

Hematological and Serum Biochemical Changes in Cats Naturally Infected with Feline Leukemia Virus and Feline Immunodeficiency Virus in Thonburi district, Bangkok, Thailand

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ABSTRACT

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are highly infectious, particularly among stray cats, with reported cases documented worldwide. This retrospective study analyzed cats infected with FeLV and FIV or co-infected with both in the Thonburi area of Bangkok from 2018 to 2022. A total of 830 cats were admitted to the animal clinic exhibiting clinical signs of depression, emaciation, ataxia, pale mucous membrane, gingivitis and corneal opacity. Twenty-nine cats tested positive for FeLV (3.49%), ranging in age from 2 to 60 months, while 16 cats tested positive for FIV (1.93%), ranging in age from 5 to 96 months and 30 cats tested positive for both FeLV and FIV (3.61%) with ages ranging from 4 to 108 months. None of the infected cats had received vaccination against FeLV or FIV and they were raised in an open husbandry system. Co-infection with FeLV and FIV was associated with a higher incidence of *Mycoplasma* spp. infection compared to cats infected with FeLV ($P < 0.001$) or FIV ($P < 0.001$) alone. Anemia was observed in co-infection with FeLV-FIV ($24.24 \pm 9.97\%$), leukocytosis in co-infection with FeLV-FIV ($21.38 \pm 21.66 \times 10^3$ cell/ μ L), neutrophilia in FeLV ($64.28 \pm 16.28 \times 10^3$ cell/ μ L), FIV ($67.19 \pm 11.92 \times 10^3$ cell/ μ L) and FeLV+FIV ($64.03 \pm 17.09 \times 10^3$ cell/ μ L). Thrombocytopenia was found in FeLV+FIV ($247.13 \pm 148.98 \times 10^3$ cell/ μ L), with increased BUN in FeLV, FIV and FeLV+FIV, increased creatinine in FIV and elevated ALT in FeLV+FIV infected cats.

Key words: Cats, FeLV, FIV, Hematology, Serum biochemistry.

INTRODUCTION

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are highly infectious, particularly in stray cats, with cases of infection reported worldwide. Both viruses belong to the RNA virus family known as retroviruses; FeLV is classified as a γ -retrovirus, while FIV is classified as a lentivirus (Little et al. 2020). Transmission of FeLV and FIV occurs through both vertical and horizontal routes including transmission from infected queens to their kittens and among cats that live together or engage in fights (Hartmann 2012).

Clinical signs in FeLV or FIV-infected cats vary and may include fever, lethargy, stomatitis, dermatitis, conjunctivitis, respiratory tract disorders and generalized lymph node enlargement (Hartmann 2012; Marcondes et al. 2018). Previous reports found that cats co-infected with

FeLV-FIV exhibited more severe clinical signs, with shorter survival times than FeLV or FIV alone (Spada et al. 2018). Diagnosing infected cats based solely on clinical signs requires the use of additional diagnostic methods such as hemato-serology, immunochromatographic assay (FeLV/FIV Test kit) and PCR (Lacerda et al. 2020; Capozza et al. 2021; Sakundech et al. 2021).

The immunochromatographic assay (FeLV/FIV Test Kit) is frequently used in animal clinics or animal hospitals as a rapid and cost-effective test. Some commercial test kits have reported specificities for the detection of FeLV at 100% and for the detection of FIV at 99.7% (Sand et al. 2010). These rapid test kits are suitable for use in veterinary practices because they are reliable and easy to use (Little et al. 2020). The PCR method is more specific but requires expertise and the testing process is more expensive (Lacerda et al. 2020; Capozza et al. 2021).

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Currently, in Thailand, the method for examining FeLV/FIV in cats at animal clinics or hospitals involves using commercial test kits in conjunction with an assessment of hematology and serum biochemistry (Nedumpun et al. 2015; Aiyaranoi et al. 2018; Sakundech et al. 2021). This study evaluated the incidence, hematology and serum biochemical changes among cats presented to the clinic that were naturally infected with FeLV and FIV in Thonburi district, Bangkok Province, Thailand.

MATERIALS AND METHODS

Ethical approval

This study was approved by Suan Sunandha Rajabhat University, Thailand (IACUC 66-003/2023). Secondary data were obtained from the records of the animal clinic and were permitted by the owners.

Study area

This study was conducted at an animal clinic in Dao Khanong subdistrict, Thonburi district, Bangkok, Thailand (establishment license 01-957/2562, latitude 13.707529, and longitude 100.478054).

Data collection

Between 2018 and 2022, 830 cats were admitted for treatment at the clinic, with 76 testing positive for FeLV, FIV or FeLV-FIV. The virus diagnosis was conducted using a commercial test kit (Bionote Antigen Rapid FIV Ab/FeLV Ag Test Kit, Korea). Clinical signs observed in positive cats included depression, emaciation, ataxia, fever, dehydration, hypersalivation, dyspnea, pale mucous membrane, gingivitis and corneal opacity. Data regarding age, gender, breed and husbandry system of the infected cats were collected from the records of the animal clinic.

Hematology and serum biochemistry collection

Blood from the cephalic vein was drawn into a 0.5mL EDTA (Ethylenediaminetetraacetic acid, Fushino, China) tube for hematology and blood smear. Evaluation of hematology in infected cats was performed through the assessment of complete blood count (CBC). The measured parameters included total white blood cells (WBC), neutrophils, band neutrophils, eosinophils, basophils, monocytes, lymphocytes, hematocrit (Hct) and platelets (PLT), using an automated hematology cell counter (MS4, Melet Schloesing laboratories, Cergy-Pontoise Cedex, France). Serum biochemistry evaluation was performed by collecting 1mL of blood into a heparin tube. The assessed parameters included blood urea nitrogen (BUN), creatinine and alanine aminotransferase (ALT) using an automated analyzer (BT 2000, Biotechnica Instruments, Rome, Italy). Blood smears were tested at the standard laboratory using the thin blood smear method (Laboratory of Vet Clinical Center, Bangkok, Thailand).

Statistical analysis

Age, gender, breed, blood parasites and husbandry systems were presented as percentages and averages. The incidence of blood parasite infection and FeLV, FIV and FeLV±FIV-infected cats was compared using Pearson's Chi-Square. Hematology and serum biochemistry were

evaluated against reference values using mean±SD. Hematology and serum biochemistry were compared among FeLV-infected, FIV-infected and FeLV±FIV infected cats using one-way ANOVA. All statistical analyses were conducted using IBM SPSS statistics version 29 (USA).

RESULTS

Gender, age and husbandry system of infected cats

During the study period, 29 cats were found to be infected with FeLV, 16 cats with FIV and 30 with both FeLV and FIV as 3.49, 1.92 and 3.61%, respectively. Cats infected with FeLV included 13 males and 16 females, while FeLV±FIV infected cats numbered 11 males and 19 females and FIV-infected cats comprised 9 males and 7 females (Table 1). All infected cats were domestic short-haired. The average age of cats diagnosed with FeLV was 13.93±13.86 months (ranging from 2 to 60 months), for FIV it was 33.25±29.32 months (ranging from 5 to 96 months) and for FeLV-FIV it was 26.23±26.40 months (ranging from 4 to 108 months). Seventy-three infected cats were found in an open husbandry system and FeLV cats were found in a closed husbandry system.

Table 1: Information on the number of cats infected with FeLV, FIV and both FeLV and FIV, including their gender and husbandry system.

Virus		Husbandry system		Total	Incidence (n=830)
		close	open		
FeLV	Gender Male	1	12	13	3.49%
	Female	1	15	16	
	Total	2	27	29	
FeLV±FIV	Gender Male	0	11	11	3.61%
	Female	0	19	19	
	Total	0	30	30	
FIV	Gender Male	0	9	9	1.92%
	Female	0	7	7	
	Total	0	16	16	
Total	Gender Male	1	32	33	
	Female	1	41	42	
	Total	2	73	75	

Close husbandry system: cats have been raised indoors since birth and have never been let outside: Open husbandry system: cat may have escaped from home, been intentionally released outside by the owner, or is a stray cat.

Hematology, serum biochemistry, and blood parasites in FeLV cats

Hematological parameters were analyzed in 29 cats. Results revealed average values for hematocrit (Hct) 31.31±8.43%, total WBC 18.77±14.51×10³ cell/μL, neutrophils 64.28±16.28%, band neutrophils 0.38±0.86%, lymphocytes 28.41±15.07%, monocytes 3.14±1.6%, eosinophils 3.48±3.14% and platelets 359.62±154.93×10³ cell/μL, with no basophils found (Table 2). Serum biochemistry revealed average BUN of 37.90±40.14 mg/dL, creatinine 1.82±2.91mg/dL and ALT 93.90±78.26 IU/L (Table 3). The thin blood smear found *Mycoplasma* spp. in two cats (Fig. 1). Hct compared with FeLV±FIV cats gave P=0.003. Platelet FeLV±FIV cats gave P=0.004, while BUN compared with FIV cats yielded P=0.04 (Table 4).

Table 2: Hematological parameter in cats infected with FeLV, FeLV±FIV, and FIV

Parameters	Virus	Mean	S.D.	Minimum	Maximum	Normal* Range
Hct (%)	FeLV	31.31	8.43	11.20	48.00	
	FeLV±FIV	24.24 ↓	9.97	5.50	46.70	30-45
	FIV	35.41	7.16	22.30	48.00	
WBC (×10 ³ cell/μL)	FeLV	18.77	14.51	3.56	71.20	
	FeLV±FIV	21.38 ↑	21.66	1.48	98.80	5.5-19.50
	FIV	14.16	5.84	7.60	28.40	
Neutrophils (%)	FeLV	64.28 ↑	16.28	30.00	85.00	
	FeLV±FIV	64.03 ↑	17.09	14.00	85.00	45-64
	FIV	67.19 ↑	11.92	39.00	85.00	
Band Neutrophils (%)	FeLV	0.38	0.86	0	3.00	
	FeLV±FIV	0.33	0.88	0	4.00	0-2
	FIV	0.13	0.50	0	2.00	
Lymphocytes (%)	FeLV	28.41	15.07	13.00	58.00	
	FeLV±FIV	29.83	15.44	13.00	64.00	27-36
	FIV	26.31	10.66	13.00	53.00	
Monocytes (%)	FeLV	3.14	1.60	1.00	7.00	
	FeLV±FIV	2.67	1.45	0	7.00	0-5
	FIV	3.25	1.29	1.00	5.00	
Eosinophils (%)	FeLV	3.48	3.13	0	11.00	
	FeLV±FIV	2.33	2.48	0	11.00	0-4
	FIV	3.13	2.42	0	9.00	
Basophils (%)	FeLV	0	0	0	0	
	FeLV±FIV	0.03	0.18	0	1.00	0-1
	FIV	0	0	0	0	
Platelet (x10 ³ cell/μL)	FeLV	359.62	154.93	174.00	771.00	
	FeLV±FIV	247.13 ↓	148.98	98.00	628.00	300-800
	FIV	365.69	111.88	180.00	570.00	

*Reference values are used according to the Laboratory of Veterinary Clinical Center in Bangkok, Thailand (Modified from March 2012: Reference ranges, 10th Ed. The Merck Veterinary Manual).

Table 3: Serum biochemistry parameter in cats infected with FeLV, FeLV±FIV, and FIV

Parameter	Virus	Mean	S.D.	Minimum	Maximum	Normal* Range
BUN (mg/dL)	FeLV	37.90 ↑	40.14	16.00	223.00	
	FeLV±FIV	32.37 ↑	26.89	15.00	160.00	19-34
	FIV	66.25 ↑	68.62	16.00	200.00	
Creatinine(mg/dL)	FeLV	1.82	2.91	0.51	15.96	
	FeLV±FIV	0.97	0.47	0.30	2.20	0.90-2.20
	FIV	3.12 ↑	4.54	0.48	15.96	
ALT (IU/L)	FeLV	93.90	78.26	20.00	294.00	
	FeLV±FIV	169.33 ↑	217.28	24.00	800.00	25-97
	FIV	85.50	48.22	40.00	219.00	

*Reference values are used according to the Laboratory of Veterinary Clinical Center in Bangkok, Thailand (Modified from March 2012: Reference ranges, 10th Ed. The Merck Veterinary Manual).

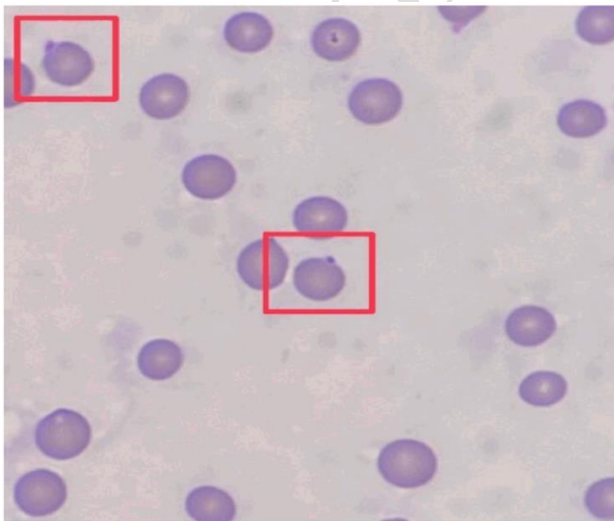


Fig. 1: The red square shows *Mycoplasma Spp.* from an infected cat, adhering to the surface of red blood cells. The microorganism has a spherical shape. Detected using the thin blood smear method with Giemsa staining at 1000× magnification.

Hematology, serum biochemistry and blood parasites in FIV cats

FIV was identified in 16 cats, with average Hct and platelet counts $35.41 \pm 7.16\%$ and $365.69 \pm 111.88 \times 10^3$ cell/μL respectively. Average values for WBC, neutrophils and band neutrophils were $14.16 \pm 5.84 \times 10^3$ cell/μL, $67.19 \pm 11.92\%$ and $0.13 \pm 0.5\%$ respectively, while average values for lymphocytes, monocytes and eosinophils were 26.31 ± 10.66 , 3.25 ± 1.29 and $3.13 \pm 2.42\%$ respectively (Table 2). BUN, creatinine and ALT levels were 66.25 ± 68.62 mg/dL, 3.12 ± 4.54 mg/dL and 85.5 ± 48.22 IU/L respectively (Table 3). (One cat was found to be infected with *Mycoplasma spp.*) (Table 4). Hct comparison with FeLV±FIV cats gave $P < 0.001$. Platelet comparison with FeLV±FIV cats resulted in $P = 0.01$. BUN comparison with FeLV±FIV cats yielded $P = 0.014$ and creatinine comparison with FeLV±FIV cats revealed $P = 0.015$ (Table 4).

Hematology, serum biochemistry and blood parasites in FeLV±FIV cats

Thirty cats infected with FeLV±FIV exhibited average hematological parameters as Hct $24.24 \pm 9.97\%$, total WBC

Table 4: Comparison of the incidence of blood parasites infection and FeLV, FIV, FeLV±FIV-infected cats, and comparison of hematology and serum biochemistry among FeLV-infected, FIV- infected, and FeLV±FIV infected cats

Virus	Blood parasites		Total (n=75)
	<i>Mycoplasma Spp.</i>	Not found	
FeLV	2	27	29
FeLV±FIV*	19	11	30
FIV	1	15	16
Pearson's Chi-Square=27.886, P value <001			
Hematology and Serum biochemistry	Virus	Virus)compare(P-value
Hct	FeLV	FeLV±FIV	0.003*
		FIV	0.141
	FeLV±FIV	FIV	<0.001*
Platelet	FeLV	FeLV±FIV	0.004*
		FIV	0.893
	FeLV±FIV	FIV	0.010*
BUN	FeLV	FeLV±FIV	0.628
		FIV	0.040*
	FeLV±FIV	FIV	0.014*
Creatinine	FeLV	FeLV±FIV	0.246
		FIV	0.136
	FeLV±FIV	FIV	0.015*
WBC	FeLV	FeLV±FIV	0.551
		FIV	0.377
	FeLV±FIV	FIV	0.167
Neutrophils	FeLV	FeLV±FIV	0.953
		FIV	0.556
	FeLV±FIV	FIV	0.522
Band neutrophils	FeLV	FeLV±FIV	0.828
		FIV	0.317
	FeLV±FIV	FIV	0.409
Lymphocytes	FeLV	FeLV±FIV	0.707
		FIV	0.641
	FeLV±FIV	FIV	0.433
Monocytes	FeLV	FeLV±FIV	0.225
		FIV	0.808
	FeLV±FIV	FIV	0.206
Eosinophils	FeLV	FeLV±FIV	0.112
		FIV	0.677
	FeLV±FIV	FIV	0.354
Basophils	FeLV	FeLV±FIV	0.273
		FIV	1.00
	FeLV±FIV	FIV	0.356
ALT	FeLV	FeLV±FIV	0.054
		FIV	0.856
	FeLV±FIV	FIV	0.071

*The mean difference is significant at the 0.05 level.

21.38±21.66×10³ cells/μL, neutrophils and band neutrophils 64.03±17.09 and 0.33±0.88%, with lymphocytes, monocytes and eosinophils 29.83±15.44, 2.67±1.45 and 2.33±2.48% respectively. Basophils were present at 0.03±0.18% and the platelet count was 247.13±148.98×10³ cells/μL (Table 2). BUN, creatinine and ALT levels were 32.37±26.89 mg/dL, 0.97±0.47 mg/dL and 169.33±217.28 IU/L respectively (Table 3). Nineteen cats were found to have *Mycoplasma* spp. and this relationship was statistically significant when compared with cats infected only with FeLV (P<0.001) or FIV (P<0.001)(Table 4).

DISCUSSION

The most common cases of retrovirus involved cats infected with both FeLV and FIV, followed by cats infected with either FeLV or FIV alone. This result differed from a study conducted in 2013-2014 that surveyed infected cats in some areas of Thailand and

reported that most cats were infected with FeLV, followed by FIV and co-infected with FeLV and FIV (Nedumpun et al. 2015). In this study, both male and female cats infected with FeLV, FIV and co-infected with FeLV and FIV were identified. However, a 2019 study conducted in Brazil revealed more retrovirus outbreaks in male than in female cats. This difference was attributed to the more aggressive nature and territorial behaviour exhibited by males compared to females (Biezus et al. 2019). Seventy-three infected cats were found in an open husbandry system. The cause of infection in these cats was attributed to fighting and transmission from the bite of a cat infected with retrovirus (Little et al. 2020). Two FeLV-infected cats were found in a closed system, with transmission assumed to result from infected queens to their kittens, as previously reported (Little et al. 2020). Clinical signs in infected cats were non-specific but previous studies (Capozza et al. 2021) reported that cats infected with FeLV often presented abnormalities in their oral mucosa (Capozza et al. 2021).

For hematological changes, average Hct in cats infected with FeLV±FIV indicated anemia (24.24%). This condition involves viral infection of the bone marrow, causing abnormalities in the production of red blood cells (Little et al. 2020). Although leukopenia was found in cats infected with retrovirus, as reported previously (Hartmann 2012), this study found that co-infection with FeLV and FIV showed leukocytosis (21.38%), possibly caused by different stages of clinical signs and varying virus strains (Gleich and Hartmann 2009). Neutrophilia was observed in cats infected with FeLV, FIV and FeLV±FIV aligning with research conducted by Tatyana et al. (2019), who attributed this phenomenon to the early detection of infection and inflammation in virus-infected cells within the body (Tatyana et al. 2019). Lymphopenia was also found in FIV-infected cats, concurring with Hartmann (2012). This condition is commonly observed in retrovirus-infected cats due to immunocompromised conditions (Hartmann 2012). Thrombocytopenia (247.13×10^3 cells/ μ L) was found in cats co-infected with FeLV and FIV. This condition results from bone marrow suppression, affecting the immune system and leading to secondary blood parasite infections (Marcondes et al. 2018).

Evaluation of serum biochemistry revealed an increase in BUN in cats infected with FeLV (37.9 ± 40.14 mg/dL) and FIV (66.25 ± 68.62 mg/dL) compared to the reference values. Elevated BUN levels are mostly indicative of renal function abnormality (Piyarungsri et al. 2020). Creatinine levels increased beyond the reference values in FIV-infected cats (3.12 mg/dL), consistent with results reported by Piyarungsri et al. (2020). These studies noted that FIV may lead to chronic kidney disease by inducing glomerulonephritis (Piyarungsri et al. 2020). An increase in ALT (169.3 ± 217.28 IU/L) was found in cats co-infected with FeLV and FIV. Pare et al. (2022) observed elevated ALT levels in FeLV-infected cats but no specific information regarding the virus pathogenesis was provided (Pare et al. 2022). It may be associated with hepatic disease in cats.

Mycoplasma spp. were found in some cats infected with FeLV (2 cats), FIV (1 cat) and FeLV±FIV (19 cats). This finding aligned with Marcondes et al. (2018), who detected *Mycoplasma* spp. in cats infected with FeLV and FIV (Marcondes et al. 2018). Blood parasite infections in cats may result from immunocompromised conditions (Hartmann 2012). Using a blood smear to detect blood parasites is not as accurate as the PCR test (Marcondes et al. 2018). However, due to the relatively high price of the PCR test, owners may choose not to use this detection method.

Currently, vaccines for FeLV and FIV in cats have been reported as 80% to 93% effective in preventing FeLV (Tizard 2021) and 56% effective in preventing FIV (Westman et al. 2016). The vaccinated cat must not have been exposed to FeLV or FIV, as this renders the vaccine ineffective (Little et al. 2020). In this study, the infected cats had no history of receiving FIV or FeLV vaccines.

Conclusion

Cats infected with retroviruses that were raised in an open husbandry system and not vaccinated presented as

FeLV±FIV (3.61%), followed by FeLV (3.49%) and FIV (1.92%).

Hematological and serum biochemistry changes including anemia, leukocytosis, neutrophilia, thrombocytopenia, increased BUN and ALT were observed. Anemia was most commonly found in cats infected with FeLV±FIV when compared with FeLV ($P=0.003$) and FIV ($P<0.001$) cats. Thrombocytopenia was most commonly found in FeLV±FIV infected cats when compared with FeLV ($P=0.004$) and FIV ($P=0.01$) cats. Co-infection with FeLV and FIV was associated with a higher incidence of *Mycoplasma* spp. infection compared to cats infected with FeLV ($P<0.001$) or FIV ($P<0.001$) alone.

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Conflict of interest

The authors declare no conflicts of interest related to this study.

Author's contribution

NK designed the study, drafted the manuscript, and wrote it. AL collected and analyzed the data and contributed to writing the manuscript. NP provided advice and revise the manuscript. All authors read and approved the submission to the journal.

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