

Feline Leukemia Virus (FeLV) in Captive Wild Felids in Thailand during 2004-2005

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Abstract

Retrospective study for prevalence of feline leukemia virus (FeLV) infection was examined in captive felids in Wildlife Conservation Division of National Park, Wildlife and Plant Department of Thailand. During 2004-2005, these captive wild felids were recovered from smuggling and out-law trading. Thirty blood samples were collected from 12 tigers, 4 leopards, 2 clouded leopards, 3 fishing cats and 9 leopard cats, and examined the presence of FeLV p27 antigen in the serum samples by the rapid immunomigration (RIM) test kit. Two (6.67%) of 30 feline blood samples were positive for FeLV by RIM. Furthermore, proviral DNA was amplified and examined by nested PCR. Twenty of the samples (66.67%) were positive. Also, in this study a total of 8 spleen and/or lymph-node tissues were collected from 8 death felids over several months in freezer. Proviral DNA was amplified and it was found that one of 8 post-mortem samples (12.5%) was positive by nested PCR.

Keywords: wild felids, feline leukemia virus, FeLV, nested PCR

Feline leukemia viruses (FeLV) are horizontally transmitted oncoretroviruses that are widespread in domestic cat populations and induce both suppressive and neoplastic diseases of the feline lymphoreticular system. FeLV belongs to the oncogenic Retroviridae, a family of viruses that inserts a double-stranded DNA copy of its single-stranded RNA genome into the host cells' chromosomes. Once stably inserted, the viral genes behave like cellular genes and maybe transcribed into functional protein products or may remain latent for long periods of time.⁶ The FeLV transmits among cats by contagion. The main sources of infection are persistently infected carrier cats which continuously excrete virus. Dissemination of FeLV among cats may be prevented by identifying infected carrier cats and removing them from contact with non-infected cats.^{12,15} Depending on the infecting viral strain and on the host response, infection with FeLV may result in early viral clearance because of an effective immune response or in viremia.⁶ In cats that are viremic, multiple tissues including bone marrow, are infected and may retain the latent virus indefinitely. A proportion of viremic cats subsequently mount an immune response that results in viral clearance. More commonly, persistently viremic cats may remain clinically healthy or, develop immunosuppressive, hematologic, intestinal or reproductive disorders, neoplasm such as lymphoma or leukemia, or autoimmune diseases.¹² Thus, FeLV infection can result in a wide range of potential disease manifestations.

The specificity of FeLV in infecting cells resides in the glycoprotein envelope. Recombination of exogenous FeLV subgroup A env genes with complementary endogenous retroviral sequences can result in FeLV viruses typed as subgroup B or C.¹ Opportunities for recombination are expanded during long periods of active viral replication and may result in viruses infecting endothelial or epithelial cells.

In Thailand, the smuggling wild animals recovered from outlaw-trading were collected by forestry officers, and then most felids from these cases were quarantined

and re-habituated at Kao-Pratabchang Wildlife Breeding Center, which belongs to Department of National Park, Wildlife and Plant Conservation. They have more than 100 smuggling felids rehabilitated in 2 separate stations, the Khao-Pratabchang main center and the Kao-Son separating station, both located in Ratchaburi Province in the central-western part of Thailand. Most felids are usually exchanged or rotated between these two centers. During 2004-2005, 8 felids died in these wildlife conservation centers but did not show any dominance signs. The lymphoid tissues and skeletal muscles were collected from deceased felids and 30 whole blood samples were collected from other 5 clinical healthy cat species. The FeLV in both cases of death and of non-clinical sign group were retrospectively examined to determine the prevalence of FeLV in 2004-2005.

The clinical healthy group was selected from 5 species of captive wild felids of Thailand which were rehabilitated in Khao-Pratabchang Conventional Center. Species of these felids were identified, based on phenotypical assessment and also according to anatomical criteria.⁴ Prior to blood sampling, the large cats were anesthetized with 5-10 mg/kg dosage of Tiletamine/Zolazepam (ZoletilTM, VIRBAC laboratories, France). The small cats were also sedated with 0.5 mg/kg dosage of xylazine hydrochloride (Rumpun[®], Bayer, Ansan, Korea) before being caught by net. The Genomic DNA derived from white blood cells of 12 tigers (*Panthera tigris*), 4 leopards (*Panthera pardus*), 2 clouded leopards (*Neofelis nebulosa*), 3 fishing cats (*Prionailurus viverrinus*) and 9 leopard cats (*Prionailurus bengalensis*) were prepared and used for nested polymerase chain reaction (nested PCR). The dead felids were also investigated retrospectively by detecting the integrated proviral DNA in feline genomic DNA. Genomic DNA was derived from lymphoid tissue and skeletal muscle of 1 dead tiger, 3 dead leopards, 1 dead clouded leopard and 3 dead leopard cats.

The genomic DNA was prepared from blood samples or tissues. For blood samples, DNA was

extracted according to previously described methods, briefly, 300 µl of blood sample were incubated for 4 hours with 600 µl of lysis buffer (0.1 M NaCl, 10 mM Tris-HCl pH 8.0, 5% SDS) with 10 µg/µl of proteinase K. The genomic DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with sodium acetate and resuspended 10 mM Tris/1 mM EDTA and the concentration determined by measurement at 260 nanometer wavelength.¹³

For tissue extraction, genomic DNA was extracted from 10 mg of lymph node, skeletal muscle of all felids. The tissue pellet was incubated overnight at 37 °C in lysis buffer (50 mM Tris-HCl, 1 mM ethylenediaminetetra acetic acid (EDTA), 0.5% Tween-20) with 10 µg/µl of proteinase K (Invitrogen, USA). DNA was extracted as above described. FeLV-specific proviral sequences were amplified from approximately 0.5 µg of DNA by a nested PCR.

The nested PCR reactions were prepared as previously reported.² Briefly, the viral long terminal repeat (LTR) regions were amplified using the outer primers, 5'-TACTCAAGTATGTTCCCATG-3' and 5'-CTGGGGAGCCTGGAGACTGCT-3', and performed in Thermocycler machine (PTC-200, MJ Research, USA). They amplified a 166-bp segment in LTR in a 50-µl reaction. Cycling parameters consisted of 30 rounds of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds. Five microliters of the first-round product was subjected to a second round of amplification with primers 5'-GGTTAAGCACCTGGGCCCCGG-3' and 5'-GCA GCGGCCTTGAACTTCTG-3' with identical cycling parameters as described above except for an annealing temperature of 58 °C. The final amplicon produced a size of 85 bp. DNA sequence was analyzed using previously published FeLV sequences. Genomic DNA from FeLV-seropositive and -seronegative cats was used as positive and negative controls, respectively.

All sera from 30 clinical healthy cats were detected FeLV p27 antigen using FeLV commercial kit

(Witness FeLV test kit, Synbiotics Corporation San Diego, CA, USA) based on rapid immunomigration (RIM) technology. Sensitized particles bound to p27 antigen presenting within the samples (whole blood, serum, or plasma) migrated along a nitrocellulose strip. The complex was then captured on a sensitized reaction line where its accumulation caused the formation of a clearly visible pink/purple band. A pink/purple band must be visible in the control window to ensure that the test was performed correctly and can be read in 10 minutes.

Feline leukemia virus proviral DNA from white blood cells was detected from 20 out of 30 (66.67%) clinical healthy group of captive wild felids (Figure 1a-1c). There were 2 samples positive to rapid immunomigration (RIM) test kit (6.67%). The result was shown in Table 1. The FeLV infection in postmortem felids was found in 1 of 8 dead felids (12.5%) by nested PCR (Figure. 1d). The FeLV infection in captive wild felids at Khao-Pratapchang Wildlife Breeding Center and Khoa-Son Breeding Center was found approximately 55% prevalence (21 from 38 samples were positive). Using the test kit for the detection of FeLV, the positive result was low, meaning that the sensitivity of the test. The proviral DNA detection by using nested-PCR specific to viral LTR region appears to be a reliable method to detect FeLV provirus in the host genomes.

The current study revealed a high prevalence of FeLV infection in captive wild felid at Khao-Pratapchang Breeding Center. A previous study in Namibia shown that a captive-born cheetah at the Cheetah Conservation Fund, had lymphoma and had been infected with FeLV.⁹ These cheetahs had contacted with domestic cats as same as infected cats in this report. From the case study of Sleeman *et al.*¹⁴, FeLV infection was found in a captive bobcat (*Felis rufus*). The first study of FeLV in free-ranging felids in North America found the first report in a cougar.⁷ In North Vietnam, the prevalence study of this disease found in 8 leopard cats had no infection.¹⁰ In France, the prevalence study in free-ranging jungle cats found 23.7%. The study of Leutenegger, who collected

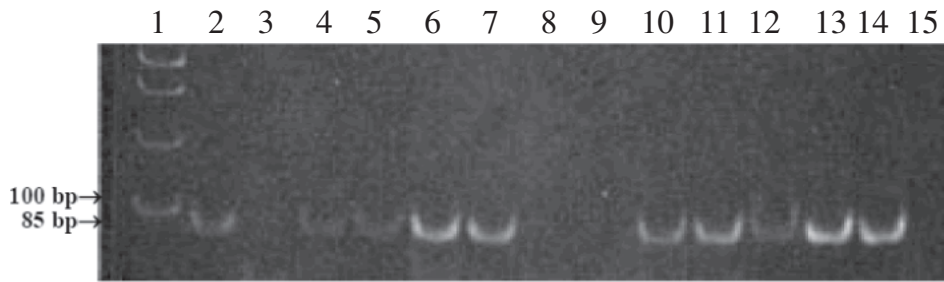


Fig. 1a

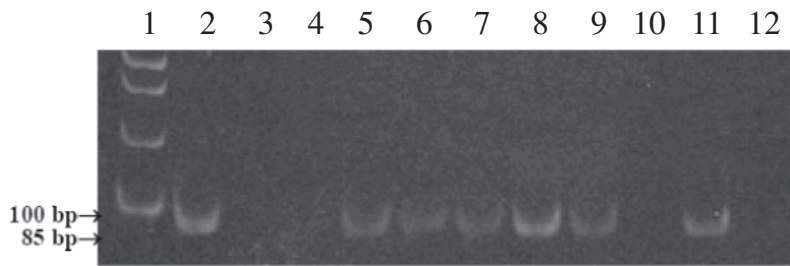


Fig. 1b

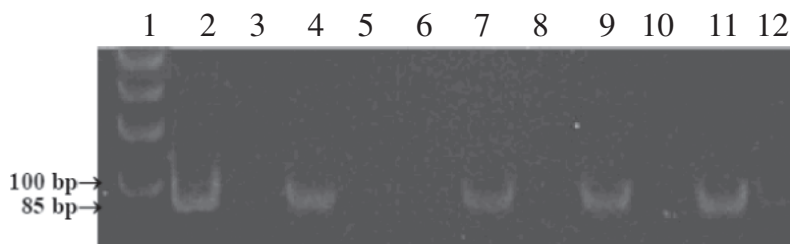


Fig. 1c

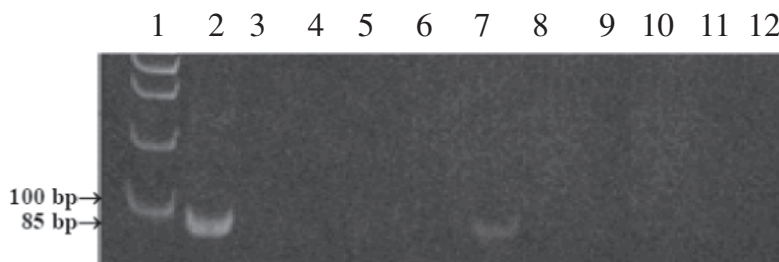


Fig. 1d

Figure 1. The polyacrylamide gel electrophoresis show nested PCR for detection of FeLV in 30 captive felids. 1a). FeLV detection of tiger 1-12 (lane 4-15), 1b). FeLV detection of clouded leopard 1-2 (lane 4-5), leopard 1-4 (lane 6-9), fishing cat 1-3 (lane 10-12) 1c). FeLV detection of leopard cat 1-9 (lane 4-12). 1d). detection of FeLV in 8 post mortem felids. Lane 1: 100-bps ladder, lane 2: positive control, lane 3: negative control, lane 4-11: post mortem felids 1-8.

Table 1. The detection of FeLV proviral DNA and p24 antigen in blood samples from 30 non-clinical sign smuggling felids and 8 dead (post-mortem) felids.

Species	no. of felid	nested PCR		FeLV p27Ag IC	
		no. of positive	% positive	no. of positive	% positive
Group I: non-clinical sign felids					
Tigers (<i>Panthera tigris</i>)	12	9	75.0	1	8.33
Leopard (<i>Panthera pardus</i>)	4	4	100	1	25.0
Clouded leopard (<i>Neofelis nebulosa</i>)	2	1	50	0	0
Fishing cat (<i>Prionailurus viverrinus</i>)	3	1	33.33	0	0
Leopard cat (<i>Felis bengalensis</i>)	9	5	55.56	0	0
Total	30	20 (n=30)	66.67	2 (n=30)	6.67
Group II: post-mortem felids					
Tiger (<i>Panthera tigris</i>)	1	0	0	ND	ND
Leopard (<i>Panthera pardus</i>)	3	1	33.34	ND	ND
Clouded leopard (<i>Neofelis nebulosa</i>)	1	0	0	ND	ND
Leopard cat (<i>Felis bengalensis</i>)	3	0	0	ND	ND
Total Group II	8	1	12.5	-	-
Total Groups I and II	38	21 (n=38)	55.26	2 (n=30)	6.67

a sample from jungle cats in France, Switzerland and Germany and found 49% positive by antigen study and 75% positive by antibody survey.^{5,8} In Scotland the prevalence was 13% and in West Saudi Arabia it was 6% positive in jungle cats and 0% in sand cats (*Felis margarita*).^{3,11} These case studies found low incidence of FeLV in free-range felids except in jungle cats.

Normally, FeLV infection is not an enzootic disease in wild felids.¹⁴ This study determined the prevalence of FeLV infection in captive wild felids at the Wildlife Breeding Centers, but did not identify the origin of disease. As the health status of these felids were not checked for any disease before coming into the recovery center, it is not clear when they were infected. There are few case reports demonstrating that nondomestic felids are susceptible to infection with domestic cat FeLV, which should be considered a potential emerging disease in large cats.¹⁴ In this study, FeLV detection in domestic cats around the breeding center area was not included. Further surveys to determine the FeLV status of free-living and captive nondomestic felids are warranted. FeLV-infected domestic/feral cats could potentially introduce this virus to populations of wild and captive felids.¹⁴

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