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Horizontal Transmission of Feline Leukaemia Virus

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As feline leukaemia virus (FeLV) is shown to be transmitted horizontally it may be possible to vaccinate cats against lymphosarcoma and other FeLV-associated diseases.

Most information on the spread of the leukaemogenic RNA viruses under laboratory and field conditions comes from the study of these viruses in the mouse and chicken. Vertical transmission (transmission of virus from parent to progeny through the gametes) is assumed to be the predominant route for the naturally occurring leukaemia viruses of the mouse[1,2]. Considerable evidence indicates that these viruses, in mice as well as fowl, are integrated in the host genome and are transmitted as Mendelian traits[3,4]. Horizontal transmission (viral spread by contact or contagion) is thought to play little or no part in the natural history of the murine leukaemia viruses. In fowl, however, contact infection has been convincingly demonstrated, showing that vertical and horizontal transmission need not be mutually exclusive[5].

The discovery of leukaemia virus in cats[6] and the development of serological methods[7–10] for identifying the feline leukaemia virus (FeLV) allow the determination of the relative importance of these two modes of transmission in animals other than mice and fowl. Serological studies have established that FeLV can be demonstrated in approximately 90% of cats with feline lymphosarcoma. In addition to lymphosarcoma, other feline diseases have shown a striking association with FeLV. These include myeloproliferative disorders, infectious peritonitis, fibrosarcomas, and certain non-responsive anaemias[7–9]. Furthermore, as in the mouse and chicken, clinically normal cats have been found to harbour FeLV[9].

Recent epidemiological studies on feline lymphosarcoma gave no evidence for horizontal transmission of FeLV[11–13] but cases of apparent contact infections with FeLV have been observed in laboratory cat colonies[14,15]. We have previously reported the occurrence of numerous household clusters of feline lymphosarcoma (two or more cases in cats from one household[7,9,16]). Most of the cluster cases occurred in unrelated cats, a fact that suggested horizontal transmission of FeLV, with subsequent development of lymphosarcoma. The use of a simple serological test for the detection of FeLV has now made possible the testing of a large population of clinically normal cats. Our findings indicate that horizontal transmission of FeLV is common and that cats naturally infected in this fashion have a high risk of developing lymphosarcoma.

Occurrence in Normal Cats

Peripheral blood smears from cats were tested for FeLV by indirect immunofluorescence using absorbed rabbit antiserum to FeLV group specific antigens[9]. Figure 1 illustrates the characteristic cytoplasmic fluorescence seen in the leukocytes and platelets of FeLV infected cats. Table 1 shows the results of parallel tests on specimens using three methods for FeLV detection; the immunofluorescence on peripheral

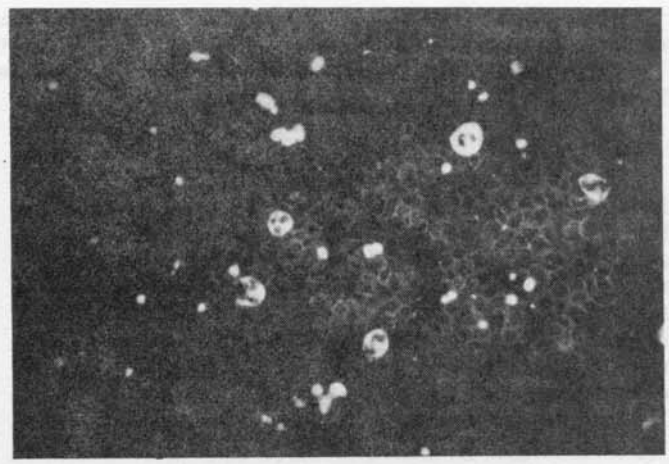

Fig. 1 Punctate granular cytoplasmic fluorescence representing FeLV gs antigen in peripheral blood leukocytes and platelets from a normal cat living in a household with a history of feline lymphosarcoma.

blood smears[9], the detection of FeLV group specific antigens by immunodiffusion[7] and the recovery of infectious FeLV in tissue culture[8]. The methods all gave similar results, indicating that demonstration of FeLV group specific antigens in peripheral blood cells by immunofluorescence identifies FeLV infected cats.

Thirty-eight household clusters of feline lymphosarcoma were studied (Table 2). One hundred and seventeen cats developed lymphosarcoma in the cluster households (average of 3.1 cats with lymphosarcoma per household), two-thirds of the cases occurring in unrelated (not parent, offspring or sibling) cats (Table 2).

Table 1 Comparison of FeLV Detection Methods

Diagnosis	Number of cats	FeLV status determined by Immunofluorescence	Immunodiffusion	Infectious FeLV isolated in tissue culture from Tumour	Plasma	Salivary gland	Urine
Lymphosarcoma	5	+	+	5	5	5	3
Normal	5	+	+	No tumour	5	5	2
Normal	5	−	−	No tumour	0	0	0

Tumour tissue and salivary glands were extracted as 20% homogenates in phosphate buffered saline. The extracts (clarified by centrifugation), undiluted plasma and undiluted urine were filtered through a 220 μm filter and 1 ml of the filtrate was added to freshly passaged cultures of feline embryo fibroblasts (FEF). FeLV infection was assayed by development of FeLV gs antigen (demonstrated by immunofluorescence[9]) in acetone fixed FEF cells or by isolating FeLV from culture media by density gradient centrifugation and demonstrating FeLV gs antigen by immunodiffusion[7].

Table 3 shows the results of immunofluorescence tests on the peripheral blood of 543 apparently normal cats from households with a history of cats with lymphosarcoma or other FeLV-associated diseases. 33% of these normal animals were infected with FeLV. Normal cats living in households already having two or more cats with lymphosarcoma were twice as likely to be infected with FeLV than those from households having only one cat with lymphosarcoma or cats with other FeLV-associated diseases.

Table 4 summarizes our experience with immunofluorescence tests on 2,005 apparently normal cats, 543 living in households with a known history of an FeLV-associated disease and 1,462 living under other circumstances. Normal cats living with infected cats have a high risk of becoming infected. Overt FeLV infection in normal cats living under other circumstances is extremely rare. Thus only two of 1,462 (0.14%) normal cats from environments other than households with a history of FeLV-associated diseases were infected with FeLV.

FeLV-Related Disease in Carriers

What is the subsequent course of FeLV$^+$ normal cats? Do they have a heightened incidence of lymphosarcoma or other diseases? We have followed up 148 of the 177 FeLV$^+$ normal cats identified in our studies (Table 5). 16.2% have developed histologically confirmed lymphosarcoma and 7.5% of the cats have died of non-responsive anaemias with no histological evidence of lymphosarcoma. The average time elapsing between FeLV detection and lymphosarcoma development was 5.3 months, with a range of 1 to 24 months. The expected lymphosarcoma incidence in the general cat population for a 5.3 month period is 18.3 cases per 100,000 cats at risk[17], thus we would have expected 0.018 cases to have developed in these 148 cats. The observed incidence of lymphosarcoma in FeLV$^+$ normal cats was 888 times greater than the expected incidence.

The natural development of lymphosarcoma was followed radiographically in a normal cat shown to be infected with FeLV. Initial radiographs of the cat showed no evidence of lymphosarcoma (Fig. 2*a*). Three months later, radiographs of the thoracic cavity revealed a large lymphoid tumour mass of the anterior mediastinum (Fig. 2*b*).

A syndrome of FeLV-associated non-responsive anaemias in cats has been recognized and appears similar to the disease induced in Balb/c mice by certain strains of Rauscher leukaemia virus (ref. 18 and W. D. H., jun., unpublished). Epidemiologists looking only for lymphosarcoma as evidence for horizontal infection with FeLV have not included deaths due to anaemia in their data. If we combine the FeLV$^+$ normal cats that developed lymphosarcoma and those that developed non-responsive anaemias, then a total of 23.7% of all FeLV$^+$ normal cats developed an FeLV-related disease. In this connexion, many young cats die of an ill defined syndrome known as the "fading kitten syndrome". Recent experiments on kittens experimentally infected with FeLV have produced a very similar syndrome which is associated with thymic atrophy[19]. Thus, FeLV may be the aetiological agent for

Table 2 Kinship and FeLV Status of Cats with Lymphosarcoma from Cluster Households

Total number of clusters	Number of cats with lymphosarcoma Total	Unrelated cats	Related cats	FeLV status of cats with lymphosarcoma Number examined	FeLV$^+$	FeLV$^+$(%)
38	117	78	39	56*	51	91

Cluster household cats were considered related if lymphosarcoma developed in either parent and offspring, or two or more siblings. FeLV status was determined by immunofluorescent detection of FeLV gs antigen in leukocytes and platelets in peripheral blood smears.

* Only fifty-six of the 117 cats that developed lymphosarcoma were examined for FeLV. The remaining sixty-one cats died before the study began.

at least two, and possibly more, non-neoplastic diseases of cats.

FeLV Transmission

How is FeLV transmitted from cat to cat? Three routes should be considered: saliva, urine, and blood. FeLV was found in the parotid salivary glands of thirty-two $FeLV^+$ lymphosarcoma cats but was not found in salivary glands of six $FeLV^-$ lymphosarcoma cats nor in eleven normal $FeLV^-$ cats. Saliva may be the most likely vehicle for spreading FeLV as cats are social animals using their suitably bristly tongues for grooming themselves and companion cats. Aerosolization of FeLV through salivary and nasal secretions may play an important role in disseminating the virus. In support of this it may be noted that Rauscher murine leukaemia virus can be experimentally transmitted to Balb/c mice by exposure to aerosol virus[20].

FeLV group-specific antigen can be detected in the kidneys of 70% of cats with lymphosarcoma[8,9]. Antigen was present in the infiltrating neoplastic lymphocytes as well as in discrete deposits on the glomerular basement membranes. Infectious FeLV and neoplastic cells infected with FeLV can pass through damaged kidneys into the urine (Table 1). The significance of this is evident when one considers the use of communal litter pans in urban households with several cats.

As FeLV is found free in the plasma, as well as budding from leukocytes and platelets in the blood of infected cats, it is conceivable that blood sucking parasites of cats might acquire and transmit FeLV. In warm months flea infestation is very high in the cat population, especially in households with several cats. A hamster reticulum cell sarcoma has been transmitted through the mosquito by transfer of tumour cells[21,22]. We were able to recover $FeLV^+$ leukocytes from a mosquito that sucked blood from an anaesthetized $FeLV^+$ cat with lymphosarcoma. Other cat parasites to be considered as possible vectors are nematodes, coccidia, and rickettsia. It is also quite possible that therapeutic blood transfusions of $FeLV^+$ blood to $FeLV^-$ anaemic cats may account for occasional iatrogenic horizontal transmission of FeLV with the attendant high risk of developing lymphosarcoma. In support of this, we have discovered two $FeLV^+$ normal cats that have repeatedly served as blood donors (see Table 4), but we were unable to identify those cats that received their viraemic blood.

Table 3 Detection of FeLV in Normal Cats from Households with a History of FeLV Associated Diseases

	Number of households in which normal cats were examined	Number of households with $FeLV^+$ normal cats	Households with $FeLV^+$ normal cats (%)	Number of normal cats tested	Number of $FeLV^+$ normal cats	$FeLV^+$ normal cats (%)
Households with a history of feline lymphosarcoma	78	44	56.4	395	154	38.9
Non-cluster households (one case of lymphosarcoma)	50	22	44.0	118	33	19.5
Cluster households (two or more cases of lymphosarcoma)	28 *	22	78.5	277	121	43.6
Households with FeLV-associated diseases other than lymphosarcoma	25	8	32.0	148	23	15.5
FeLV-associated disease households Total	103	52	50.5	543	177	32.6

* Twenty-eight of the thirty-eight lymphosarcoma cluster households (see text) had clinically normal cats.

Table 4 Detection of FeLV in Normal Cats and Frequency of FeLV Infection Correlated with Exposure to Cats with FeLV-Associated Disease

Environment of normal cats	History of FeLV-associated disease in household	Number studied	Number of households with $FeLV^+$ normal cats	Number of normal cats tested	Number of $FeLV^+$ normal cats
Multiple cat households	Yes	103	52	543	177
Multiple cat households	No	47	0	130	0
Single cat households	No	497	0	497	0
Stray cats	—	638	—	638	2 *
Experimental colonies	—	4	0	197 †	0
			Total	2,005	179

* Cats used repeatedly as blood donors.

† Including fifty-four cats from a colony receiving chronic administration of ^{90}Sr.

Implications

Two conclusions emerge from our study. Non-infected cats living in close proximity to cats shedding FeLV have a greatly increased risk of becoming infected and, once infected, the cat has an approximately 900 times greater risk of developing lymphosarcoma.

Whether the exogenous FeLV induces lymphosarcoma, or activates an endogenous virus which does so, is an open question. The important point is that it should be possible to break the cycle of horizontal transmission by vaccination or by selectively segregating or eliminating $FeLV^+$ cats from the cat population. We are now attempting to protect cats at high risk by vaccinating them with attenuated FeLV.

As FeLV replicates so well in human cell tissue culture[23] and as cats live in such close proximity to humans, the question of human infection with FeLV is important. Epidemiological evidence concerning this is inconclusive[24–26]. We have not found any antigen related to FeLV in more than 148 specimens of normal or neoplastic human tissues[7,9] and FeLV antigen has not been found in blood smears from 130 humans, including forty-three individuals living in households with FeLV+ cats, fifty-seven veterinary surgeons and thirty employees of veterinary hospitals. Much additional evidence needs to be obtained, however, before concluding that human infection with FeLV does not occur. For instance, does FeLV-neutralizing antibody exist in human serum? We have recently found that some cats are highly resistant to FeLV and have high titres of neutralizing antibody. A similar response to FeLV infection may occur in humans.

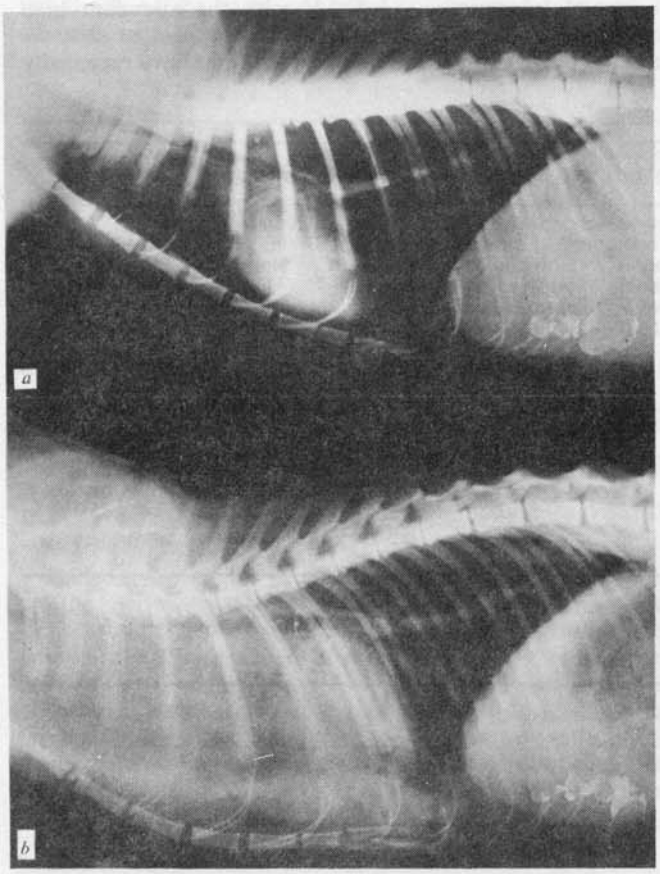

Fig. 2 *a*, Thoracic radiograph of an FeLV+ normal cat. There is no evidence of lymphosarcoma. *b*, 3 months later radiographs reveal thoracic effusion and a large anterior mediastinal lymphoid mass.

Table 5 Summary of Disease Development in FeLV Infected Normal Cats

Result	Number of cats	Cats followed up (%)
Total FeLV positive normal cats	177	—
Follow ups available	148	100
Alive and well	91	61.5
Death due to		
Lymphosarcoma	24*	16.2
Anaemia	11	7.5
Panleukopaenia	13	8.7
Feline infectious peritonitis	5	3.4
Other	4	2.7
Total	57	38.5
Follow-up not available		
Killed while healthy	14	—
Lost contact with owners	14	—
Cat lost	1	—
Total	29	

* The average time required to develop lymphosarcoma after detection of FeLV infection was 5.3 months (range 1 to 24 months).

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1 Gross, L., in *Oncogenic Viruses* (Pergamon, New York, 1970).
2 Huebner, R. J., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1087 (1969).
3 Emmelot, P., and Bentvelzen, P., *RNA Viruses and Host Genome in Oncogenesis* (North-Holland, Amsterdam, 1971).
4 Old, L. J., and Boyse, E. A., *Harvey Lect.*, **67**, 273 (1973).
5 Rubin, H., Cornelius, A., and Faushier, L., *Proc. natn. Acad. Sci. U.S.A.*, **47**, 1058 (1961).
6 Jarrett, W. F. H., Crawford, E. M., Martin, W. B., and Davie, F., *Nature*, **202**, 567 (1954).
7 Hardy, jun., W. D., Geering, G., Old, L. J., de Harven, E., Brodey, R. S., and McDonough, S., *Science*, **166**, 1019 (1969).
8 Hardy, jun., W. D., *J. Am. vet. med. Ass.*, **158**, 1060 (1971).
9 Hardy, jun., W. D., Hirshaut, Y., and Hess, P., *Fifth Int. Symp. Comp. Leukaemia Res. Bibl. Haematol.*, **39**, 778 (Karger, Basel, and New York, in the press).
10 Sarma, P., Baskar, J. F., Huebner, R. J., Old, L. J., and Hardy, jun., W. D., *Proc. Fourth Int. Symp. Comp. Leukaemia* (Karger, Basel, 1970).
11 Schneider, R., *J. Am. vet. med. Ass.*, **157**, 1753 (1970).
12 Schneider, R., *J. Am. vet. med. Ass.*, **158**, 1125 (1971).
13 Schneider, R., *Int. J. Cancer*, **10**, 345 (1972).
14 Rickard, C. G., *J. small Anim. Pract.*, **10**, 615 (1969).
15 Jarrett, W., *J. clin. Path.*, **25**, 43 (1972).
16 Brodey, R. S., McDonough, S., Frye, F. L., and Hardy, jun., W. D., *Proc. Fourth Int. Symp. Comp. Leukaemia* (Karger, Basel, 1970).
17 Dorn, C. R., Taylor, D. O. N., and Hibbard, H. H., *Am. J. vet. Res.*, **28**, 993 (1967).
18 Camiscoli, J. F., LoBue, J., Gordon, A. S., Alexander, P., Schultz, E. F., and Weitz-Hamburger, A., *Cancer Res.*, **32**, 2843 (1972).
19 Anderson, L. J., Jarrett, W. F. H., Jarrett, O., and Laird, H. M., *J. natn. Cancer Inst.*, **47**, 807 (1971).
20 McKissick, G. E., Griesemer, R. A., and Farrell, R. L., *J. natn. Cancer Inst.*, **45**, 625 (1970).
21 Banfield, W. G., Woke, P. A., MacKay, C. M., and Cooper, H. L., *Science*, **148**, 1239 (1965).
22 Woke, P. A., and Konwinski, N., *J. natn. Cancer Inst.*, **48**, 219 (1972).
23 Jarrett, O., Laird, H. M., and Hay, D., *Nature*, **224**, 1208 (1969).
24 Bross, I. D. J., and Gibson, R., *J. Med.*, **1**, 180 (1970).
25 Bross, I. D. J., Bertell, R., and Gibson, R., *Am. J. Publ. Hlth.*, **62**, 1520 (1972).
26 Schneider, R., *Int. J. Cancer*, **10**, 338 (1972).

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