Detection of Feline Leukemia Virus Infection in Tokyo Area by Enzyme-linked Immunosorbent Assay (ELISA)

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Abstract. Plasma samples from household cats in Tokyo area were examined for feline leukemia virus (FeLV) infection by enzyme-linked immunosorbent assay. Out of 642 cases examined, 37 (5.8%) were found FeLV-positive. Cats with some clinical disorders were more frequently FeLV-positive showing mortality as high as 54% during a 14-month survey. The FeLV-positive cats showed a wide variety of disorders, most frequently renal diseases and anemia.

Feline leukemia virus (FeLV) infection is one of the most important problems in the domestic cat population in many countries. The etiologic association of FeLV with lymphosarcoma and myeloproliferative neoplasms has been well established [2, 5]. Furthermore, FeLV is known to cause either directly or indirectly a wide variety of disorders which are commonly found in domestic cats [2, 3]. In the United States and Europe, cats are widely tested by indirect fluorescent antibody (IFA) technique for presence of FeLV group specific antigen (gsa) in the peripheral blood leukocytes and platelets [6]. And recently, enzyme-linked immunosorbent assay (ELISA) test for detection of FeLV gsa p27 has been devised. This ELISA test was reported to be compatible with IFA testing [9]. In order to know the incidence of FeLV infection in Japan, we have conducted an epidemiologic survey by testing plasma samples collected at veterinary hospitals in Tokyo area employing the ELISA test.

Materials and Methods

Samples: Plasma samples were obtained from

cats presented at six private veterinary hospitals in Tokyo during the period from January to November, 1980, and stored at -20°C before ELISA testing. Each practitioner was asked to take plasma samples also from clinically normal cats. Blood smear specimens for IFA test were also taken, and the specimens stored at room temperature under unfixed state were examined within 2 weeks.

The cats diagnosed as FeLV-positive on the basis of the first ELISA testing were retested 4 and 8 weeks later. The clinical courses of FeLV-positive cats were reported by the practitioners, and mortality was determined at the end of February, 1981. Dead FeLV-positive cats were necropsied for gross and histopathologic examinations.

ELISA test: Plasma samples were examined for presence of FeLV p27 by utilizing Leukassay F test kits (Pitman-Moore Inc., NJ, USA). Reading of the test was performed on the basis of criteria described by Lutz et al. [10].

IFA test: Indirect fluorescent antibody procedure was carried out on acetone-fixed peripheral blood smears by the method described by Hardy et al. [6], using bovine anti-FeLV gsa and FITC-conjugated rabbit anti-bovine Ig sera (Antibodies Inc., Davis, CA, USA).

Results

Out of 642 cats first tested, 37 (5.8%) were FeLV-positive in the ELISA test. These included 185 clinically healthy and 457 diseased cats, and a greater population of

Table 1. ELISA for FeLV in cats presented at veterinary hospitals in Tokyo area

Cases	Examined	FeLV-positive (%)	
Healthy	185	6	(3.2)
Diseased	457	31	(6.8)
Total	642	37	(5.8)

Table 2. Retesting of 23 FeLV-positive cases after 4 and 8 weeks

Retesting	Positive/Tested (%)	
4 weeks	20/23	(87)
8 weeks	18/23	(78)

Table 3. Follow-up study in two veterinary clinics

	Clinic A		Clinic B	
Examined	305		78	
FeLV-positive	14	(4.6%)	9	(11.5%)
Mortality	32/281*	(11.4%)	18/73	(24.7%)
FeLV-positive	8/14	(57.1%)	4/9	(44.0%)
FeLV-negative	24/267	(9.0%)	14/64	(21.9%)
FeLV/All	8/32**	(25.0%)	4/18	(22.0%)

* Dead/Followed.

** FeLV-positive dead/Total dead.

diseased cats were found infected with FeLV (Table 1). Those diagnosed FeLV-positive at the first testing were retested after 4 and 8 weeks. Among 23 FeLV-positive cases retested, 18 (78%) were still positive after 8 weeks, and five cases turned negative after 4 or 8 weeks (Table 2).

A follow-up study was carried out in two of the clinics during a 14-month period. As shown in Table 3, the incidence of FeLV-positive cases varied between the two. However, mortality rate of infected cats was almost equal, and about one fourth of total dead cases were positive for FeLV in both clinics.

A total of 110 cases were comparatively examined by both ELISA and IFA tests. As is presented in Table 4, all of 93 ELISA-negative samples were shown to be IFA-negative. While 12 of 17 ELISA-positive specimens were also positive in the IFA

Table 4. Comparison of ELISA and IFA for detection of FeLV

I F A Test result	E L I S A		Total
	+	—	
+	12	0	12
—	5	93	98
Total	17	93	110

Table 5. Health status of 37 FeLV-positive cases*

Status	No. of cases (%)	
Dead	20	(54)
Surviving		
with illness	12	(32.5)
without illness**	5	(13.5)

* Positive at the first ELISA testing.

** Turned negative 4 or 8 weeks after the first test.

Table 6. Diseases in FeLV-positive cases

Diagnosis	No. of cases	(No. of dead)
Lymphosarcoma	2	(2)
Myeloid leukemia	3	(3)
Anemia	8	(4)
Panleukopenia	2	(1)
Renal diseases	10	(7)
Infectious peritonitis	3	(1)
Upper respiratory infection	2	
Bacterial pneumonia	3	(3)
Septicemia	1	(1)
Dermatophytosis	1	
Hemobartonellosis	1	

test, the remaining five samples from clinically healthy cases were IFA-negative and turned ELISA-negative upon retesting 4 or 8 weeks later.

Table 5 summarizes the health status of all 37 FeLV-positive cats. Mortality was 54% as determined in February, 1981, at the end of 14-month survey, and 32.5% of the infected cats were surviving with some clinical disorders. Diseases found in FeLV-positive cats are summarized in Table 6. Some individuals concurrently had two or more disorders listed on the Table. The

most frequent disorders were renal diseases and anemia. Anemia was also found in the lymphosarcoma and leukemia cats. Out of seven fatal cases with renal diseases, four were necropsied and revealed to have membranous glomerulonephritis. Two FeLV-positive lymphosarcoma cases were of anterior mediastinal type. All the three cases of myeloid leukemia were of granulocytic type while varying in differentiation of neoplastic cells.

Discussion

It has been reported that FeLV is prevalent in urban areas and in multiple-cat households in the United States or England [2, 12]. In Tokyo area, lymphosarcoma was reported to comprise 25% of all feline tumor cases [13], and it is obvious that the virus has been present among the pet cat population for a long time. Therefore, the FeLV positivity rate of 5.8% is not unexpectedly high, being almost compatible with the data obtained in the Netherlands with IFA testing [14].

Although FeLV-positive cats were more frequently found in a group of cats with some clinical disorders, 3.2% of cats that had no signs of illness were also found infected. In some large cities, approximately 70% of cats were reported to be transiently infected by 3 years of age [4, 12]. In fact, in the present study, five of six FeLV-positive cats lacking signs of illness turned negative 4 or 8 weeks after the first testing in the ELISA.

Such transiently FeLV-positive cats in the ELISA were usually IFA-negative. Some discrepancies between the ELISA and IFA tests have been reported [9, 10]. Lutz et al. [10] demonstrated using experimentally exposed cats that the ELISA test could detect transient infection which failed to be detected by the IFA test. This may be related

to difference in sensitivity of antigen detection between the two tests. Furthermore, the transient FeLV infection might produce only antigenemia without viremia, since not all ELISA-positive specimens were positive for the virus isolation [8].

On the other hand, those persistently infected more often had some clinical signs of illness. The difference in incidence of FeLV infection between the clinics A and B may be explained by a larger number of cases with clinical diseases and higher mortality in FeLV-negative cats in clinic B. However, it is very interesting that FeLV-positivity in all dead cats were similar in the two clinics even if the incidence itself was quite different.

In this study, the mortality rate of FeLV-positive cats during a 14-month survey was significantly higher as compared with that reported by Hardy et al. [7], probably because the present follow-up study mostly concerned with cats having some clinical disorders. The disorders FeLV-positive cats presented were those commonly seen in the domestic cats including neoplastic diseases. Leukemia, aplastic anemia and feline panleukopenia-like syndrome have been reported to be associated with FeLV infection [2]. Furthermore, FeLV is known to cause immunosuppression or bone marrow granulocyte hypoplasia which may predispose the cat to secondary infections [5, 11]. The most common in the present FeLV-positive cases was renal disease characterized by membranous glomerulonephritis. These findings may support earlier assumption of Anderson [1] that FeLV might cause immune complex glomerulonephritis in the cat.

The FeLV-positive cats in the first ELISA test were immediately isolated either in clinics or in house, and diagnosis of persistent infection was made on the basis of

two successive positive tests at 4- to 8-week intervals. Test and removal program [5] has been proven effective in order to prevent prevalence of FeLV in many countries, and the ELISA test might be useful for this program.

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要 約

東京地区におけるネコ白血病ウイルス感染の酵素抗体法による検出：石田卓夫・河合三郎・藤原公策（東京大学医科学研究所獣医学研究部）——1980年1月～11月に東京都内で採取したネコ血漿材料について酵素抗体法（ELISA）でネコ白血病ウイルス（FeLV）抗原の検出を行ない、642例中37例（5.8%）がFeLV陽性であった。これらの陽性例では何らかの疾患、とくに腎疾患および貧血を示すものが多く死亡率は54%であった。