

日本の飼い猫における猫Tリンパ球指向性レンチウイルス (FTLV)感染症の検出

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Detection of Feline T-Lymphotropic Lentivirus (FTLV) Infection in Japanese Domestic Cats

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ABSTRACT. Antibodies to feline T-lymphotropic lentivirus (FTLV) were detected in the Japanese domestic cat population. The antibodies were shown to react with a 26 kd protein seen in the FTLV-infected lymphocytes. Almost all the 86 antibody-positive cats had chronic disease signs such as stomatitis/gingivitis, emaciation, upper respiratory diseases and lymphadenopathy in the absence of feline leukemia virus (FeLV) infection. The infected cats were found in all age groups, and most were either outdoor cats or previous free-roaming cats introduced into the house. The infection seemed common in multiple-cat households, and the overall infection rate in such contaminated households was 52.7%.—**KEY WORDS:** AIDS, cat, retrovirus.

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Feline T-lymphotropic lentivirus (FTLV), a new feline retrovirus, has been found in an outbreak of acquired immune deficiency-like diseases in a multiple-cat household in northern California, U. S. A. [9]. Although the disease problems associated with FTLV infection in this cattery resembled those caused by feline leukemia virus (FeLV) infection [2, 3], none of the cats in this cattery was FeLV-positive. The infection was apparently brought into this cattery with one kitten which suffered from a number of chronic diseases and eventually died, and the similar disease problems started to develop in other cats with close contact to this cat [9].

In Japan, pet cats are kept in households in a quite similar manner as in the U. S., and the apparent density is quite high. The FeLV infection is seen in the pet cat population being a major cause of mortalities [4]. During the course of an epidemiologic study of FeLV infection in this country, however, we have seen a substantial number of cats suffering from immune deficiency-like diseases not associated with FeLV

infection.

The present paper describes detection of FTLV-related antibodies in Japanese pet cats, and presents some information on clinical diseases associated with the infection as well as on the epidemiology of this virus infection.

MATERIALS AND METHODS

Serum samples: Cat serum samples with known FeLV p27 [7] and feline infectious peritonitis virus (FIPV) antibody [6] status were used in this study. These samples were originally submitted for testing of these viruses by private veterinary practitioners in Tokyo and other prefectures. Two hundred-sixty samples were selected from FeLV-negative cats with chronic disease signs. Additional 50 samples from FeLV-free healthy cats and five specific pathogen-free (SPF) cats, negative for FeLV and FIPV infection, were also used. Additional serum samples were collected from multiple-cat households where FTLV antibody-positive cases were found.

Virus and cell culture: A UCD isolate of FTLV [9] was provided by Dr. N. C. Pedersen, University of California, Davis. The virus was maintained on peripheral blood lymphocytes from SPF cats as described previously [9]. Briefly, the Ficoll-isolated (Histopaque-1077, Sigma, St. Louis, MO, U. S. A.) cat lymphocytes were first stimulated with 5 $\mu\text{g}/\text{ml}$ of concanavalin A (Con A; Sigma) and then with 100 units/ml of human recombinant interleukin-2 (rIL-2; Cetus Corp. Emeryville, CA, U. S. A.) in RPMI 1640 medium containing 10% fetal bovine serum, 10 mM HEPES, 10 M 2-mercaptoethanol, and 2 $\mu\text{g}/\text{ml}$ polybrene, and infected with FTLV. Fresh stimulated feline lymphocytes were added at a ratio of 1:1 at a weekly interval.

Indirect immunofluorescence and Western blotting assays: The infected cells with cytopathic effect characterized by syncytium formation were dried on a glass slide, fixed with acetone, and used as the antigen for indirect immunofluorescence assay (IFA). The serum samples were diluted 1:10 with phosphate-buffered saline (PBS, pH7.2), and incubated with the antigen at 37°C for 30 min. The washed slides were then incubated with goat anti-cat IgG fluorescein isothiocyanate (FITC) conjugate (Cappel Laboratories, PA, U. S. A.) at a 1:50 dilution at 37°C for additional 30 min, washed three times with PBS, and post-stained with 0.1% evans blue. For Western blotting analysis, the infected or uninfected lymphocyte proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then were electrophoretically transferred to nitrocellulose paper [11]. The blotted paper was sliced and incubated with appropriately diluted serum samples at 4°C for overnight, washed three times, and again incubated with anti-cat IgG peroxidase conjugate (Cappel Laboratories) at 37°C for 1 hr. The reaction was visualized by addition of a

diaminobenzidine-hydrogen peroxide substrate [5].

Antibodies: An FTLV antibody-positive reference serum, anti-feline syncytium forming virus (FeSFV) serum and a mouse monoclonal antibody to FIPV p45 were provided by Dr. N. C. Pedersen. A rat monoclonal antibody (17-4) to FeLV p27 has been previously described [5].

RESULTS

Detection of antibody-positive cats: In the initial study, a total of 260 serum samples from FeLV-free cats with chronic diseases were selected. The diseases included chronic stomatitis/gingivitis, chronic intermittent diarrhea, chronic upper respiratory diseases, lymphadenopathy, chronic skin diseases, emaciation, fever and other nonspecific signs. The cats with apparent acute viral signs such as feline viral rhinotracheitis, feline calicivirus infection or feline panleukopenia, as well as a prominent chronic viral disease like FIP were excluded from this study. Some were from multiple-cat households, but one cat per household was tested in this initial phase of the study. The samples were from Tokyo and five prefectures around the metropolitan area.

Out of 260 cats with some chronic diseases, 59 (22.7%) were positive for antibody reactive to FTLV-infected lymphocytes by IFA. In the healthy cat population, on the other hand, two out of 55 (3.6%) were antibody-positive with none detected in five SPF cats. These positive samples did not react with uninfected lymphocytes in the IFA. The antibody-positive cats were found in all six prefectures involved in this study, which included Tokyo, Kanagawa, Chiba, Saitama, Gunma, and Fukushima.

The age of the antibody-positive cats varied from less than 1 year to over 15 years, with positive cases seen in all age groups (Fig. 1). Although the sex ratio of the test

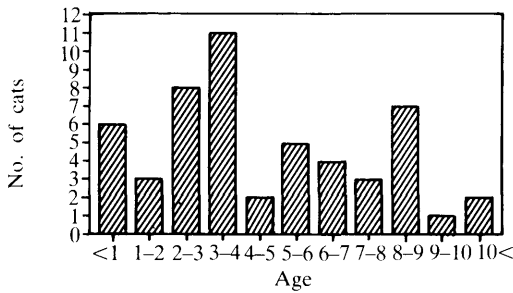


Fig. 1. Age distribution of FTLV antibody-positive cats.

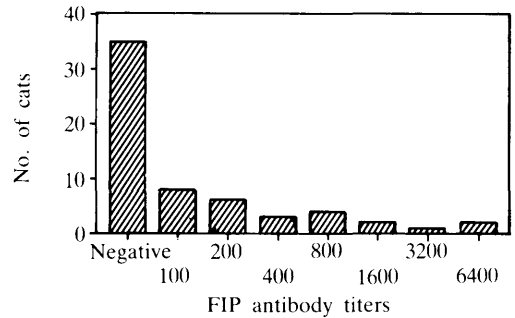


Fig. 2. FIP antibody titers in FTLV antibody-positive cats.

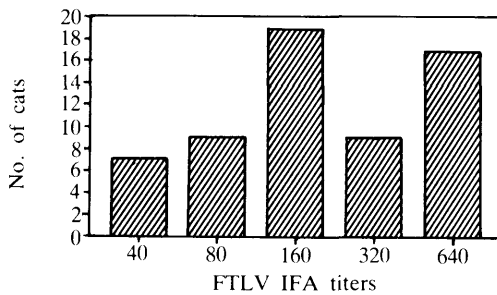


Fig. 3. FTLV IFA antibody titers seen in the antibody-positive cats.

population was almost 1:1, male cats (40 out of 61; 65.6%) had a greater incidence of infection, and in the antibody-positive males all but one were intact males. The complete history of the FTLV-positive cats were obtained from households, which revealed all 40 male cats were either allowed to go out freely or free-roaming before introduced into the houses. There was no significant relation between the clinical status and FIP antibody titers in the FTLV-positive cats. The FIP titer was generally low or negative with the exception of five which had titers of 1:1600 or greater (Fig. 2).

FTLV antibody titers and antibody specificity: The 61 positive samples were serially diluted two-fold for antibody titration by IFA. The titer which is the reciprocal of the highest dilution showing positive reaction varied from 40 to 640, with more cases showing titers of 160 or greater (Fig. 3). The

all IFA-positive samples reacted with an FTLV protein of apparent molecular weight of 26kd (p26) (Fig. 4), while none reacted with uninfected lymphocytes (data not shown). The additional reactivities were observed at two or three proteins around 45kd, and another at 14kd. The FTLV-infected cells were confirmed to be free from FeLV, FeSFV or FIPV by using the specific antiserum or monoclonal antibodies to these viruses.

Infection in multiple-cat households: Of 61 antibody-positive cats initially detected, 14 were from multiple-cat households. Blood sampling from housemate cats were requested, and IFA test for FTLV was performed. The smallest number kept in the multiple-cat households was two and the largest 70. All housemate cats were tested except the house with 70 cats. In total, 60 housemate cats were newly examined, and additional 26 FTLV-positive cats were found. As shown in Table 1, the overall positivity including initially detected cases in these multiple-cat households was 39 out of 74, which is 52.7%. One of the FTLV antibody-positive housemate cats was concurrently infected with FeLV, and five were free from clinical illness.

Clinical diseases associated with FTLV infection: A total of 86 FTLV antibody-positive cats were detected in this study. As shown in Table 2, chronic stomatitis/gingivi-

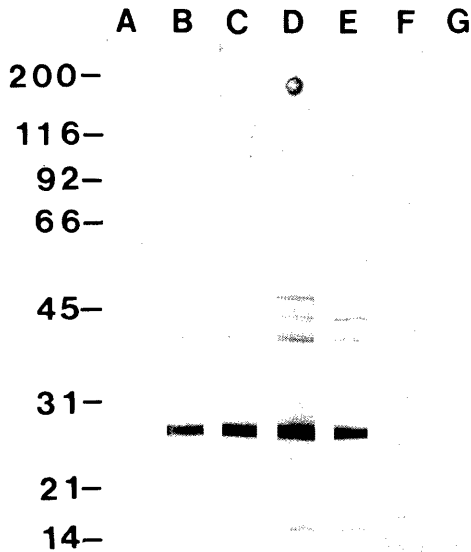


Fig. 4. Western blot of FTLV-infected lymphocyte proteins reacted with various serum samples. A: SPF cat, B-E: FTLV IFA antibody-positive cats, F, G: FTLV antibody-negative healthy cats.

tis, emaciation, chronic respiratory diseases and lymphadenopathy were the popular signs. Many cats were showing multiple signs in combination. All but five cats are still surviving with variable degrees of clinical signs.

DISCUSSION

The present study showed the first evidence of FTLV infection in this country. Although the population studied here was not a randomly selected one, it is possible to state that FTLV infection may be found in a substantial number among sick cats.

The FTLV antibody-positive cats were mostly outdoor cats or free-roaming cats of varying ages. Although the largest number

Table 1. Detection of FTLV antibody-positive cats in multiple-cat households.

House	No. Tested	No. Positive
A	4	1
B	4	2
C	4	4
D	5	4
E	2	2
F	3	1
G	2	2
H	9	8
I	4	2
J	2	1
K	2	1
L	2	1
M	2	2
N	29 ^{a)}	8
Total	74	39

a) 29 out of 70 were randomly selected and tested.

of infected cats was observed in the age group of 2 to 4 years old, this may be due to the relatively large number of test samples from this age group. Some cats were brought into house after free-roaming life. Male cats, who have greater tendency to go out, were found more frequently infected. Therefore, the source of infection seems to be outside the house, and those free-roaming cats may play an important role in transmitting the infection in areas with a heavy cat population.

The infection was seen in many multiple-cat households. These houses were not closed breeding catteries but animal shelter-type homes introducing free-roaming cats, or those keeping multiple cats both in and out. Although FeLV infection used to be common in closed breeding catteries [3], the above observation may indicate a slightly different epidemiologic pattern associated with FTLV infection. The infected households frequently had chronic or recurrent disease problems even without FeLV infection which is another cause of such problems [2] because of the suspected immunosup-

Table 2. Clinical signs seen in 86 FTLV antibody-positive cats^{a)}.

Signs	No. of cats
Chronic stomatitis/gingivitis	45
Emaciation	30
Chronic upper respiratory diseases	26
Lymphadenopathy	16
Fever	9
Renal disease	7
Vomiting	7
Chronic skin disease	6
Chronic diarrhea	6
Others ^{b)}	14

a) Many cats showed multiple signs.

b) Other signs include hypersalivation, ocular disease, dehydration, and jaundice.

pressing properties [8].

The observation by Pedersen *et al.* [9] that FTLV antibody is usually associated with chronic illness was confirmed in this study. It is interesting that the Japanese cats with antibodies reactive to an American FTLV isolate showed an almost identical group of disorders reported with that isolate. Therefore, it may be possible to consider a chronic disease syndrome associated with FTLV infection.

The disease problem most frequently encountered in FTLV-positive cats was chronic stomatitis/gingivitis. About 30% of the cats showing such severe signs were found FTLV-positive in the population studied here. Emaciation and chronic respiratory illness were also seen in many FTLV-positive cats. The pathogenesis of these conditions are not currently understood, and relationship with FTLV infection is also unclear. However, altered immunologic functions are highly likely as the predisposing factor for these conditions as in the human acquired immune deficiency syndrome (AIDS) [1, 10]. It should be noted that generalized lymphadenopathy was seen in 16 (18.6%) out of 86 antibody-positive cases. This condition is also seen in ex-

perimentally-infected kittens [9], and may be closely or directly related to the FTLV infection *per se*.

The antibody-positive cats were not followed for a long period of time in this study, since the purpose of the study was demonstration of infection. The longest period followed was 6 months after blood sampling, and the mortality was not very high. Although the follow-up was not long enough, this observation may be related to the chronicity of the disease syndrome. In addition, the fact that infected cats were evenly distributed among all age groups may be an indication of the chronicity and a low mortality.

It was confirmed that the antibody activity was directed against at least one of the protein (p26) seen only in FTLV-infected cells, which is likely to be a viral protein. Pedersen *et al.* [9] reported antibody activities to at least three different molecular weight proteins, which might be corresponding to HIV p24, p32 and pr55^{gag}. In our observations, however, the band at a 26kd protein was most intense, and additional reactivities were seen in three proteins at around 45kd, and another at 14kd. The significance of these latter findings are unknown, but those proteins weakly recognized by cat sera have apparent molecular weights similar to HIV gp41 and p17 [12]. It should be necessary to confirm these observations by using a purified virus preparation. With regard to the 26kd protein, a similar molecular weight *gag* protein (p24) is demonstrated with human immunodeficiency virus (HIV) [12]. The IFA antibody titers were between 1:40 and 1:640, but there was no correlation between severity of clinical disease and antibody titer.

At this moment, it is not known whether the presence of antibody titer simply demonstrates previous exposure to FTLV or active ongoing infection. The greater incidence of antibody-positive cats in the dis-

eased population, however, indicates the latter possibility. Virus isolation is currently underway to answer this question.

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要 約

日本の飼い猫における猫 T リンパ球指向性レンチウイルス (FTLV) 感染症の検出: 石田卓夫・鷺巢月美・鳥谷部一成・本好茂¹⁾ (日本獣医畜産大学臨床病理学教室, ¹⁾内科学教室) —— 日本の飼い猫から猫 T リンパ球指向性レンチウイルス (FTLV) に対する抗体を検出した。本抗体は FTLV 感染リンパ球に認められる分子量 26kd の蛋白を認識していた。今回検出された抗体陽性 86 例中ほとんどは、口内炎/歯齦炎、削癬、慢性上部気道疾患、リンパ節腫大などの慢性疾患症状を示し、猫白血病ウイルス (FeLV) は陰性であった。FTLV 抗体陽性猫はすべての年齢において認められ、ほとんどが屋外飼育あるいは野良猫から飼育されるようになったものであった。感染は多頭飼育家庭でよくみられ、1 例以上の陽性猫が存在する多頭飼育群の感染率は 52.7% であった。