due to feline immunodeficiency virus (FIV) in captive Pallas' cats.

FIV causes immune dysfunction in domestic cats, resulting in depletion of CD4+ cells, increased susceptibility to opportunistic infections, and sometimes death (Pedersen et al., 1989). FIV is also found in nondomestic felids; a serosurvey of over 3000 specimens from 35 felidae species identified 11 free-ranging felid species infected with FIV (Troyer et al., 2005). Monophyly of FIV proviral sequence within distinct Felidae species suggests that FIV transfer between cat species is an infrequent event (Carpenter et al., 1996; Troyer et al., 2008). FIV is endemic, in African cat species and in species of Hyaenidae and infects nearly all South American felid species (Carpenter et al., 1996; Troyer et al., 2005). Within populations in the wild, seroprevalence is highest in African felids (68–74%), lower in South American felids (5–28%) and nearly absent in Asia and Europe (Troyer et al., 2005). Free-ranging Pallas' cats are the only known species from Asia that have a species-specific strain of FIV (Barr et al., 1995). Only one other case of FIV has been reported in free-ranging Asian cats; a Japanese leopard cat population was infected with a domestic cat FIV_{Fca} strain (Nishimura et al., 1999) through suspected cross-species transmission.

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Pallas' cat FIV, designated FIV_{Oma}, was first isolated from a wild-born male Pallas' cat imported into the United States from Kazakhstan (termed Oma-Barr herein) (Barr et al., 1995). As in recent reports of immune depletion associated with FIV infection in lions and pumas (Roelke et al., 2006, 2009), the infected Pallas' cat also exhibited a low CD4+/CD8+ T-cell ratio and was co-infected with opportunistic infections of *Trypanosoma* species and *Hepatozoon canis*. *In vitro* characterization of this FIV_{Oma} isolate found it to be highly cytopathic in Crandell feline kidney cells in contrast to other isolates of domestic cat FIV (Barr et al., 1995).

In this study, samples from wild Pallas' cats living in central Mongolia were assessed for FIV seroprevalence. Proviral DNA was amplified from Pallas' cats, and cloned FIV sequences from three wild Pallas' cats were analyzed phylogenetically in relation to other known FIV_{Oma} and FIV sequences isolated from other species. FIV_{Oma} was found to be monophyletic with little genetic distance among FIV isolates from disparate geographic locations, suggestive of either a 20th century introduction, a re-emergence of a new strain of FIV, and/or a selective adaptation leading to a unique monophyletic lineage within Pallas' cat populations. In addition, spleen and lymph node from normal and infected Pallas' cats were compared to assess the impact of FIV_{Oma} on immune function of the animal.

2. Materials and methods

2.1. Sample collection and FIV status

Blood samples and necropsy tissues were collected from 28 free-ranging Pallas' cats monitored in a long-term ecology study in Altanbulag, Central Province in Mongolia from 2000 to 2007 (Brown et al., 2005; Ross, 2009). 28 free-ranging Pallas' cats (15 males, 13 females) were identified as Oma 27–32, 35–38, 60–65, 101–1–2, 106–107, 114–115, and 117–122 (Table 1). Sample collection and animal handling was performed as previously described (Brown et al., 2005). Serum and buffy coat aliquots were stored at −70 °C. Fifteen domestic cat serum samples from the region were also included along with sample Oma-34, a wild-caught (Gobi, Mongolia) captive FIV positive Pallas' cat held from 1999 to 2001 at Wildlife on Easy Street Big Cat Rescue (Tampa, FL, USA). Seroprevalence was determined on serum samples by enzyme-linked immunoassays (ELISA) for feline immunodeficiency virus (Petchek FIV ELISA, Idexx Laboratories, Westbrook, Maine, USA) and verified by western blot using the three-antigen detection method using FIV_{Fca}, FIV_{Pco}, and FIV_{Ple} (Troyer et al., 2005) for samples from 10 cats (Oma 27–Oma 38) and the FIV_{Oma} antigen was used for western blots run on eighteen cats

Table 1
FIV-ELISA and FIV-western blot[a] results and demographic information for 28 free-ranging, three wild-born captive, and two captive Pallas' cats.

ID	Sex	Age	Sample year	FIV-ELISA	FIV-WB[a]	Range	Status	GenBank number
Oma-27	F	2 yrs	2000	N	N	Altanbulag	Wild	
Oma-28	F	1–2 yrs	2000	N	N	Altanbulag	Wild	
Oma-29	F	1–2 yrs	2000	N	P	Altanbulag	Wild	
Oma-30	F	1–2 yrs	2000	N	N	Altanbulag	Wild	
Oma-31	M	2 yrs	2000	N	N	Altanbulag	Wild	
Oma-32	M	1–2 yrs	2000	N	P	Altanbulag	Wild	
Oma-35	M	2 yrs	2001	N	N	Altanbulag	Wild	
Oma-36	F	1–2 yrs	2001	N	N	Altanbulag	Wild	
Oma-37	F	1–2 yrs	2001	N	P	Altanbulag	Wild	
Oma-38	F	2 yrs	2001	N	N	Altanbulag	Wild	
Oma-60	M	1–2 yrs	2004	N	N^	Altanbulag	Wild	
Oma-61	M	1–2 yrs	2004	P	nd	Altanbulag	Wild	Reported here
Oma-62	F	3 yrs	2005	N	N^	Altanbulag	Wild	
Oma-63	F	1–2 yrs	2005	N	N^	Altanbulag	Wild	
Oma-64	M	1–2 yrs	2005	N	N^	Altanbulag	Wild	
Oma-65	M	1–2 yrs	2005	N	N^	Altanbulag	Wild	
Oma-101	F	3–5 yrs	2006	N	N^	Altanbulag	Wild	
Oma-102	F	3–5 yrs	2006	N	N^	Altanbulag	Wild	
Oma-106	F	3–5 yrs	2006	N	N^	Altanbulag	Wild	
Oma-107	M	3–5 yrs	2006	N	N^	Altanbulag	Wild	
Oma-114	F	3–5 yrs	2007	N	N^	Altanbulag	Wild	
Oma-115	M	8 mo	2007	N	N^	Altanbulag	Wild	
Oma-117	M	1.5 yrs	2007	N	N^	Altanbulag	Wild	
Oma-118	M	1.3 yrs	2007	P	P^	Altanbulag	Wild	Reported here
Oma-119	M	1.5 yrs	2007	P	nd	Altanbulag	Wild	Reported here
Oma-120	M	10 mo	2007	N	N^	Altanbulag	Wild	
Oma-121	M	1.5 yrs	2007	P	P^	Altanbulag	Wild	Reported here
Oma-122	M	2 yrs	2007	N	N^	Altanbulag	Wild	
Oma-34	M	10 yrs	2001	P	P	South Gobi	Wild-born captive	AY878240
Oma-12	M	6 yrs	1992	nd	P	Russia	Wild-born captive	AY878239
Oma-21	F	8 yrs	1998	nd	P	Russia	Captive-born	AY878241
Oma-22	M	5 yrs	1998	nd	P	China	Captive-born	AY878238
Oma-Barr	M	1–2 yrs	1992	P	P	Kahzakstan	Wild-born captive	U31349

ID: Pallas' cat identification number. FIV positive Pallas' cats are highlighted in grey. F: Female; M: male; yrs: years; mo: months; N: negative; P: positive; nd: not done. FIV-WB: Western blot. Three-antigen detection method using FIV_{Fca}, FIV_{Pco}, and FIV_{Ple} (Troyer et al., 2005) run on Oma-27 through Oma-38; FIV_{Oma} (Barr et al., 1997) antigen run on Oma 60–Oma 122 (^). Additional sequences of FIV_{Oma} from wild-born captive Pallas' cats from disparate geographic regions are also listed (Oma-12: Zoo accession number {ZAN A00318}, Moscow Zoo Dr. Vladimir Spitsin, Oma-21: ZAN 900236, studbook number 243 Brookfield Zoo, Dr. Mike Briggs, Oma- 22: ZAN 950012 studbook number 273 Brookfield Zoo, Dr. Mike Briggs, Oma-Barr) (Troyer et al., 2005).

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(Oma 60–Oma 122; see ^ in Table 1) (Cornell University Animal Health Diagnostic Center, Ithaca, NY, USA).

2.2. PCR amplification of proviral DNA

Genomic DNA was isolated from buffy coat samples from the 28 wild Pallas' cats and Oma-34 (Table 1). Briefly, the buffy coat was digested in proteinase K followed by standard DNA extraction using the QIAGEN DNeasy tissue DNA extraction kit (QIAGEN, Valencia, CA, USA). Isolated DNA was visualized by electrophoresis on a 1% agarose gel using ethidium bromide loading buffer and quantified by using a UV spectrophotometer (Bio-Rad, Hercules, CA, USA). The viral gene region of interest was amplified from 50 ng of genomic DNA using PCR primers (Forward/Reverse primers: 5′-TTTAAAAGCTTGCCCACCAC-3′/5′-CATTCCCCAATGTCCT-TTTG-3′) designed from *RT-Pol* FIV_{Oma} (Oma-Barr: accession number U56928; Barr et al., 1997). Amplification was performed in a 50 μL reaction using 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, with 0.25 mM concentrations of dATP, dCTP, dGTP, and dTTP, 2 mM concentrations of each primer, and 2.5 units of Platinum Taq polymerase (Applied Biosystems). Reactions were performed by GeneAmp PCR system 9700 thermocyclers (Applied Biosystems) with the following touchdown conditions: 2 min at 95 °C followed by 3 cycles of 20 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C; annealing temperature was then dropped 2 °C every 5 cycles until it reached 50 °C, where it was kept for 22 cycles; followed by a final elongation at 72 °C for 2 min. PCR products were cloned using TOPO-TA cloning kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA was isolated using a QIAGEN Miniprep Kit. Sequences were obtained from clones by using internal primers in standard ABI BigDye terminator (Applied Biosystems) reactions.

2.3. Phylogenetic analysis

Nucleotide sequences were compiled and aligned for subsequent phylogenetic analysis by ClustalX 2.0.11 (Thompson et al., 1997) and verified visually. Phylogenetic analyses in PAUP4.0 (Swofford, 2002) were performed as previously described (Troyer et al., 2005) for the following methods: minimum evolution, maximum parsimony, and maximum likelihood. Modeltest 3.7 (Posada and Crandall, 1998) was used to estimate the optimal model of sequence evolution, and these settings were incorporated into subsequent analyses. Genetic distances were calculated in MEGA 3.0 (Kumar et al., 2004) by using the Tajima-Nei (nucleotide) and Pam-Dayhoff (amino acid) algorithms. The sequences of FIV_{Oma} were deposited in GenBank under accession numbers GQ370820–GQ370824.

2.4. Pathology

Tissues sampled from spleen, liver, lymph node, intestine, and kidney from FIV-negative Oma-107, a deceased wild Pallas' cat from the Altanbulag study site, were cut into sections approximately 1 cm^3 thick and stored in 10% neutral buffered formalin and routinely processed and embedded in paraffin. Sections (5 μm) were stained with haematoxylin and eosin (HE) (National Cancer Institute Laboratory Animal Sciences Program, Frederick, MD, USA) and examined histologically by a board-certified veterinary pathologist (RS). Similar tissues were obtained, processed, and evaluated from FIV-positive Oma-34 in 2001.

3. Results

Seroprevalence of FIV in twenty-eight free-ranging Pallas' cats found in the central province of Mongolia (Altanbulag) sampled from 2000 to 2007 was 25% based on FIV ELISA and western blot results (Table 1). While western blots run with FIV_{Oma} antigen were concordant with FIV ELISA tests (Table 1), the three-antigen detection method (using FIV_{Fca}, FIV_{Ple}, and FIV_{Pco}) was more sensitive than the ELISA, picking up a signal in three cats (Oma-29, Oma-32, and Oma-37) that were negative by ELISA. Of the seven FIV seropositive wild cats, 5 were male. Additionally, 15 of 15 (9 males) domestic cats found in the rocky steppe around and within the village of Altanbulag were FIV negative by ELISA.

Histopathological examination of lymphoid tissues for FIV positive (Oma-34) and FIV negative (Oma-107) Pallas' cats revealed several histopathological changes in the FIV positive individual. These included loss of normal tissue architecture and the absence of follicles indicative of severe lymphoid depletion in the spleen (Fig. 1) and moderate depletion of small lymphocytes within the lymph nodes (not shown).

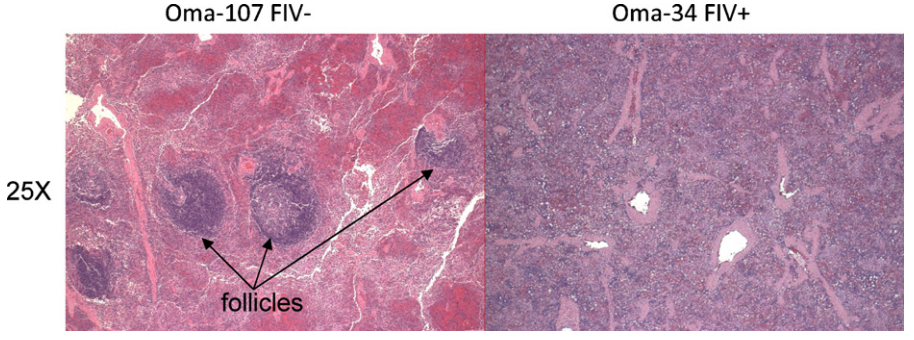

Fig. 1. Histopathology of spleen from an FIV positive (Oma-34) versus FIV negative (Oma-107) Pallas' cat from Mongolia. Note the loss of normal tissue architecture and lack of large follicles in Oma-34. HE slides shown at 25× magnification.

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A 494 bp fragment of proviral *RT-Pol* FIV sequence was obtained from three of the free-ranging Pallas' cats (Oma-61, Oma-118, and Oma-121) and from one FIV-positive wild-born captive Pallas' cat (Oma-34). PCR fragments were cloned and a total of 78 cloned sequences from these 4 cats were produced, resulting in 23 unique sequences.

FIV sequence from four additional wild-born captive and captive-born Pallas' cats from Russia (Oma-12, Oma-21), China (Oma-22) and Kazakhstan (Oma-Barr) were included for phylogenetic analysis. The FIV_{Oma} sequences from these eight cats, representing disparate geographic ranges, were monophyletic within the Pallas' cat species relative to other FIV species. There was no significant structure relating to geographic distribution within the Pallas cat viral sequences (Fig. 2). In comparison to FIV isolated from other felid species, FIV_{Oma} is most similar to FIV_{Ppa} (leopard) and FIV_{Aju} (cheetah) (Fig. 2) with a mean genetic distance of 11% and 14.6%, respectively. Mean percent DNA sequence differences among individual Pallas' cat FIV *RT-Pol* cloned sequences were calculated and found to be minimal (Table 2). The genetic variation among 27 available FIV_{Oma} sequences was 1.9%.

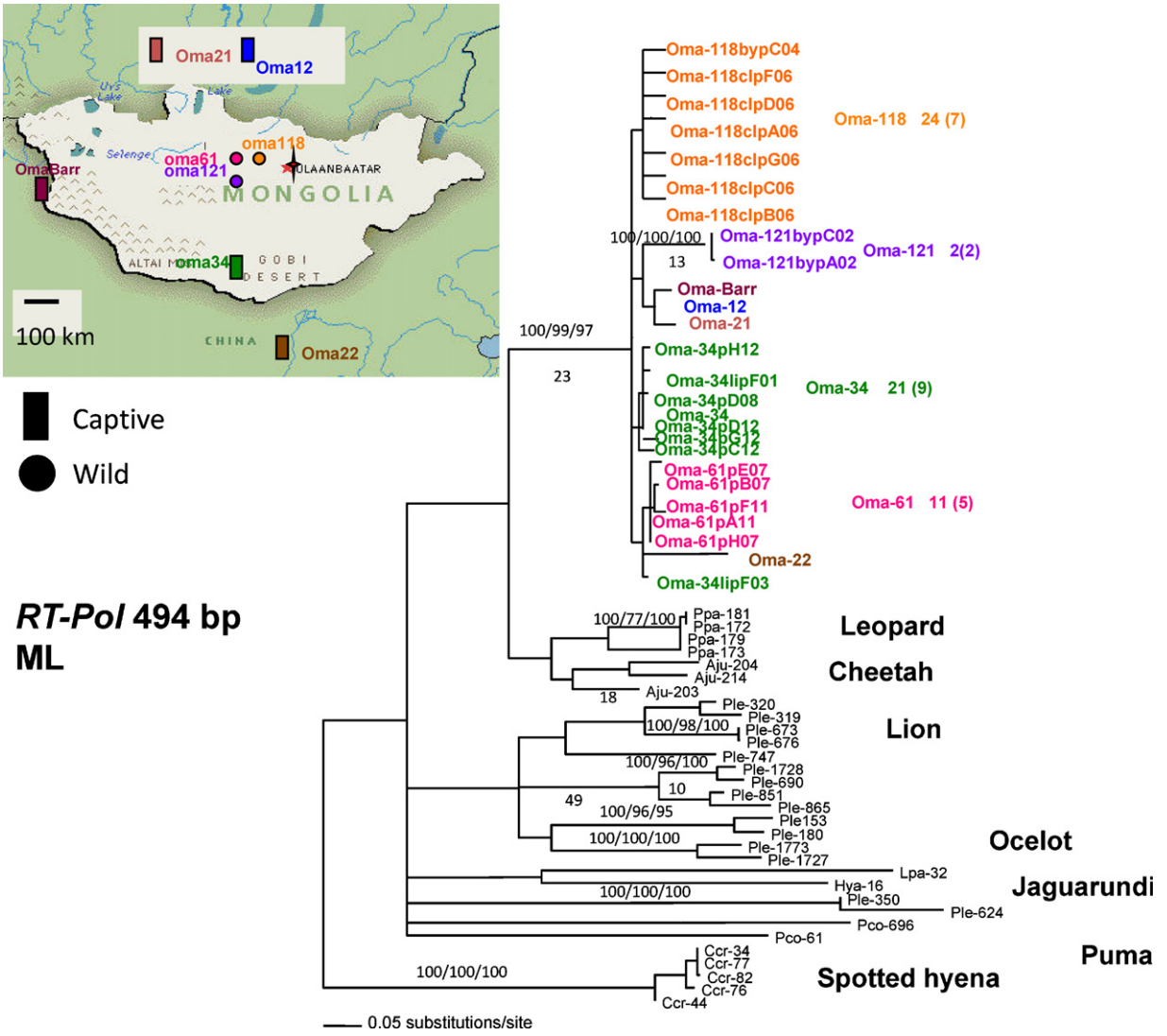

Fig. 2. Phylogenetic tree of proviral *RT-Pol* (494 bp) FIV sequence highlighting the monophyletic clade of the eight FIV_{Oma} and geographical origin of FIV_{Oma}-infected Pallas' cats reported in this study. Total number of clones and the number of unique clones (in parentheses) generated is shown for each Pallas' cat (see Table 2). Maximum likelihood tree is shown. Bootstrap values (maximum parsimony/minimum evolution/maximum likelihood) are reported when greater than 70. When maximum parsimony tree topology is concordant with maximum likelihood tree, number of steps is indicated below the branches. The score (−ln likelihood) of the best maximum-likelihood tree was 3723.037761, consistency index [CI] = 0.321, retention index [RI] = 0.701. Maximum likelihood parameters specified by MODELTEST selected the general time-reversible model of substitution; they included empirical base frequencies and estimated rate matrix and corrected for among-site rate variation (γ distribution). GenBank accession numbers used in this analysis: for FIV_{Ple} (lion) (AY878208–AY878222), FIV_{Pco} (puma) (AY878236–AY878237), FIV_{Ccr} (spotted hyena) (AY878196–AY878200), FIV_{Aju} (cheetah) (AY878201–AY87203), FIV_{Ppa} (leopard) (AY878204–AY878207), FIV_{Lpa} (AY878194) (ocelot), FIV_{Hya} (jaguarundi) (AY878195), FIV_{Oma}-22,34,12,21,Barr (Pallas' cat) (AY878238–AY878241, U31349). On map, circle indicates wild Pallas' cat while rectangular bar indicates wild-born captive or captive Pallas' cat (see Table 1).

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Table 2
Mean percent nucleotide differences among individual cloned FIV_{Oma} isolates in the *Pol-RT* region.

	Genetic distance (%)	No. of clones	No. of unique sequences
Oma-34	0.3	21	9
Oma-61	0.4	11	5
Oma-118	0.3	24	7
Oma-121	0.2	2	2
Total[a]	1.2		27

[a] Includes FIV sequences from Oma-12, Oma-21, Oma-22, and Oma-Barr.

4. Discussion

This is the first report of FIV isolated from a free-ranging species in Asia: the wild Mongolian Pallas' cat. Serosurvey of 28 wild Pallas' cats sampled from 2000 to 2007 detected a 25% (7 of 28) seroprevelence of FIV by ELISA and western blot. Phylogenetic analysis of 27 unique cloned 494 bp FIV *RT-Pol* sequences established a monophyletic grouping and low genetic distance among all available FIV_{Oma} sequences from disparate geographic locales. Histolopathologic evaluation of necropsy tissue from an FIV_{Oma} positive Pallas' cat and an uninfected wild Pallas' cat is suggestive of immune dysfunction related to FIV_{Oma} infection (Fig. 1). Further investigation of the clinical and pathological effects of FIV_{Oma} infection in both captive and wild populations of this threatened species is recommended.

It has been proposed that FIV arose in Africa, and may have been introduced to Asia as early as the late pleistocene, approximately 100,000 years ago, when FIV positive individuals were present among those lions that migrated from Africa (Antunes et al., 2008) to range throughout Eurasia and into North America (Pecon-Slattery et al., 2008). However, our data suggest a more recent re-emergence of a single strain of FIV_{Oma} throughout the Pallas' cats reported in this study. Low genetic variation among all FIV_{Oma} sequences (1.9%) is comparable to the 2.2% genetic variation, based on the same genetic segment of FIV *RT-Pol*, found in a population of 23 feral domestic barn cats, an isolated population descending from a small group of founders approximately 60 years before FIV sampling. (Carpenter et al., 1998). This low genetic diversity in FIV_{Oma} is in contrast to much larger genetic distances observed for FIV_{Ple} within lion populations and for FIV_{Pco} in pumas, which diverge 28–34% within lions (Troyer et al., 2004) and also within pumas (Biek et al., 2006; Carpenter et al., 1998). The monophyletic grouping and low genetic distance observed for FIV_{Oma} is suggestive of a recent 20th century emergence or re-emergence of FIV_{Oma} into the Asian Pallas' cat population.

Several opportunities for more recent cross-species transmission from African felids into Asia exist and would be consistent with the findings reported here. It is possible that the Asiatic cheetah (*A. j. venaticus*), which currently only exists in Iran, was previously connected to African populations. Historic records of cheetah within the last 100 years include all regions between Iran and the surviving populations in northern and southern Africa (Nowell and Jackson, 1996), providing evidence of a connection between Asian and African populations until construction of the Suez Canal started in 1859. A review by Krausman and Morales (2005) also included cheetahs from the northern Sahara in Asiatic subspecies. It is therefore possible that the FIV was transmitted from Africa to Asia via a low density but contiguous cheetah population. The transmission from cheetah to Pallas' cat could have happened where the two populations met in the region east of the Caspian Sea. Similarly the leopard or some as yet unidentified carnivore species from Africa or Asia may have been the source of FIV_{Oma} introduction to Pallas' cats.

The clinical effects of FIV in free-ranging species are controversial. The previously accepted paradigm, based on lion and puma studies, was that these viruses were less pathogenic (Carpenter et al., 1996). However, there is evidence indicating immune suppression may still occur, as recent reports show CD4+ depletion in both wild and captive pumas and lions (Bull et al., 2003; Roelke et al., 2006). Further, a recent study of over 60 lions from Botswana showed that relative to uninfected lions, FIV_{Ple}-infected lions displayed a significant elevation in clinical health conditions such as lymphadenopathy, gingivitis, tongue papillomas, dehydration, and poor coat condition that were attributed to chronic FIV infection (Roelke et al., 2009). Additionally, lymph node laparoscopic biopsies from free-ranging FIV_{Ple} infected lions revealed evidence of lymphoid depletion, the hallmark pathology documented in immunodefieciency virus infections of humans (HIV-1), macaques, and domestic cats (Roelke et al., 2009).

Similarly, histopathological changes in wild-born captive Pallas' cat (Oma-34) observed in this study were consistent with FIV caused immune depletion (Fig. 1). However, the effects of FIV_{Oma} in the wild population are uncertain. We recommend that wild Pallas' cat populations, now known to be infected with a potentially immune debilitating virus, continue to be monitored for FIV_{Oma} and that the clinical correlates to FIV_{Oma} infection, as observed in FIV_{Ple}-infected lion populations, be further investigated in this threatened cat species.

Conflict of interest

The authors report no conflict of interest.

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