The Feline Leukemia Virus

William D. Hardy, Jr, VMD

The feline leukemia virus (FeLV) is a contagious oncogenic RNA virus (oncovirus) that causes both neoplastic and non-neoplastic diseases in pet cats. During FeLV replication, a DNA copy of the viral RNA genome is made and inserted into the DNA of infected cells. The integrated FeLV DNA (provirus) codes for the replication of more virus, can cause cell transformation or, by recombining with cat cellular gene(s) can generate the highly oncogenic feline sarcoma virus (FeSV). The fate of an FeLV exposed cat depends on its immunological response to the FeLV envelope antigens and to the FeLV and FeSV-induced tumor-specific antigen, FOCMA. About 30% of FeLV exposed cats do not mount an adequate immune (antibody) response to FeLV and become persistently infected. However, about 40% of exposed cats produce high titers of antibody to the FeLV envelope antigens (neutralizing antibody) and become immune to infection. The remaining 30% of exposed cats become neither infected nor immune to FeLV and thus remain susceptible to FeLV infection. Since cats can become immune to FeLV infection it is probable that an effective FeLV vaccine will be developed. FeLV can infect the cells of nonfeline species, including human cells in culture, and it is therefore possible that FeLV may be a public health hazard. However, the evidence that FeLV can infect people is contradictory and the public health questions are still unresolved.

From the Laboratory of Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

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Origin of Feline Leukemia Virus

Of all the species with naturally occurring retroviruses (reverse transcriptase containing RNA viruses) only the cat has such a large diversity of naturally occurring nonlaboratory retrovirus induced diseases.[1] These diseases occur in pet cats around the world and collectively account for the majority of deaths due to disease in pet cats.

Ancient History – About one to 10 million years ago in the arid deserts of northern Africa an ancestor of the present day rat roamed freely. At the same time and in the same region, ancestors of the present day cat roamed and hunted prehistoric rats. It is now apparent that after a fierce battle between an ancestral rat harboring and replicating its endogenous oncovirus (an RNA tumor virus present in the chromosomes of all individuals of that species) and an ancestral cat, the cat became infected with the rat virus, either via the bite of the rat or by consuming the rat's body. Ever since that prehistoric battle the descendants of that ancestral cat, which have evolved to our present day pet cats, have transmitted the new oncovirus, now known as the feline leukemia virus (FeLV), contagiously from one cat to another.[2] Of course this is a simplified dramatization based on information which scientists have hypothesized via complex molecular biological studies. For example, the FeLV RNA genome has been found to be closely related to the cellular DNA of the present day rat indicating the rodent origin of FeLV.[3] The transmission of oncoviruses between species appears not to be an isolated event since it is known that other

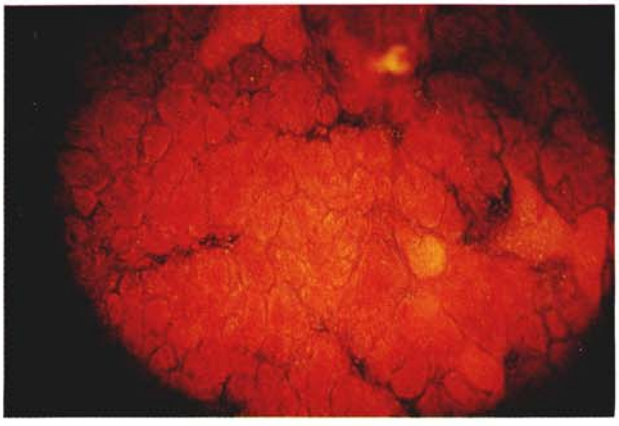

Figure 1A— Salivary gland from an FeLV-uninfected cat. The frozen section of the salivary gland is negative in the immunofluorescent antibody test for FeLV and thus no green fluorescence is visible.

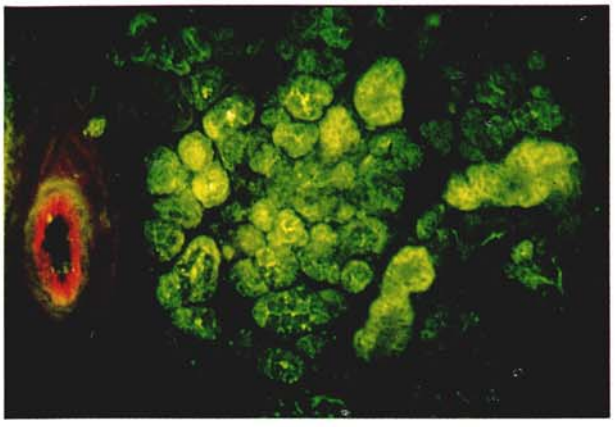

Figure 1B— Salivary gland from an FeLV infected cat. Note the strong granular fluorescence in the cytoplasm of the salivary acinar cells.

oncoviruses were transmitted between species. For example the endogenous RD-114 virus of cats came from old world primates, the gibbon ape leukemia virus originated in ancestors of the Asian mouse *Mus caroli* and the bovine leukemia virus originated in an unknown nonbovine species, a species which may now be extinct.[4]

Modern History – It was not until 1964 when W.F. Jarrett, and his colleagues in Scotland, discovered FeLV in a cat that lived in a cluster-household where several cats had developed lymphosarcoma (LSA).[5] FeLV was first isolated from the serum of a cat with LSA by Kawakami and his colleagues in 1967.[6] In 1969, the author and his colleagues produced the first anti-FeLV serum[7] and subsequently in 1973 developed the indirect immunofluorescent antibody (IFA) test for the detection of FeLV antigens in peripheral blood leukocytes.[8] Using this test the author, *et al* discovered that FeLV is transmitted contagiously between pet cats and that FeLV induces several neoplastic and non-neoplastic diseases of cats.[2,7] The major vehicle for transmitting the virus is saliva which can contain as many as 5×10^3 to 2×10^6 viable virus particles per milliliter.[2,9,10] Licking or biting enables the virus to infect cats via the ocular, oral and nasal membranes.[11] After penetrating the mucous membranes, the virus infects the lymphocytes in the local lymph nodes of the head and neck and then spreads to the bone marrow. However, more FeLV exposed cats become immune to the virus [Table 1] before the infection spreads from the local lymph nodes of the head and neck to the bone marrow, than become persistently infected.[12]

Once the rapidly dividing bone marrow cells become infected, FeLV proliferates rapidly and most cats soon become persistently viremic having approximately 10^3 to 10^6 infectious FeLV per ml of blood.[10] Within a few weeks the virus spreads to, and replicates in the normal salivary glands [Figures 1A, 1B] and respiratory epithelium from where it is shed and thus transmitted to other cats.[11] In persistently infected cats, FeLV replicates in several different cell types including lymphocytes, neutrophils, megakaryocytes, erythroblasts, salivary epithelial cells, respiratory and pharyngeal mucosal cells, pancreatic and intestinal mucosal cells, and can cause proliferative or degenerative diseases of each of the cell types that it infects.[8,11,13,14] Although the period of disease development is highly variable, 83% of FeLV infected healthy cats die within three years [Table 2].[15]

The feline leukemia virus is an example of an exogenous oncovirus (oncogenic RNA virus).[1] Both endogenous and exogenous oncoviruses are known to exist. Endogenous oncoviruses are incor-

Table 1

Consequences of FeLV Exposure

Result of Exposure to FeLV	Per Cent of Exposed Cats
Persistently infected	30%
Immune to FeLV	42%
Not infected nor immune	28%

Table 2

Survival of FeLV-Infected and Uninfected Pet Cats

FeLV Status	Number of Cats	Number of Cats That Died	Per cent of Cats That Died
Uninfected	512	82	16%
Infected	96	80	83%
	608	162	27%

Table 3

Examples of Cross Species Transmission of Oncoviruses

Infected Recipient Species	Virus	Source or Donor of Virus	How Virus is Transmitted in the Recipient Species
Cat	FeLV	Rat ancestor	Contagiously
	RD-114	Old world monkey	Genetically
Pig	PoLV	Mouse ancestor	Genetically
Cow	BLV	Unknown (may be extinct species)	Contagiously
Gibbon ape	GaLV	Mouse ancestor	Contagiously

Adapted from reference 4.

porated into the genome of all uninfected host cells and are transmitted from the parents to the offspring as genes via the egg or sperm (*ie* – as inherited Mendelian traits), whereas the exogenous oncoviruses are not incorporated into the genome of uninfected cells and are transmitted contagiously among individuals of the host species.[16] During evolution some endogenous oncoviruses escaped from host control and infected members of other species [Table 3].[17]

In some cases the virus is transmitted vertically (genetically) in its new species, but in other cases it is transmitted contagiously. FeLV, for example, may have been transmitted from an ancestor of the rat to the ancestor of the domestic cat approximately one to 10 million years ago and may have been transmitted contagiously between cats ever since.[3] There are three known oncoviruses of domestic cats: (1) the nondisease producing endogenous RD-114 virus which is present as multiple complete proviral copies (DNA sequences), but not as complete replicating virus, in all cells of domestic cats;[18,19] (2) the disease producing FeLV; and (3) the sarcomagenic feline sarcoma virus (FeSV) which is a recombinant virus, that is, its genetic material arose from a recombination between FeLV and cat cellular DNA sequences.[20-23] Unlike RD-114, FeLV and FeSV are not present as complete proviral DNA in normal uninfected cat cellular DNA.

The Replication of FeLV and FeSV

The FeLV Genome

All oncoviruses contain a 60-70S single stranded RNA genome [Figure 2], consisting of a *gag* (group specific antigen) gene that codes for the internal viral proteins, the major protein being the p27, so called because it has a molecular weight of 27,000 daltons, and the other structural proteins p15, p12, and p10; a *pol* gene which codes for the viral RNA dependent DNA polymerase (reverse transcriptase), the enzyme responsible for copying the viral RNA into DNA and thus permitting virus replication; and an *env* gene that codes for the viral envelope components gp70 (a glycoprotein with a

molecular weight of 70,000 daltons) and p15E.[24,25] These three FeLV genes, the *gag*, *pol* and *env* genes, account for over 90% of the viral genome. Additional minor components (less than 2%) of the virogene are the terminal regions (TRs in Figure 2) which control the replication of the virus but which do not code for any of the viral structural proteins.[25] Although these TR oncovirus genes have not been positively identified in the feline oncoviruses they have been found in avian oncoviruses.[25] Regions coding for the initiation of DNA synthesis, designated PB for primer binding site, and for the control of RNA synthesis, designated U_3 (or C), have also been identified in avian oncoviruses.[25]

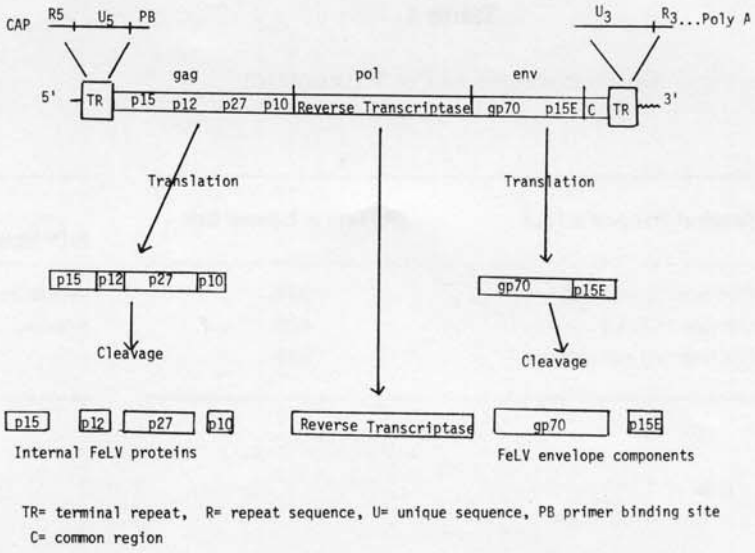

Figure 2— A diagram of the feline leukemia virus genome. The integrated provirus codes for polyproteins which are cleaved to individual viral proteins.

An important feature of oncoviruses is that their genes are stably integrated into the DNA of the host cell during viral replication and, thus, can cause transformation of the cell at any time after infection.[26,27] Unlike some acute leukemia viruses, FeLV induces leukemias after a long latent period and the FeLV genome is not known to contain oncogenic (*onc*) or leukemogenic (*leuk*) genes in addition to the viral *gag*, *pol*, and *env* genes. In contrast, the highly oncogenic, acute transforming leukemia and sarcoma viruses of mice (Abelson MuLV), chickens (ASV), rats (RaSV), hamsters (HaSV), cats (FeSV) and primates (SSV), do possess a transforming gene, the *leuk* or *src* gene.[28] The *src* gene of sarcoma viruses induces transformation of fibroblasts resulting in fibrosarcomas. Except for some avian sarcoma viruses,[29] sarcoma viruses are replication defective, that is they do not possess the complete virogene and cannot replicate without the help of a replication competent helper leukemogenic virus.[30] FeSV, for example, differs from FeLV in that when it is formed by recombination of FeLV genes with a cat cellular gene, it loses the *env*, *pol* and part of the *gag* viral genes and thus loses its ability to replicate [Figure 3]. FeSV incorporates an additional gene from the infected cell called *sarc* which is present in all cat cells and which is similar to, but not identical with, the FeSV *src* gene.[23,31]

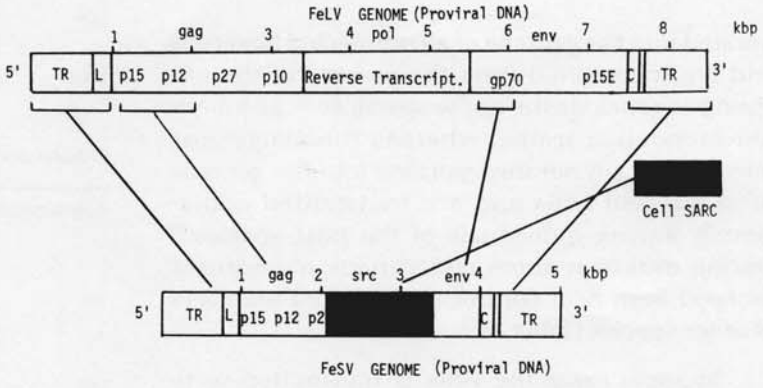

Figure 3— Generation of feline sarcoma viruses by recombination of the FeLV genome with a cat cellular gene called *sarc*. Note that some of the FeLV genes are deleted and thus the FeSV is unable to replicate ("replication defective").

Since FeSV is a defective virus it is probably not very contagious. However, FeLV can 'rescue' FeSV, that is, supply the missing envelope necessary for FeSV replication.[32] Theoretically, FeSV can be generated in every FeLV infected cat cell by recombination of FeLV genes with cat cellular genes. Thus, FeLV infection may result not only in the induction of FeLV diseases but also in the possibility that the highly oncogenic FeSV may be generated during the FeLV replication process.

Table 4

Species Host Range of FeLV Subgroups

Species Cells Susceptible to FeLV	Replication of FeLV Subgroups		
	FeLV-A	FeLV-B	FeLV-C
Cat	+	+	+
Dog	–	+	+
Mouse (Swiss)	–	–	–
Rat (Fischer)	–	–	–
Mink	–	+	+
Hamster	–	+	–
Guinea pig	–	–	+
Pig	–	+	–
Bovine	–	+	+
Monkey (Vero)	–	+	–
Human	–	+	+

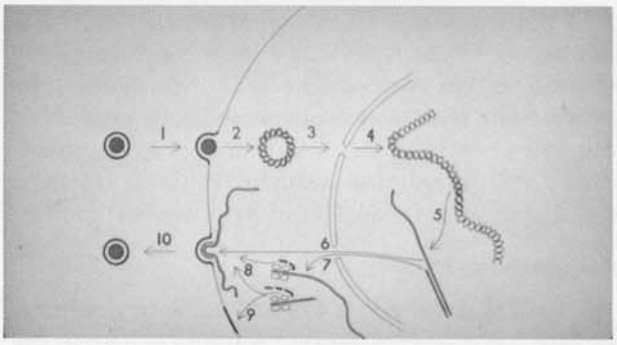

Figure 4— Diagram of the replication cycle of the feline leukemia virus: 1) cell attachment and viral penetration, 2) FeLV RNA is copied into DNA by the viral reverse transcriptase, 3) integration of the viral-specified DNA, 4) FeLV proviral DNA integrated in the cell's chromosomes, 5) translation of the FeLV DNA provirus, 6, 7, 8) synthesis of viral RNA, proteins and reverse transcriptase, 9) transformation protein (FOCMA) is produced only if cell is transformed and 10) FeLV budding from the cell membrane.

FeLV Replication Cycle

The first step in the FeLV replication cycle is cellular infection, but not all cells are susceptible to FeLV infection, since only cells with receptors on their surfaces which are complementary to the FeLV envelope can be infected with the virus.[33,34] However, unlike the murine and avian oncoviruses, FeLV can infect various feline cell types as well as cultured cells from many different species.[33,34] In fact, only rat and mouse cells are known to be resistant to infection by all three serotypes of FeLV [Table 4]. After infecting the cell, a complementary DNA copy of the FeLV RNA is made in the cell cytoplasm by the FeLV reverse transcriptase.[26,27] Using this newly synthesized single stranded DNA as a template, double stranded DNA is formed in the cell nucleus and is integrated into the DNA of the chromosomes of the host cell [Figure 4]. The integrated virogene (provirus) may remain part of the cellular genetic material without producing infectious virus or transforming the host cell. However, the virogene can be activated at any time to produce virus.[35] In virus producing cells, messenger RNA is synthesized which, in turn, produces viral proteins in the cell cytoplasm. Complete infectious virus particles are assembled from the viral proteins and newly synthesized viral RNA and bud [Figure 5] from the cell membrane, thus completing the infectious cycle. FeLV is not cytopathic, but

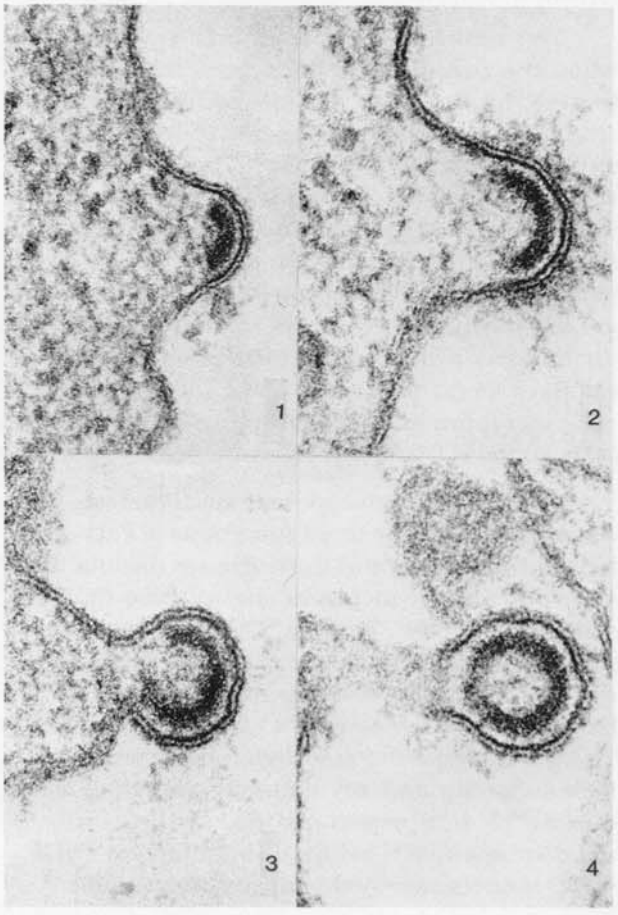

Figure 5— Electron micrograph of different stages in the process by which FeLV buds from the membrane of an infected cell. The inner circular electron dense doughnut-shaped core contains the FeLV RNA (Courtesy, E. deHarven, Sloan Kettering Institute, published with permission from Experimental Leukemia, M.A. Rich, editor, Appleton-Centry Croft, New York, 1968).

FeSV induces foci of transformed cells in fibroblasts in culture.[20-22] Both FeLV and FeSV can induce cell transformation with or without concomitant virus production, and these transformed cells express an antigen known as the feline oncornavirus associated cell membrane antigen (FOCMA) on their cell membranes regardless of virus replication.[36,37]

Structure of FeLV

FeLV is spherical, is 110 millimicrons in diameter and has a single stranded genome of RNA in the viral core. The envelope of the virus is composed of spikes supporting knobs which are composed of glycoproteins. Bolognesi and his coworkers have proposed a model of oncoviruses which relates their structure to individual viral components [Figure 6].[38]

The FeLV Antigens

Envelope Antigens

The replication cycle of FeLV is completed when the mature virus buds from the cell membrane. The *env* gene of FeLV codes for the two structural envelope components of FeLV, the gp70 and p15E [Figures 2, 6A, 6B].[39] The viral envelope consists of a basement membrane from which project spikes (p15E) supporting knobs composed of the major envelope antigen, the gp70. It is likely that cat cell membrane proteins and glycoproteins are also incorporated into the FeLV envelope during the budding process. For example, Azocar and Essex have found that FeLV, when grown in human cells, incorporates the major human histocompatibility antigens (HLA) into its envelope.[40,41]

Viral interference and neutralization tests have shown that there are three subgroups of FeLV envelope antigens (A, B and C) which are distinct from each other and which give rise to three different subgroups of FeLV [Table 4].[42-44] The occurrence of the three FeLV subgroups in cats is markedly different. For example, FeLV-A is found in 100% of infected cats, whereas FeLV-C is rarely found.[42-45] There is no apparent association between any one FeLV subgroup and any naturally occurring feline disease[45,46] but, experimentally FeLV-C induces aplastic anemias[47] while one isolate of FeLV-A (F422) induces mainly thymic lymphosarcoma.[48]

The different FeLV subgroups differ in their ability to infect cells of various species [Table 4]. For example, FeLV-B and -C readily infect cultured human cells but FeLV-A does so only poorly or not at all.[42,43] FeLV-B appears to make FeLV-A more contagious and is found in more cats with LSA than in healthy cats.[46] If the immune system of the cat responds to the viral envelope antigens it will produce antibody that can neutralize the infecting strain of virus and, if high titers of this antibody are produced quickly after FeLV infection, the cat may be able to reject the infecting virus before becoming persistently viremic and will then be immune to further infection by the same subgroup of FeLV.[45]

Internal FeLV Antigens

Geering, in Old's group, first identified the p30 group-specific antigen (the major gag gene product) in laboratory strains of murine leukemia viruses (MuLV).[49] Geering, Old and the author later found a shared interspecies antigen, which we called gs-3 (group specific antigen 3), in MuLV and FeLV.[50] The gs-3 antigen was subsequently found to be common to the p30 (p27 in FeLV) of all mammalian C-type oncoviruses except the bovine leukemia virus.[51,52] The internal group-specific viral proteins of FeLV are coded for by the gag gene and consist of the p15, p12, p27, and p10 in that order from the 5′ to the 3′ end of the viral genome [Figure 2].[24]

The internal structural proteins of FeLV are found at specific sites in the virus [Figure 6].[38] The p15 is found in association with the exterior of the viral core, p12 is a component of the inner coat which is located just inside the viral envelope, p27 is a component of the shell of the viral core while p10 is a nucleoprotein associated with the viral RNA genome.

The internal FeLV structural proteins are produced in great excess in the cytoplasm of the cell during the replication cycle [Figure 6B].[7,53] Most of these antigens are never packaged into FeLV particles and remain inside the cell or become solubilized in plasma. Detection of FeLV antigens by means of the indirect immunofluorescent antibody (IFA) test or the enzyme linked immunosorbent assay (ELISA) can be used to detect FeLV infection in pet cats. In the IFA test, alcohol fixed cells are reacted with a rabbit or goat anti-disrupted-FeLV serum in an indirect test. Positive cytoplasmic fluorescence indicates that the cell is replicating FeLV and that the cat is infected with the virus.[54] In contrast to the MuLV system, FeLV antigen positive cells do not exist without replicating FeLV. Positive IFA test results correlate 98% of the time with the ability to isolate FeLV from the serum of infected cats.[54]

Cats are not tolerant to their FeLV gs antigens as would be expected from the postulates of the oncogene theory.[35] Noronha and his coworkers

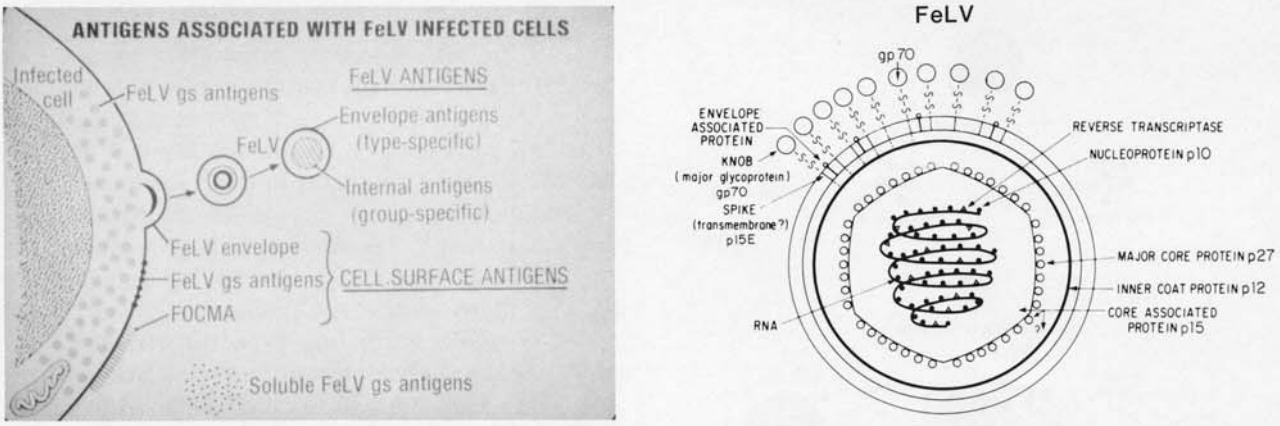

Figure 6— A (Left): Antigens produced, or induced by, FeLV in infected and transformed cells. B (Right): A model of the structure of the feline leukemia virus. (Adapted from reference 38 and reprinted with permission. Copyright 1978 by the American Association for the Advancement of Science).

first showed that cats immunized with disrupted FeLV produced antibodies to the group-specific antigens.[56] In addition, FeLV uninfected and, even occasionally, FeLV infected pet cats can produce antibody to the internal FeLV p15, p12, p27 and p10 antigens.[57-59] In some cats we have found FeLV gs antigen and antibody to FeLV gs antigens in immune complexes which were deposited in the glomeruli.[60] It is apparent that natural antibodies to FeLV gs antigens serves no beneficial role in cats and in fact are probably detrimental in that they form immune complexes in persistently viremic cats.

Reverse Transcriptase

FeLV, like other replication competent oncoviruses, possesses the RNA dependent DNA polymerase (reverse transcriptase) enzyme.[26,27] This enzyme is coded for by the *pol* gene and its function is to copy the viral RNA into single stranded complementary DNA (cDNA), a process called reverse transcription. The enzyme is found in association with the coiled viral RNA in the FeLV core [Figure 6]. Recent studies by Jacquemin and coworkers have shown that some cats (8 of 68) exposed to FeLV produced antibodies to the FeLV reverse transcriptase.[61] These antibodies were only found in FeLV uninfected cats.

The Feline Oncornavirus Associated Cell Membrane Antigen (FOCMA)

The feline oncornavirus-associated cell membrane antigen (FOCMA) was initially defined by Essex and his coworkers, as an antigen detected by a fluorescent antibody reaction of certain cat sera on viable cell membranes of cultured feline lymphosarcoma (LSA) cells (FL74) which produce all three subgroups of FeLV.[62-64] The positively reacting cat sera were obtained from cats who had rejected FeSV-induced sarcomas.

FOCMA Antibody and Immune Surveillance

Shortly after the discovery of FOCMA, Essex and his coworkers began to study the immune response of cats to FOCMA. He studied the FOCMA antibody response of cats inoculated with FeSV and found that cats who regressed their FeSV induced fibrosarcomas or cats that did not develop any palpable tumors had high titers of FOCMA antibody, whereas those cats who developed progressive sarcomas had very low FOCMA antibody titers.[65,66] The geometric mean titer of "regressor" or "no tumor" cats was 20 fold higher than cats with progressor tumors. There was also a good correlation between maternally transmitted FOCMA antibody in the serum of neonatal kittens and their ability to resist FeSV-induced fibrosarcomas.

In addition to the correlation between FOCMA antibody and regression of FeSV induced sarcomas, Essex found a correlation between high FOCMA antibody titers and the resistance to LSA development in FeLV inoculated cats.[67,68] Similarly, under natural household conditions, all pet cats that developed LSA or leukemia had low or nonexistent FOCMA antibody titers.[45,68-70] In one study, healthy exposed cats from two households where multiple cases of feline LSA had occurred were tested for FOCMA antibody.[45,67,71] Of the 140 cats tested, 82%

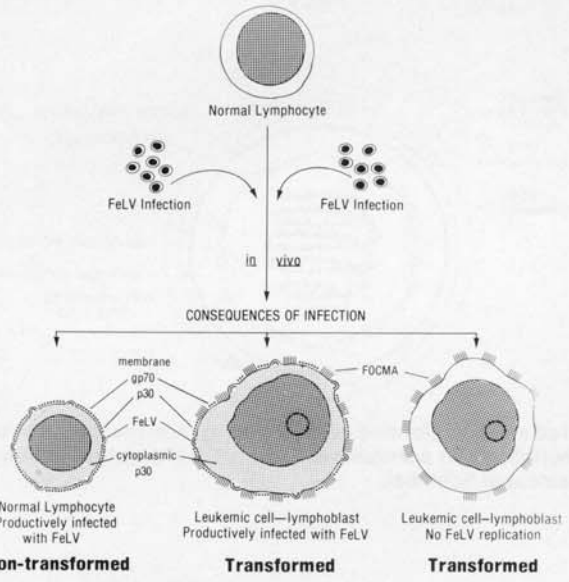

Figure 7— Consequences of FeLV infection and transformation of cat lymphocytes. FOCMA is expressed on both FeLV positive and negative lymphosarcoma cells. (Reproduced with permission, Cold Spring Harbor Laboratory, New York, reference 78).

had FOCMA antibody. Fourteen of the FeLV-infected cats developed LSA during the two year observation period and none of these cats had FOCMA antibody titers higher than four. In contrast, none of the infected cats who had titers above four developed LSA.

Most healthy pet cats are not persistently infected with FeLV, but most have low titers of antibody to FOCMA indicating that they were transiently infected with FeLV at some time in their lives.[31] However, most of these cats with low FOCMA antibody titers do not have protective titers of FeLV neutralizing antibody.[45] The only group of cats which appears to have no FOCMA antibody at all are specific pathogen free (SPF) cats.[72]

FOCMA – An FeLV and FeSV Induced Tumor-Specific Antigen

FOCMA is Not an FeLV or FeSV Structural Antigen

We first reported that the occurrence of FOCMA antibody and FeLV serum neutralizing (SN) antibody were discordant in many cats.[45,73] Persistently FeLV infected cats with high FOCMA antibody titers have no SN antibody.[45,67,73] It has been shown by radioimmunoprecipitation assays that viremic cats with high FOCMA antibody titers have no antibody to purified FeLV gp70 or p27, and that purified gp70 or p27 could not remove FOCMA antibody by *in vitro* absorption.[57,58] Similarly, intact or disrupted FeLV does not absorb out FOCMA antibody reactivity.[75,76] Snyder and my group have isolated FOCMA from FL-74 cells and have shown it to be a protein with a molecular weight of 70,000 daltons which is distinct from the 70,000 dalton FeLV envelope glycoprotein, gp70.[76] Also, FeLV infected normal nontransformed cells which have both FeLV gp70 and p27 on their membranes do not express FOCMA,[73-77] but conversely, FeLV negative (nonproducer) LSA cells which do not have FeLV gp70 or p27 do express FOCMA.[74-76,78] Similarly, FeSV transformed nonproducer mink fibroblasts do not have FeLV gp70 or p27 but do express FOCMA.[77] It is clear then that although FOCMA is an FeLV and FeSV induced tumor-specific antigen, it is not a structural component of either virus [Figure 7].

FOCMA from Lymphosarcoma (FOCMA-L) and FeSV Transformed Cells (FOCMA-S)

FOCMA was first detected on cultured LSA cells by reaction with sera from cats who had rejected FeSV induced fibrosarcomas.[63,64] Although there may be no difference between FOCMA expressed on LSA cells (FOCMA-L) and FOCMA expressed on FeSV transformed fibroblasts or sarcoma cells (FOCMA-S) we will differentiate them here for this discussion.

FOCMA-L

FOCMA has been found to be present on the surface of every feline LSA cell [Figure 8] tested whether or not is was naturally occurring or experimentally induced, FeLV positive or FeLV negative.[74-76,78] Our laboratory has examined 44 LSA cats and, in all of these cats, FOCMA was expressed on the tumor cells. Both B and T cell LSAs express FOCMA [Table 5].[74,75,78] However, normal lymphocytes that were obtained from cats with FOCMA positive LSAs were negative for FOCMA.[74,78] A total of 91 nontransformed normal cell preparations from 22 FeLV uninfected and 15 FeLV infected cats were also tested for FOCMA. Thirty-seven cell preparations were FeLV infected and 54 were negative for FeLV [Table 5]. None of the normal nontransformed cells were positive for FOCMA [Figure 9].

Snyder and we have characterized FOCMA-L from both FeLV positive and negative feline LSA

Table 5

Occurrence of FOCMA on Feline Cells

Cell	FeLV Positive	FeLV Negative	Total	FOCMA Positive
Normal	37	54	91	0
Lymphosarcoma	31	13	44	44

cells.[76,79] The ^{125}I-labelled cell surface proteins precipitated from LSA cultures by a FOCMA typing antiserum from a viremic cat were analyzed by SDS-PAGE. Analysis of FOCMA-L showed that it is a non-glycosylated 70,000 dalton protein which is present on both FeLV positive and negative LSA cells. The protein is a component of the cell membrane and is distinct from FeLV and RD-114 *gag* proteins and from the FeLV and RD-114 glycoprotein molecules.[79] Unlike the transformation-specific proteins of the avian sarcoma virus and Abelson MuLV, FOCMA-L does not appear to have protein kinase activity.[79-81]

FOCMA-S

Feline Cells

Although FeLV does not transform fibroblasts in culture, the recombinant replication defective FeSV can transform fibroblasts from a number of species including cats.[20,21,81,82] Sliksi and Essex have reported that FeLV infected feline fibroblasts have undergone rare "spontaneous transformation" with the release of transforming virus and with the expression of FOCMA.[83] This spontaneous transformation has only been observed after infection with FeLV subgroups B or C and may represent *in vitro* generation of FeSV. We have detected FOCMA on the surface of fibrosarcoma cells from three cats with naturally occurring multicentric fibrosarcomas.[78,84]

The Gardner-Arnstein strain of FeSV is able to induce melanomas in the skin and eyes of cats when inoculated intracutaneously or intraocularly.[85-87]FOCMA-S is expressed on the surface of the ocular melanoma cells.[87] That observation is significant since it shows that FeSV can transform not only cells of the mesodermal embryonic germ layer but also cells of ectodermal origin.[88] It is also evidence against the hypothesis that FOCMA-type transformation related proteins represent a differentiation-related cellular gene acquired by FeSV that acts at a specific stage of transition of cells committed to a specific line of differentiation.[89]

Nonfeline Cells

FeSV can transform fibroblasts from nonfeline species including the dog, mouse, guinea pig, rat, mink, sheep and primates and FOCMA is expressed on these transformed cells.[78,83,90] The induction of FOCMA on heterospecies cells clearly indicates that FeSV can code for FOCMA. Many of these heterospecies cells are nonproductively transformed, that is, no FeLV or FeSV are produced by these cells. However, FeSV can be rescued from these nonproducer (NP) cells by FeLV, the endogenous baboon oncovirus and the amphotropic MuLV.[91]

Cloned FeSV NP transformed cells express FOCMA as a polyprotein covalently linked to the amino-terminal *gag* gene peptides.[58,79,91-94] The polyprotein (pp) contains the FeLV *gag* p15, p12 and variable amounts of p27 covalently linked to FOCMA, and varies in size from 85,000 to 180,000 daltons depending on the strain of FeSV and the clone of cells selected.[24,92,93] The polyprotein can be immunoprecipitated with either anti-FeLV *gag* p15, or p12 or with cat anti-FOCMA sera.[59,88] The polyprotein is cleaved into the individual proteins; p15, p12 and a molecule of 65,000 to 70,000 daltons which contains the FOCMA determinant [Figure 10].[24]

The pp 85,000 to 180,000 dalton FOCMA-containing molecule is called the gag-X protein and is found in the cytoplasm of transformed cells as well as on the cell membrane.[79,91] FOCMA has never been detected as a gag-X polyprotein in feline LSA cells although it is present as a polyprotein in FeSV transformed feline fibroblasts and melanoma cells.[79,88,89]

FOCMA is the most extensively characterized tumor-specific antigen known. Since tumor viruses possess definable amounts of genetic material that encode for viral replication and cellular transformation, they are more suitable agents for the study of the mechanism of transformation than are chemical or physical carcinogens. It may also be possible to isolate FOCMA for use as an immunogen as one component of an anti-FeLV and anti-LSA vaccine. However, such a vaccine must also include an FeLV immunogen which will induce SN antibody since FeLV infected cats with protective FOCMA antibody titers are resistant to LSA and other FeLV and FeSV induced tumors but are still susceptible to all of the non-neoplastic FeLV diseases.

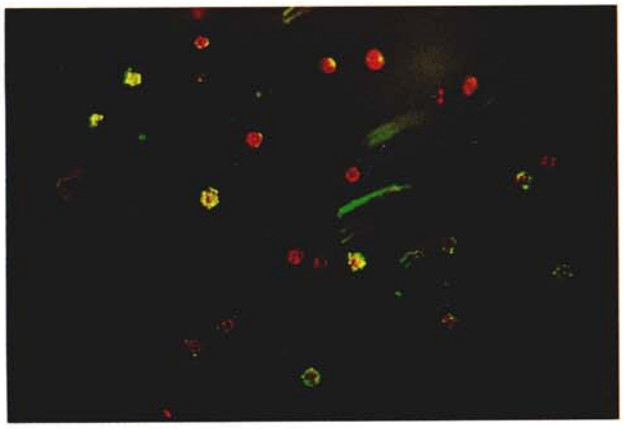

Figure 8— Detection of FOCMA in a viable cell immunofluorescent antibody test on FeLV negative lymphosarcoma cells.

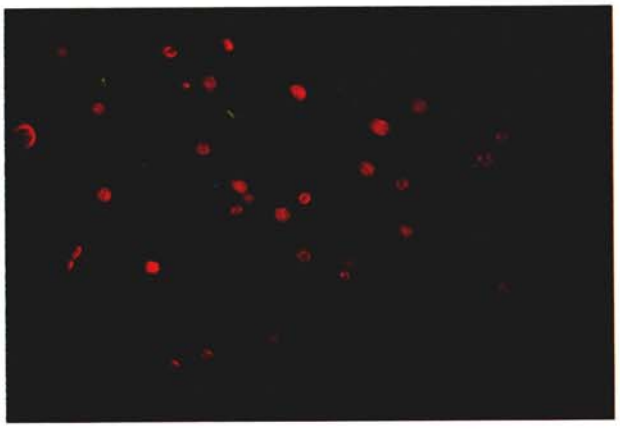

Figure 9— A FOCMA negative viable cell immunofluorescent antibody test of FeLV positive normal cat lymphocytes.

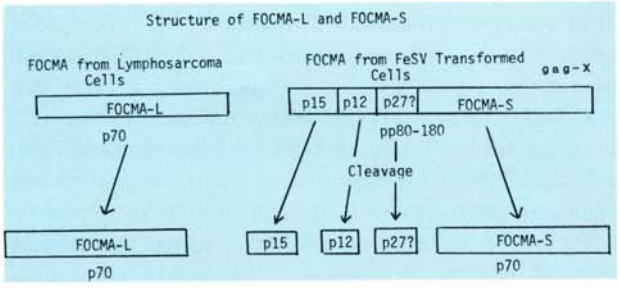

Figure 10— A diagram of the structure of FOCMA-L derived from lymphosarcoma cells and FOCMA-S derived from FeSV-transformed cells.

Table 6

Comparison of the Immunofluorescent Antibody Test for FeLV with Tissue Culture Isolation of FeLV

Reference	FeLV Immunofluorescent Antibody Test Results	Number of Cats Tested	Results of Tissue Culture Isolation of FeLV	Percent Concordance of the Two Tests
Hardy[8,55]	Negative	153	3	98%
	Positive	121	118	97.5%
Jarrett[99]	Negative	563	4	99%
	Positive	49	4	92%

Epidemiology of FeLV

FeLV Testing Methods

FeLV Immunofluorescent Antibody (IFA) Test

Until 1972 only immunodiffusion,[53,95] complement fixation[96] and electron microscopy[97] were used to detect FeLV in cat tissues. In 1972, the author and his colleagues developed the sensitive, rapid and inexpensive indirect immunofluorescent antibody (IFA) test for detection of FeLV antigens in leukocytes of the peripheral blood.[2,8] This test can be done on two to three drops of blood and was quickly adopted by the veterinary profession to aid in the diagnosis of FeLV diseases and to screen exposed cats for FeLV infection.[54]

The specificity of the IFA test has been evaluated by comparing the results obtained in the test with the ability to isolate infectious FeLV from the plasma of the tested cat. We found that virus could be isolated from the plasma of 118 of the 121 (97.5%) cats that were IFA test positive. Conversely, FeLV could not be isolated from 150 of the 153 (98%) IFA test negative cats [Table 6].[54,55] Those results showed that the IFA test is specific for FeLV and that the test can be used to detect the presence of infectious virus. IFA test positive cats excrete FeLV in their saliva.[2,8,10,99]

The IFA test is a highly specific, but relatively simple, test that requires only a small blood sample. The blood is smeared on a microscope slide and air dried before being fixed in alcohol or acetone for three minutes. The fixative disrupts the outer membrane of the white blood cells and enables the rabbit anti-FeLV serum to come into con-

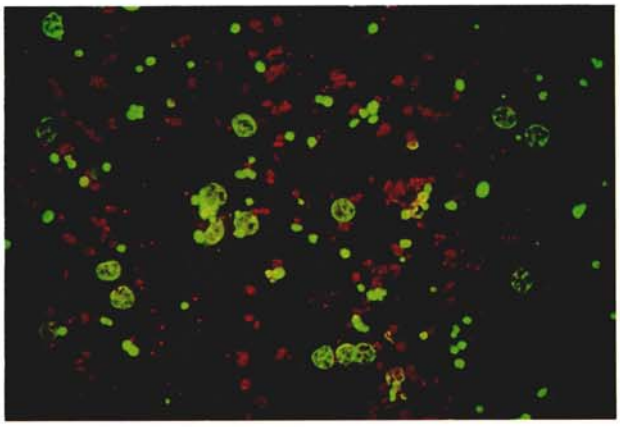

Figure 11— A positive FeLV immunofluorescent antibody test. Positive apple-green cytoplasmic fluorescence in neutrophils and platelets indicates that FeLV is replicating in the cells.

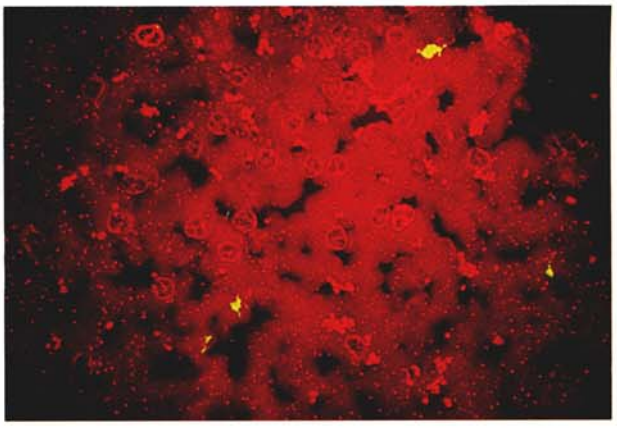

Figure 12— A negative FeLV immunofluorescent antibody test. No fluorescence is present in the uninfected neutrophils.

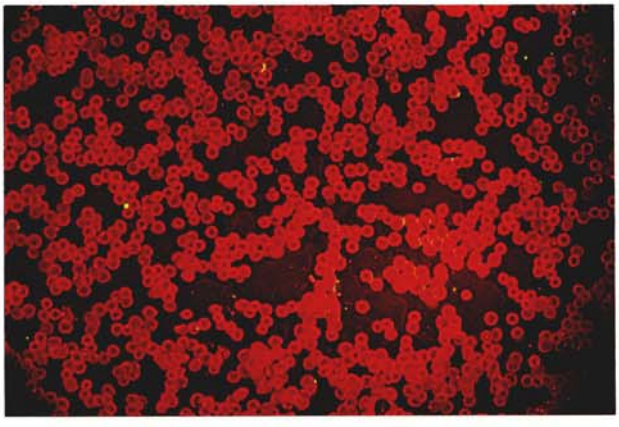

Figure 13— An FeLV immunofluorescent antibody test on blood smears from a cat with severe leukopenia. No leukocytes are present in the test and thus no evaluation is possible.

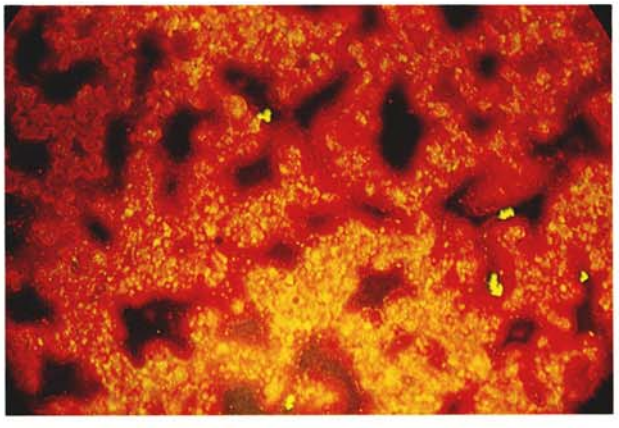

Figure 14— An FeLV immunofluorescent antibody test. The nonspecific reaction caused by an improperly prepared blood smear, which was too thick, made an evaluation impossible.

tact with any FeLV antigens that may be present in the cytoplasm of the cells. After adding the rabbit serum to the blood smear, the slide is incubated at 37 C for one hour to permit the serum to react with any FeLV antigens, and is then washed. Fluorescein-conjugated goat antiserum to rabbit gamma globulin is then used to identify any rabbit serum that may remain on the slide attached to FeLV antigens in the cells. After counterstaining with Evans blue the slides are examined with a fluorescent microscope. Because of the fluorescein conjugate, FeLV-infected cells exhibit a strong apple-green fluorescence whereas uninfected cells appear red in the ultraviolet light [Figures 11,12]. If there are few white cells on the slide (this is common in blood smears from cats with the FeLV panleukopenia-like syndrome for example) or if the blood smear is so thick that it nonspecifically traps the fluorescein conjugate on the slide, it may not be possible to evaluate the slide for the presence of FeLV antigens [Figures 13,14]. In these cases new blood smears must be made and the test must be repeated on the new smears before it can be determined whether or not FeLV antigens are present.

Studies have shown that a positive IFA test result is equivalent to the presence of infectious FeLV in the cat [Table 6].[2,55,99] Provided that the anti-FeLV serum is specific for the FeLV antigens, and thus does not recognize cat proteins in general, the test is very accurate. A positive IFA test indicates that the cat is infected with FeLV and is shedding the virus in its saliva. About 97% of the IFA test positive cats remain infected for life whereas 3% of these cats reject the virus and become immune to FeLV and IFA test negative.[55]

Uses of the IFA Test

The IFA test *cannot* be used to diagnose any FeLV disease since the test can only detect FeLV. FeLV infected cats may be healthy or may have any one of the FeLV diseases. The test, however, can be used as an aid in the diagnosis of the FeLV diseases and can enable veterinarians to advise their clients about the disposition of a sick cat. For example, the IFA test result will have a significant bearing on whether or not the cat should be treated or euthanized, and on whether or not it is necessary to test other cats in the household for FeLV. The IFA test, used as part of the test and removal program allows veterinarians to practice preventive medicine and eliminate the virus from multiple cat households.

FeLV ELISA Test

The enzyme-linked immunosorbent assay (ELISA test) detects soluble FeLV gs antigens in the plasma or serum of FeLV infected cats and the test is available for in-hospital use.[100] Samples of plasma or serum are added to wells in a test plate that are coated with antibody to FeLV gs antigens. If the cat is infected with FeLV, the FeLV antigens in the sample bind to the antibody in the test well. If the cat is not infected with FeLV no FeLV antigens will be available to bind to the antibody in the well. An antibody against the FeLV gs antigens, which is conjugated to an enzyme, is then added to the well and reacts with any FeLV antigen that is bound to the test well. The wells are washed and a substrate for the enzyme is added enabling an enzymic reaction to take place. If the test sample is FeLV negative, there are no FeLV antigens for the enzyme conjugate to bind to and it is therefore washed out of the well and no enzymic reaction can take place. The final step in the ELISA test procedure is the addition of a color developer which turns the FeLV positive samples in the wells an amber color. Wells containing FeLV negative samples remain colorless.

The Interpretations and Uses of the ELISA Test

Several studies have been done in which the results of the ELISA and IFA tests have been compared [Table 7]. In a study of 553 pet cats, Hardy found that 66.7% of the cats tested were ELISA test positive but IFA test negative.[101] However, only 12.8% of the cats were ELISA test negative and IFA test positive. In another study of 626 pet cats, Lutz found that 3.8% of the cats tested were ELISA test positive and IFA test negative and similar results (5.2%) were obtained by Kahn in a study of 95 cats.[100,102] Very different results were obtained by Lazcrowicz who found that 62.5% of the 48 cats he tested were ELISA test positive and IFA negative.[103] Gwalter also compared the two FeLV testing methods in 121 cats and found that 17.3% of these cats were ELISA test positive and IFA negative.[104]

The most complete study of FeLV testing methods has been done by Jarrett *et al*, in Scotland.[99,105] They compared virus isolation, the ELISA test and the IFA test in 1148 FeLV infected pet cats. He found that FeLV could be isolated from only 68% of the ELISA test positive cats.[99] However, FeLV could be isolated from 98.5% of the IFA test positive cats. He also found, in four experimentally infected kittens, that the IFA test detected FeLV infection earlier (at 21 days after infection) than the ELISA test (only two kittens were positive at 21 days, one was positive at 56 days and one was positive 170 days after infection).[105] Jarrett has concluded that the IFA test should be used for routine testing of cats for persistent FeLV infection and that it was not advisable to euthanize ELISA test positive cats until they are confirmed to be positive by the IFA test.

The basic biological research into FeLV was done using the IFA test.[2,8] The IFA test has proven to be invaluable for both researchers and for helping veterinarians to prevent the spread of the virus in the test and removal program.[89,106] In view of the fact that many ELISA test positive cats appear, in fact, not to be persistently infected with FeLV, it is this author's recommendation that ELISA positive cats NOT be euthanized in a test and removal program, but instead be retested using the fluorescent antibody test.[99,107]

Pathogenesis of FeLV Infection

Using the IFA test, Rojko and her coworkers studied the pathogenesis of FeLV infection.[11,108] The virus gains entry, primarily via saliva from infected cats, through the ocular, nasal or respiratory membranes. After penetrating these membranes the

Table 7

Comparison of the FeLV Immunofluorescent Antibody (IFA) Test and Virus Isolation (VI) with the ELISA FeLV Test

	Total Number of Cats Tested	Overall Agreement of IFA and VI Tests with the ELISA Test	ELISA Negative Tests			ELISA Positive Tests		
			Total Number of ELISA Negative Tests	Total Number of IFA or VI Negative Tests	% Agreement	Total Number of ELISA Positive Tests	Total Number of IFA or VI Positive Tests	% Agreement
Jarrett[99]	1148*	80%	466	466	100%	682	465	68%
Hardy[101,107]	553**	48%	148	129	87%	405	135	33%
Lutz[102]	626	87%	416	408	98%	210	146	69%
Kahn[100]	95	93%	52	50	96%	43	38	88%
Gwalter[104]	121	83%	93	0	100%	28	21	75%
Lazarowicz[103,104]	48	38%	0	0	—	48	30	38%
Totals:	2591	73.6%	1175	1053	89.6%	1359	796	58.6%

*Compared by Virus Isolation Test—all other comparisons were done with the immunofluorescent antibody test.

**405 of the 553 samples submitted by veterinarians were from ELISA positive cats, thus the sample is biased toward testing mostly ELISA positive cats by IFA.

virus replicates in the lymphoid cells of the local lymph nodes of the head and neck and in the tonsils. Most cats mount an adequate immune response against the virus (neutralizing antibody) while FeLV is confined to the local lymph nodes of the head and neck, reject the virus and become immune.[45] Cats who do not mount an adequate immune response become persistently infected and the virus spreads to the bone marrow where it replicates in all nucleated cells. It is in the marrow that FeLV replication proceeds rapidly enabling the virus to enter the blood via infected leukocytes or as free circulating virus (viremia). The virus then spreads to various tissues such as the intestinal lamina propria, epithelial cells of the salivary gland and pancreas and the respiratory membranes. The cycle of infection in the cat is complete when infectious FeLV is shed in the saliva [Figure 1B]. Infected cat saliva can contain as much as 5×10^3 to 2×10^6 infectious FeLV per ml.[9,10,53] The urine also contains FeLV, but at lower concentrations.[98,109,110] Thus, mutual grooming, sneezing and the sharing of feeding bowls and litter pans are the most likely ways in which the virus is transmitted from one cat to another. Since FeLV is also present in the peripheral blood, cat fleas may act as a vector for the virus. FeLV is also spread iatrogenically by blood transfusions and we have found that 101 of 852 (12%) of the blood donor cats that we tested were FeLV infected. In addition to contagious transmission, FeLV is transmitted epigenetically, that is, via the milk or via the placenta as infectious virus to the kittens or fetus.[45] We have found FeLV in several litters of unborn fetuses or newborn kittens as well as in the uterus of viremic pregnant queens. There is no evidence that FeLV is transmitted as an inherited Mendelian trait (genetically).

The Occurrence of FeLV in Healthy Pet Cats

Persistent FeLV viremia is not widespread in healthy cats in the natural environment.[107] Persistently infected cats are found predominantly in exposure environments, such as multiple cat households in which there is a history of feline LSA, and much less frequently in nonexposure environments such as multiple cat households with no history of FeLV diseases or in households containing only one cat [Table 8].[45,98] Approximately 30% of healthy FeLV exposed cats are persistently infected with FeLV, whereas very few nonexposed cats (less than 1%), or cats whose exposure to FeLV is unknown, are infected with the virus.

Table 8

Occurrence of FeLV in Healthy Pet Cats

Environment and FeLV Exposure History	Number of Cats Tested*	Number of Cats FeLV Positive	Percent of Cats FeLV Positive
Single cat household (no known FeLV exposure)	280	2	0.7%
Multiple cat household			
Exposed to FeLV	1612	441	27.4%
Not exposed to FeLV	465	0	0%

*Immunofluorescent antibody test for FeLV

Table 9

Occurrence of FeLV in Stray Cats

Geographic Origin of Stray Cats	Number of Cats Tested*	Number of Cats FeLV Positive	Percent of Cats FeLV Positive
New York City	1290	13	1%
San Diego	158	1	0.6%
Boston	102	0	0%
	1550	14	0.9%

Immunofluorescent antibody test for FeLV

Table 10

Summary of Pet Cat Immune Response to FeLV Exposure

Immune Response	FeLV Status	Consequence of FeLV Exposure	Percent of Exposed Pet Cats
None	Infected	Persistently FeLV infected (viremic)	28%
None	Not infected	Susceptible to FeLV (not exposed to FeLV by contact)	30%
Protective titers of FeLV neutralizing antibody	Not infected	Immune to FeLV	42%

We have tested 1550 stray cats obtained from animal shelters in New York City, San Diego and Boston for persistent FeLV infection [Table 9]. Only about 1% of these stray cats were persistently infected and thus stray cats are not a significant source of FeLV infection.

Most cats become infected as a result of prolonged contact with an infected cat while living in the same household. Prolonged direct contact between cats is required for effective transmission of FeLV since the virus cannot survive long under adverse conditions. For example, we and others have found that FeLV loses its infectivity in only two to three minutes when it dries and can survive for only two to three days in a moist environment such as tissue culture fluid without cells.[111] It is thus unlikely that FeLV can be transmitted from one cat to another via the hands or clothing of people since the virus would die quickly upon drying. Ordinary household and hospital detergents quickly kill FeLV by lysing its lipid envelope.

Immune Response to FeLV

Consequences of FeLV Exposure

As was mentioned previously, 28% of FeLV exposed cats become persistently infected and never reject the virus. These cats do not produce FeLV neutralizing antibody. However, not all cats exposed to FeLV become persistently infected with the virus [Table 10].

Most pet cats (42%) exposed to FeLV under natural household conditions become immune to FeLV by producing protective titers of FeLV serum neutralizing (SN) antibody.[45] In our FeLV neutralization assay, a titer of 1:10 or greater is protective against FeLV challenge, both under household conditions and experimentally by FeLV inoculation. Many cats also produce protective titers of FOCMA antibody, that is, 1:32 or above in our FOCMA assay. Most cats that produce SN antibody also produce protective titers of FOCMA antibody.[45] In those cats, small numbers of transformed lymphocytes, which express FOCMA, stimulate the FOCMA antibody response. The majority of cats that become immune do not test positive for FeLV by the IFA test, and thus are not persistently infected. In these cats, FeLV replication is localized and transformed lymphocytes are present only in the lymphoid tissues of the head and neck. These cats reject FeLV and the LSA cells at this early stage before becoming persistently infected and before developing LSA. The remaining 30% of pet cats who neither become persistently infected nor immune remain susceptible to FeLV since, even though they lived in the same household as infected cats, they were not exposed enough to become even transiently infected. These cats are often the "anti-social" cats who do not mingle with their house mates.

FeLV Neutralizing Antibody in Healthy Cats

The author's group has tested 808 healthy cats for serum neutralizing antibody to FeLV serotypes A and B, the most commonly occurring FeLV serotypes in pet cats (99% of all FeLV isolates from pet cats have been FeLV-A or FeLV-A and -B).[46,107] Each

Table 11

Protective FeLV-A,B Neutralizing Antibody Titers in Healthy Cats (1:10 or above)

FeLV Exposure History	FeLV Status	No. of Cats Tested	No. of Cats with Protective Neutralizing Antibody
Unexposed cats	–	58	0
Unknown exposure cats (strays)	–	353	5 (1.4%)
Exposed cats	–	283	120 (42.4%)
		694	125 (18.0%)
Unknown exposure cats (strays)	+	5	0
Exposed cats	+	109	0
		114	0

Table 12

FOCMA Antibody Titers in Healthy Cats

FeLV Exposure	FeLV Status	No. of Cats Tested	No. of Cats with Protective FOCMA Antibody (≥ 1:32)*	
Unexposed	–	48	0	0%
Unknown exposure (stray cats)	–	156	12	7.7%
Exposed	–	203	78	38.4%
		407		
Exposed	+	72	18	25%

**A FOCMA antibody titer of 1:32 in our laboratory is protective and is equivalent to a titer of 1:8 in the laboratory of Dr. Essex.*

cat was characterized as to its FeLV exposure history and its FeLV status [Table 11]. Six hundred and ninety-four of the 808 cats were not infected with FeLV. Of the 58 uninfected, unexposed cats tested none had protective SN antibody titers and only five of the 353 (1.4%) uninfected stray cats tested had protective titers. In contrast, 120 of the 283 (42.4%) uninfected but exposed healthy cats had protective FeLV SN titers of 1:10 or above. In addition to uninfected cats, we tested 114 healthy persistently infected cats but found none of these cats to have protective SN antibody to FeLV-A and -B. Our SN antibody titer results corresponded well with the occurrence of persistent FeLV infection in cats from different exposure environments. Only a few unexposed cats are infected with FeLV and only a few have protective titers of SN antibody, whereas 28% of the exposed cats are persistently infected, but not immune, and 42% have protective SN antibody and are not infected.

FOCMA Antibody in Healthy Cats

The author's group has extended the previous FOCMA antibody household studies of Essex's group[62,65,67,70] by testing 479 healthy pet cats for FOCMA antibody [Table 12]. Antibody to FOCMA at titers of 1:32 or above protects cats from the development of FeLV induced neoplasia (lymphosarcoma, myelogenous leukemia etc) and FeSV-induced multicentric fibrosarcomas.[45] Persistently infected cats with protective FOCMA antibody titers will not develop these neoplastic diseases but they are susceptible to the development of all the non-neoplastic FeLV diseases such as nonregenerative anemias and FeLV immunosuppressive diseases (see FeLV-Non-Neoplastic Disease paper). Antibody to FOCMA does not neutralize FeLV.[58,73]

Of the 48 unexposed healthy uninfected cats tested, none had protective FOCMA antibody titers and only 12 of the 156 (7.7%) stray cats had FOCMA antibody. In contrast, 38% of the uninfected exposed and 25% of the infected healthy cats had protective FOCMA antibody titers. Grant and his coworkers have shown that FOCMA antibody will lyse feline LSA cells *in vitro* and we have shown that cat sera containing FOCMA antibody will lyse LSA cells in cats as well (see paper on Hematopoietic Tumors of the Cat).[112]

Immune Classes of Healthy Cats

The immune status of healthy cats in relation to FeLV can be ascertained by determining the: 1) FeLV status; 2) FeLV serum neutralizing antibody titer (1:10 or above) and; 3) FOCMA antibody titer (1:32 or above).[45,73] Determining these three parameters allows one to determine the exposure history and the consequences of current or future FeLV exposures and enables one to classify all healthy pet cats into six immune classes [Table 13].

Cats in class 1 have not been exposed to FeLV since they are not infected and they do not have

Table 13

Immune Classes of Healthy Pet Cats

Class	Exposure History	FeLV Status	Protective FeLV Neutralizing Antibody (≥1:10)	Protective FOCMA antibody (≥1:32)	Susceptibility or Resistance to: FeLV Infection	LSA Development
1	Unexposed	–	–	–	Susceptible	Susceptible
2	Exposed	–	–	+	Susceptible	Resistant
3	Exposed	–	+	–	Resistant	Susceptible
4	Exposed	–	+	+	Resistant	Resistant
5	Exposed	+	–	–	Infected	Very Susceptible
6	Exposed	+	–	+	Infected	Resistant

*Adapted from reference 45

protective SN or FOCMA antibody titers. They are thus susceptible to FeLV infection and LSA development. Cats in class 2 have been exposed to FeLV but they are not persistently infected. These cats are susceptible to FeLV infection since they do not have protective titers of SN antibody but are resistant to LSA development because they have protective titers of FOCMA antibody. Class 3 cats are uninfected cats which have been exposed since they have protective titers of FeLV SN antibody. However, class 3 cats are susceptible to LSA because they do not have protective FOCMA antibody titers. Class 4 cats have been exposed to FeLV but are immune to FeLV infection and LSA development because they have protective antibody titers to both FeLV and to FOCMA. Cats in classes 5 and 6 are cats that have been exposed to FeLV and are persistently infected. We have never found a persistently infected cat to have free circulating FeLV SN antibody against the same FeLV serotype with which it is infected. Class 5 cats are very susceptible to the development of LSA because they are viremic and they do not have protective titers of FOCMA antibody.[45,70] Finally, cats in class 6 are chronic shedders of FeLV who are resistant to LSA development because they have protective titers of FOCMA antibody. However, cats in class 6 are susceptible to all of the non-neoplastic FeLV diseases.

The author's group have classified a total of 492 cats (420 FeLV uninfected and 72 FeLV infected) based on their FeLV status and protective SN and FOCMA antibody titers [Table 14]. All of the 48 FeLV uninfected, unexposed cats tested were class 1 cats, that is, they were susceptible to FeLV infection and LSA development. Of the 156 uninfected stray cats with an unknown history of FeLV exposure 144 (92.4%) were class 1 cats, nine (5.8%) were class 2 cats, that is, were susceptible to FeLV infection but not LSA development, and three (1.9%) were class 4 cats, that is, were resistant to both FeLV infection and LSA development. Of the 203 FeLV exposed but uninfected cats, 99 (48%) were in class 1, 13 (6.4%) were in class 2, 26 (18%) were in class 3, that is, were resistant to FeLV infection but susceptible to LSA development and 65 (32%) were in class 4. All 13 wild cats tested were in class 1. Of the 72 FeLV infected exposed cats 54 (75%) were in class 5, that is, were susceptible to LSA and 18 (25%) were in class 6, that is, were resistant to LSA.

It should be noted that the classification of healthy pet cats according to their FeLV status and their immune response to FeLV depends on the environmental exposure to the virus as well as on the cat's immune response and may therefore change with time. For example, many (42%) healthy susceptible unexposed class 1 cats become class 4 (immune) cats after natural exposure to FeLV in their household environments [Table 10]. In addition, a substantial number of class 1 cats (28%) become infected with FeLV, that is, are converted into class 5 or 6 cats and only 30% remain in class 1 even after living with infected cats, probably because of insufficient FeLV exposure. Since the FeLV immune classes are dynamic, repeated testing for FeLV and for FeLV neutralizing and FOCMA antibody is necessary to determine the current FeLV immune class, and thus, the possible consequences of FeLV exposure of healthy pet cats. However, testing for

Table 14

Exposure History and the Six Immune Classes of Healthy Cats

Exposure History and FeLV Status	Number of Cats Tested	Susceptible to FeLV & LSA Class 1	Susceptible to FeLV, Resistant to LSA Class 2	Resistant to FeLV, Susceptible to LSA Class 3	Resistant to FeLV & LSA Class 4	Infected: Very Susceptible to LSA Development Class 5	Infected: FeLV Chronic Shedder Resistant to LSA Class 6
FeLV uninfected							
Unexposed cats	48	48 (100%)	0 (0%)	0 (0%)	0 (0%)	N.A.	N.A.
Unknown exposed cats (strays)	156	144 (92.3%)	9 (5.8%)	0 (0%)	3 (1.9%)	N.A.	N.A.
Exposed cats	203	99 (48.8%)	13 (6.4%)	26 (12.8%)	65 (32.0%)	N.A.	N.A.
Undomesticated (wild) large cats	13	13 (100%)	0 (0%)	0 (0%)	0 (0%)	N.A.	N.A.
	420						
FeLV infected							
Exposed cats	72	N.A.	N.A.	N.A.	N.A.	54 (75.02%)	18 (25.0%)
Total	492						

N.A. = not applicable

antibody to FeLV and to FOCMA is not practical nor is it necessary for veterinarians, since cats with neutralizing antibody may not maintain high antibody titers and should not be housed with infected cats. In addition, cats with FOCMA antibody are not protected against the non-neoplastic FeLV diseases. The only information that is required for a veterinarian to be able to advise his clients concerning the disposition of their exposed cats is an FeLV test.

Complement and FeLV

Complement along with antibody constitutes the humoral half of the immune system. We have found that large amounts of normal cat serum, with low or no FOCMA antibody, when infused into cats with LSA, causes regression of their tumors, but heat inactivated normal cat serum is inactive.[113,114] The anti-LSA factor in normal cat serum is thought to be complement.[114] In addition, small amounts of cat serum containing a high titer of FOCMA antibody causes rapid regression of feline LSA.[115] Grant and his coworkers have found that FOCMA antibody can lyse feline LSA cells *in vitro* with feline complement.[112] Kobilinsky and his coworkers have shown that FeLV positive LSA cats are hypocomplementemic as a result of FeLV activation of the classical complement pathway.[116] These cats may be low in complement because complement is being bound to the tumor cells in a futile attempt to lyse the LSA cells.

Serum from healthy cats, who lack SN antibody, can lyse FeLV, although cat serum is only 10% to 30% as effective as human serum in this regard.[117] Kobilinsky and his coworkers have recently found that FeLV activates feline complement *in vitro* via the classical pathway when added to feline serum and that there is no difference in FeLV lysis by normal or leukemic cat serum regardless of their FeLV status.[118] In addition, we have found complement deposited as complexes with FeLV antigens and antibody in the glomeruli of some viremic cats indicating yet another possible mechanism of complement consumption.[60]

Thus, it appears that complement along with an antibody response to FeLV and to FOCMA is important in governing the outcome of FeLV exposure of pet cats.

Cell Mediated Immune Response to FeLV

The thymus and lymph nodes of kittens are well developed before birth.[119] Feline thymus-derived (T) lymphocytes form nonimmune rosettes with guinea pig red blood cells.[120] Like other species, short term feline lymphocyte cultures respond to phytomitogen and antigen-induced blast transformation.[121] However, the cat differs from most other domestic animals in that it does not manifest gross delayed hypersensitivity reactions to an intradermal dose of antigen.[122]

There are few reports of the quantitation of T and B cells from FeLV uninfected healthy cats. Taylor and her coworkers reported that in nine cats tested, 41± 7% of peripheral blood lymphocytes (PBL) were T cells while 45 ± 4% were B cell in origin.[123] Holmberg and her coworkers found that 34 ± 5% of the PBL were B cells but they did not test for T cells. They found that the lymph nodes contained 32 ± 19% T cells and 43 ± 12% B cells, the thymus contained 34 ± 22% T cells and 0.2 ± 0.1% B cells and the spleen contained 36 ± 19% T cells and 47 ± 22% B cells.[124] Zuckerman, in my laboratory, has studied FeLV uninfected healthy pet and stray cats and found 32 ± 4.4%[a] T cells and 48 ± 8.5% B cells in PBL, 40 ± 3.5% T cells and 40 ± 3.4% B cells in lymph nodes, 46 ± 5.2% T cells, 64 ± 5.2% B cells in spleen and 53 ± 5.5% T cells and 1 ± 0.63% B cells in thymus.[125]

There is only one report of the quantitation of T and B cells in FeLV infected healthy SPF cats.[126] Cockerell and his coworkers found that of PBL, 17 ± 1% were T cells and 29 ± 4% were B cells. Thus, there was a reduction in PBL due to a decrease in B cells which was either due to FeLV infection or due to the protective SPF environment. Essex and his coworkers have reported a depression of lymphocytes in naturally infected pet cats.[127] McCarty and Grant have found cytotoxic lymphocytes and activated macrophages in FeLV exposed pet cats and in cats immunized with LSA cells. These lymphocytes may be specifically activated cells or they may be NK cells.[128]

There have been several studies of the cellular origin of feline LSA. In general, most cat LSAs are T cell in origin.[36] Almost all thymic LSAs are T cell in origin while most of the alimentary LSAs are B cells. Multicentric LSA is most often a T cell disease. The finding that feline LSA is usually a T cell disease is interesting since it has been shown that a B cell function of cats, the production of FOCMA antibody, prevents LSA. Thus, FeLV may transform T cells and may also suppress FOCMA antibody production by B cells which may allow the transformed T cells to grow, resulting in clinical LSA.

There have been very few studies of the functional status of T lymphocytes in uninfected and infected cats. Perryman and his coworkers used skin allografts in FeLV infected SPF kittens and found a significantly longer allograft retention time than in uninfected control cats.[129] The infected kittens in this study also developed thymic atrophy. Unfortunately, there have been no similar *in vivo* studies reported for naturally FeLV infected pet cats. In an *in vitro* evaluation of SPF kittens inoculated at birth with FeLV, Cockerell and his coworkers demonstrated a lymphopenia and a precipitous decline in Con-A induced blast transformation.[130]

Perryman and his coworkers and Essex and his coworkers found that FeLV infected SPF and pet cats were able to produce antibody to sheep RBCs and to the panleukopenia virus equally as well as uninfected SPF and pet cats.[127,129]

FeLV Immunosuppression

FeLV is immunosuppressive in pet cats and is indirectly responsible for numerous chronic secondary diseases.[107] There are three likely mechanisms responsible for this immunosuppression: 1) FeLV induced lymphopenia and granulocytopenia;[107,127] 2) FeLV p15E inhibition of blast transformation;[131] and 3) the formation of immunosuppressive FeLV immune complexes.[60] Numerous naturally infected pet cats are immunosuppressed and develop secondary diseases due to severe FeLV induced granulocytic leukopenias and lymphopenias.[107] Another way in which FeLV can immunosuppress cats is by exfoliating p15E from the viral envelope. The p15E has been shown to abrogate feline lymphocyte blastogenesis by Mathes and his coworkers.[131] Finally, FeLV immune complexes occur in infected cats and these small circulating immune complexes are immunosuppressive.[60]

Prevention of the Spread of FeLV

The spread of any infectious organism can be prevented using knowledge of its life cycle, reservoir hosts, vectors and its susceptibility to environmental factors [Figure 15]. Thus, preventing the spread of an infectious organism can be accomplished by removal of infected carrier healthy or sick animals from contact with susceptible animals and by vaccination.

FeLV Fluorescent Antibody Test and Removal Program

Since FeLV is an infectious agent its spread in a given cat population, for example a cattery, can be prevented by completely separating the infected cats from the uninfected cats.[54,106] The best way of achieving total separation is by isolation or, preferably, by euthanasia of all FeLV-infected cats that test positive in the fluorescent antibody test for FeLV [Table 15]. I consider the FeLV test and removal program to be justified because: a) it is the most effective currently available method; b) the prognosis for all infected cats is poor; c) infected cats are a dan-

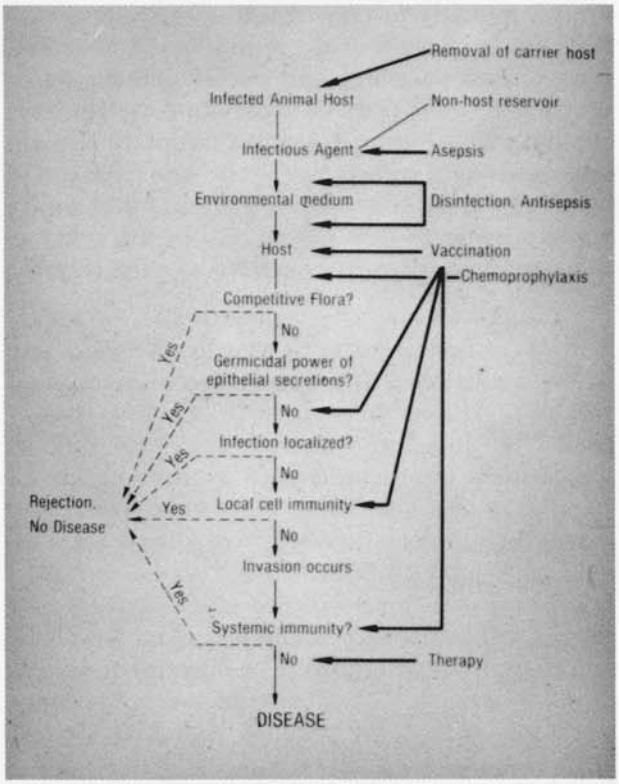

Figure 15— A diagramatic representation of the chain of events resulting in infectious diseases and the possible ways to prevent infection and subsequent disease development.

ger to uninfected cats and d) the public health risk of FeLV is unknown. However, the author only recommends euthanasia for cats who have been tested for the virus by a qualified laboratory that performs the fluorescent antibody test for FeLV, and the author and Dr. Oswald Jarrett do not recommend that in-hospital ELISA FeLV test positive cats be euthanized unless they are confirmed FeLV positive by a qualified laboratory fluorescent antibody test or FeLV isolation test.[99,105,107] After all of the infected cats have been isolated, or removed, the household should be thoroughly cleaned with detergents. Three months after the first test all of the uninfected cats remaining in the household should be retested. If all of the cats are negative in both FeLV tests the household can be considered to be free of FeLV. If, however, any cats test positive in the second test then a third test of the cats is required three months after the second test. All cats in a household must test negative for FeLV in two tests, done three months, apart for the household to be considered free of FeLV. Once the household is free of FeLV infected cats, new cats can be brought in, but they should be obtained from an FeLV-free environment and they should be tested for FeLV before they are allowed to mingle with the other cats. The FeLV fluorescent antibody test and removal program has been used successfully during the past nine years and can significantly protect uninfected cats from infection with FeLV and subsequent FeLV disease development [Table 16].

Table 15

FeLV Immunofluorescent Antibody Test and Removal Program

1. Remove all FeLV infected sick cats from the household.
2. If there are no other cats at home wait 10 days before bringing another cat into the household.
3. Immediately test all remaining cats for FeLV.
4. Remove all FeLV infected healthy cats from the household.
5. Clean all dishes, litter pans and bedding with detergents.
6. Quarantine all remaining FeLV uninfected cats in the household.
7. Retest all FeLV uninfected cats three months after the first test. The incubation period for FeLV infection can be as long as three months.
8. The household can be considered free of FeLV infected cats only when all cats have tested FeLV negative in two tests done three months apart.
9. Test all new cats for FeLV before they are introduced into the household.

Table 16

Results of FeLV Immunofluorescent Antibody Test and Removal Program

	Number of Households	Initial FeLV Test of Healthy Cats: Infected	Initial FeLV Test of Healthy Cats: Uninfected	Number of Healthy Cats Removed	Second FeLV Test of Healthy Cats: Infected	Second FeLV Test of Healthy Cats: Uninfected	Number of Cats That Became Infected	Percent of Cats That Became Infected
FeLV-infected Cats removed	51	190	657	190	3	654	3	0.46%
FeLV-infected Cats not removed	25	129	284	0	184	229	55	19.3%

Inactivation of FeLV in the Environment

Due to its lipid envelope, FeLV is a very labile virus when it is excreted in the saliva and urine into the environment. FeLV can survive for about three days if it remains in a moist environment but it usually dies within three to five minutes as a result of drying.[107,111] Ordinary household and hospital detergents and alcohol inactivate the virus within seconds. Since FeLV is not effectively transmitted by the aerosol route, direct and long cat-to-cat contact (days to weeks) is usually required for the virus to spread among cats. Thus, veterinary hospitals and cat shows are not major environments in which FeLV is efficiently spread.

FeLV Vaccine

Since cats can develop natural immunity to FeLV, the prospect for the development of an FeLV and FeSV vaccine is good. In previous studies, two research groups have attempted to develop a vaccine composed of LSA cells in order to induce FOCMA antibody and thus prevent LSA.[132,133] This approach is, however, unwise since cats who produce FOCMA antibody without concurrent FeLV SN antibody can become persistently infected with FeLV and remain susceptible to all of the non-neoplastic FeLV diseases.[107] Since the non-neoplastic diseases kill more cats than does LSA, I feel that the emphasis should be on the development of a vaccine against FeLV infection and not only against LSA.

There are three types of FeLV vaccines: 1) live FeLV; 2) killed FeLV; and 3) an FeLV envelope subunit vaccine free of the viral RNA. The advantage of a live FeLV vaccine is that it is more effective than a killed virus vaccine in producing high neutralizing antibody titers since FeLV replicates for a limited time in the vaccinated cats. However, it has the following disadvantages: a) there is the possibility that a live FeLV vaccine may induce FeLV negative LSA in certain cats[55] and b) the possible public health risks are greater with a live FeLV vaccine than with a killed virus vaccine. These disadvantages are reduced with a killed virus vaccine but such a vaccine requires higher virus doses and more frequent revaccination to produce and maintain protective antibody titers. In many respects, an FeLV subunit vaccine composed of specific viral envelope antigenic subunits, free of the viral RNA, would be the safest type of vaccine.

In 1974, William Jarrett and his coworkers vaccinated cats with live FL-74 LSA cells which replicate FeLV-A, -B and -C.[132] The immunized cats produced antibody in one month and no virus was found in the tissues of any immunized cat. Several groups have used live and paraformaldehyde or heat killed FL-74 cells to vaccinate cats. The vaccinated cats produced FOCMA antibody and resisted challenge with FeLV.[133-135] However, the use of feline LSA cells in an outbred animal population seems unwise to this author due to the possible "anti cat" immune response of the vaccinated cats.

UV and formaldehyde inactivated FeLV vaccines were not found to be effective in one study where seven pregnant queens were immunized and their kittens subsequently challenged with FeSV. The Ohio State University FeLV group found that kittens immunized with a combination of killed FeLV and heat killed FL-74 cells were more susceptible to FeLV diseases than kittens vaccinated with killed FL-74 cells alone.[134] Thus, the killed FeLV abrogated the protective effect of the FL-74 vaccine and enhanced LSA development. Live FeLV vaccines have been shown to be effective by several research groups.[107,136] Most cats vaccinated with low doses of live FeLV produce both FeLV SN and FOCMA antibody.

We have immunized 13 cats with a low dose, about 10^3 infectious FeLV, live vaccine composed of FeLV-A and -B obtained in the author's laboratory from a cat with thymic lymphosarcoma. Twelve of the 13 cats developed protective SN (1:10 or above) and FOCMA titers six weeks after vaccination [class 4 cat, see Table 13]. The average SN titer was 1:600 with one cat developing a titer of 1:5120. However, four of the vaccinated cats became transiently viremic for one to three weeks as judged by virus isolation and by positive IFA tests of their bone marrow and peripheral leukocytes. One of these cats became persistently viremic and died nine weeks after vaccination. Nine of the 12 remaining immunized cats were challenged under natural household conditions for two years in a house containing over 10 persistently infected cats. None of the immunized cats became persistently infected and none developed disease. In contrast, all 10 of the four month old nonimmunized control cats that were placed in the house at the same time as the immunized cats developed evidence of FeLV infection, seven became persistently viremic and three of them died of FeLV induced anemia, one of LSA and three of feline infectious peritonitis.[137] Unfortunately, it is now clear that the use of a live FeLV vaccine is too dangerous since FeLV negative LSA can develop in FeLV exposed pet cats and since the author's group has observed the development of an

Figure 16— This cat developed an FeLV-negative nasal lymphosarcoma six years after it was immunized with a low dose live FeLV vaccine.

FeLV negative nasal LSA in one of our immunized cats [Figure 16].

Because live FeLV vaccines are too dangerous to be used routinely, and since killed FeLV vaccines have been poorly immunogenic and FL-74 cells seem inappropriate, the author's group and others, are attempting to develop an FeLV envelope subunit vaccine which is free of the viral RNA. Subunit viral vaccines have been successfully used against influenza viruses which are similar to oncoviruses in that they have lipid envelopes.[138] FeLV has two components in its outer envelope; the gp70 and p15E. These two proteins can be cross-linked into a (gp90) complex which is the native form of gp70 and p15E in the virion.[39] Cats immunized with purified FeLV gp70 failed to produce protective SN antibodies in one study.[139] In contrast, the Ohio State group reports successful immunization of cats with gp70 obtained from culture fluid.[140,141] The author's group, along with Pinter, is presently immunizing cats with FeLV (gp90) in various adjuvants. A safe and effective FeLV vaccine will be available in the forseeable future and will greatly reduce the deaths from this major infectious disease producing agent in pet cats.

Public Health Aspects of FeLV and FeSV

Growth in Human Cells

When FeLV was first discovered in 1964 by William Jarrett and his coworkers, both the avian leukosis viruses (ALV) and the murine leukemia viruses (MuLV) were thought to be spread only vertically by genetic means.[142,143] There was therefore no concern for the public health risks from these viruses. However, Oswald Jarrett and his coworkers reported in 1969, that natural field isolates of FeLV replicate well in normal human embryonic lung cells.[144] It is now known that only FeLV-B and -C replicate in human cells but that FeLV-A, the most commonly occurring serotype, is unable to do so. Jarrett's observation was different from those of similar studies of the infectivity of ALV and MuLV for human cells. Shortly thereafter, the growth of FeSV in human lymphoblastoid cells was reported.[145] Subsequently, there have been other reports of the growth of FeLV and FeSV in human cells.[146,147] Those reports, together with the observation that FeLV is spread contagiously among cats, has led to the fear that FeLV may be able to induce disease in people since FeLV is probably the only known tumor virus of any species to which humans are frequently exposed.[98,147-149]

Oncogenicity of FeLV in Species Other than Cats

MuLV will replicate in rat cells and when inoculated into rats causes virus positive leukemia.[150] Similarly, FeLV will replicate in dog cells in culture and when inoculated into newborn puppies will produce FeLV positive lymphosarcomas.[151] Since FeLV replicates in human lymphoid cells in culture it is possible that FeLV may be able to induce lymphoid tumors in humans.[147,152] Experimental challenge of humans with FeLV can never be done but, the oncogenicity of FeLV in humans may eventually be determined by long term observation of people living with infected cats, especially people exposed *in utero* or as children.

Oncogenicity of FeSV in Species Other than Cats

The FeSV is a rarely occurring, highly oncogenic feline virus which can induce fibrosarcomas in numerous species, including primates and can transform human cells in culture.[20-22,146,153,154] FeSV is generated by recombination of FeLV with cat cellular sequences. It therefore may be possible for FeLV to recombine with human cellular sequences to generate a human sarcoma virus.[23] There is a precedent for such an occurrence — when MuLV was inoculated into rats a new sarcoma virus, the rat sarcoma virus was generated.[155] In addition, it should be noted that by transspecies rescue, FeLV can rescue defective MuSV from hamster tumor cells.[156] One of the most significant observations in the biology of FeSV is that the transformation of heterospecies cells in culture or the induction of tumors in nonfeline species often occurs without the production of virus (FeLV-FeSV) in the transformed cells or in the tumors.[32,77,81,153] Thus, when epide-

Table 17

Epidemiologic Studies* of Cancers in Veterinarians Related to Exposure to Cats

Author	Veterinarians From:	Controls	Cancer Results
Botts, *et al*, 1966[157]	Missouri (390 white males)	Missouri population	Cancer mortality NSSD**
Fasal, *et al*, 1966[158]	California (1722 white males)	California population	Excess skin melanoma in veterinarians. Other tumors NSSD
Schnurrenberger, *et al*, 1977[159]	Illinois: 481 white males 4 non-white males 1 female	Illinois population	Cancer mortality NSSD
Gutensohn, *et al*, 1980[160]	United States, 19,000, 45 yrs. of age or over	Physicians & general U.S. population	80% increase occurrence of lymphoid cancer in veterinarians
Blair & Hayes, 1980[161]	United States (1551 white males)	United States population	Excess occurrence of leukemia & Hodgkin's disease in veterinarians in clinical practice

*Based on all types of human cancers as index cases. Mortality studies.

**NSSD=No statistically significant difference at the 5% level or less.

Table 18

Epidemiologic Studies* of Children with Leukemia Related to Exposure to Cats

Author & Reference	Population Studied	Index Cases	Type of Study	Controls	Cancer Results
Penrose,[162] 1970	28 Children	Lymphoid tumors	Retrospective	28 children with non-lymphoid tumors	Two fold increase in exposure to cats for children with lymphoid tumors.
Bross & Gibson 1970[163]	300 Children	Leukemias	Retrospective	831 Normal children	More human leukemia patients exposed to sick or dead cats.

*Based on the childhood leukemia index cases.

miologists and virologists refer to the lack of detectable FeLV-FeSV in human tumors one must remember that FeSV, under experimental conditions, can induce virus negative tumors in species other than cats.

Transspecies transmission of oncoviruses under natural conditions is now a well established fact.[3,4] Oncoviruses have been known to spread from monkeys to cats, rats to cats and mice to apes [Table 3] when these species hypothetically lived closely, millions of years ago.[3,4] Might it not now be possible for FeLV to be spread, if it has not already done so, from cats to humans who share the same household environments?

Epidemiologic Studies of Humans Exposed to FeLV

Since FeLV and FeSV replicate in human cells, since FeLV causes lymphosarcomas in dogs, since FeSV transforms cultured human cells and since cats live in close association with people, epidemiological studies were done to determine if there

Table 19

Epidemiologic Studies of Human Adult Cancers in the General Population Related to Exposure to Cats

Authors & Reference	Population Studied	Index Cases	Type of Study	Controls	Cancer Results
Schneider, 1970*[164]	675 households with people exposed to index cases of cats with all types of cancer. Total number of exposed people not given	Data not given for cats with lymphosarcoma or for cats with other neoplasms	Retrospective	675 households with cats with non-neoplastic diseases	No increased human cancer risk—NSSD***
Schneider, 1972*[165]	760 people exposed to cats with lymphosarcoma	221 cats with lymphosarcoma	Retrospective	221 cats with non-neoplastic diseases	No increased human cancer risk—NSSD
Hanes, *et al*, 1970**[167]	530 people of all ages, non-veterinarians	People with: 208 leukemias 195 lymphomas 127 sarcomas	Retrospective	1042 normal age & sex matched people	Exposure to normal or sick cats: NSSD

**Based on cat cancer index cases*

***Based on human cancer index cases*

****NSSD = No statistically significant differences at the 5% level or less.*

is an association between human disease and exposure to FeLV. A series of retrospective case-control studies were reported based on cancers occurring in veterinarians, children with leukemia and cancers in the general population. Studies were done using both the human cancer as the index case and using cats with lymphosarcoma as the index case [Tables 17, 18, 19].

Veterinarians

Veterinarians are exposed to many cats as a part of their occupation. Five studies of the cause of death in veterinarians, each with conflicting results, have been published [Table 17].[157-161] Of 390 white, male, Missouri veterinarians and 486 Illinois (mostly white male) veterinarians no statistically significant differences in the occurrence of cancer was found compared to controls.[157,159] However, an excess of skin melanomas in 1722 white, male, California veterinarians was reported in another study.[158] Recently, Gutensohn and her coworkers reported that Matanowski has found an 80% increase in the occurrence of lymphoid tumors among 19,000 U.S. veterinarians who died after the age of 46 years as compared to physicians and the general U.S. population.[160] No conclusion as to the cause of this increased risk of lymphoid tumors in veterinarians was possible, however. In a very recent study, Blair and Hayes also found a significantly elevated occurrence of leukemia and Hodgkin's disease in 1551 white, male veterinarians who were in clinical practice.[161]

Children

Since in general, children are more susceptible to infectious agents than young adults, retrospective studies of children with leukemia and their exposure to cats have been done [Table 18]. Penrose found a two-fold increased exposure to cats in houses in London where children had developed lymphoid tumors as compared to an equal number of children with other childhood cancers.[162] Similarly, Bross and Gibson studied 300 children with leukemia and 831 control children and found that the relative risk among children exposed to "sick" cats was more than doubled (2.24) and that this difference was significant at the 1% level.[163] However, they concluded that only a small proportion (15%) of the cases of childhood leukemia were related to exposure to cats.

Adults

In addition to the studies of veterinarians and children with leukemia, three studies of the occurrence of cancer in nonveterinarian adults have been done [Table 19]. Schneider's two studies were based on cat cancer as the index cases, whereas Hanes and his coworkers based their study on the

Table 20

Serological Studies of People for Antibody to FeLV

Author & Reference	Population Studied		Serologic Assay	Results
Fink *et al*, 1971[168]	200	Cancer patients	HA test for antibody to FeLV	9 of 389 (2.3%) had low titers of antibody to FeLV
	189	Non-cancer patients		
	389			
Schneider & Riggs, 1973[169]	626	Veterinarians	Cell membrane IFA test on cat lymphosarcoma cells. 1:4 dilution of serum	1 veterinarian positive (retested negative 8 months later).
	67	Other people		
	693			
Sarma *et al*, 1974[170]	36	Veterinarians	FeLV neutralization test for FeLV-A, B and C. Serum diluted 1:2	No antibody found
	33	FeLV research workers		
	69			
Olsen, *et al*, 1975[171]	378	Cancer patients	CFI test for FeLV antibody	Antibody to FeLV found in 70% of lymphosarcoma, 41% of osteosarcoma, 57% of reticulum cell sarcoma patients but in only 6% of healthy people, p=0.01
	193	Healthy people		
	571			
Caldwell, *et al*, 1976[172]	107	People exposed to known FeLV infected cats	1) FOCMA antibody 2) FeLV-SN, CFI and MCT test, Serum diluted 1:4	74 of 107 people (69.1%) positive in 1 or more test.
Hardy, *et al*, 1976[45]	78	Healthy FeLV exposed	Neutralization test of FeLV-A,B. Serum diluted 1:2	No antibody found
	7	Leukemia patients exposed to FeLV cats		
	1	Non-lymphoid tumor patients exposed to FeLV		
	86	People exposed to FeLV		
	18	Healthy people not exposed		
	104			
Krakower & Aaronson, 1978[173]	408	Healthy, not FeLV exposed	RIP for antibody to FeLV p27 and gp70. Dilution of sera not specified	No antibody found
	291	Healthy, exposed to FeLV		
	543	Lab workers exposed to oncoviruses		
	490	Leukemia & lymphoma patients		
	133	People with non-lymphoid tumors		
	209	People with non-neoplastic diseases		
	2,074			
Jacquemin, Saxinger and Gallo, 1978[174]	33	People with various leukemias	Antibody to FeLV reverse-transcriptase on the surface of human blood leukocytes	Antibody found in 8 of 9 patients with chronic myelogenous leukemia in blast crisis, none of 24 other leukemias or none of 29 healthy people had antibody
	29	Healthy people		
	62			

FOCMA = cell membrane IFA test for antibody to the feline oncornavirus associated cell membrane antigen.
SN = serum neutralization, CFI = complement fixation inhibition, MCT = microcytotoxic antibody test.
RIP = radioimmunoprecipitation assay

human cancer index case. Schneider found no increase in the occurrence of cancer in an unstated number of people exposed to 675 cats with cancer, compared to people exposed to 675 cats with non-neoplastic diseases.[164] In a similar study of 760 people who were exposed to 221 cats with lymphosarcoma no increase in the occurrence of cancer was found.[165] It should be noted that Schneider, using the same methodology, concluded that FeLV is not horizontally transmitted from cat to cat, a conclusion that was subsequently found to be incorrect.[164,166] Hanes and his coworkers found no excess exposure to cats in 530 people with cancer compared to 1042 normal controls.[167]

Table 21

Search for FeLV Antigens in People

Author & Antigen Assay	Population Studied	Results
Sutherland & Mardiney 1973.[175] IFA test for FeLV antigen in renal glomeruli	12 patients with lymphoid and myeloid leukemias	2 of 12 (17%) positive for FeLV antigens
Metzgar *et al*, 1976[176] Cytotoxic test using antibody to FeLV gs antigens	36 patients with lymphoid leukemia 53 patients with myeloid leukemia 89	35 of 36 lymphoid leukemia patients and 43 of 53 myeloid leukemia patients positive for FeLV related antigen on their leukemic cells
Hardy, *et al*, 1976[45] IFA test for FeLV antigens in leukocytes or tumor cells	Healthy exposed people: 63 cat owners 4 bitten by FeLV infected cat 52 veterinarians 41 veterinary hospital employees 7 other Healthy non-exposed people: 67 healthy people Cancer patients: 200 lymphoid tumors 59 nonlymphoid tumors	No antigen found
Krakower & Aaronson, 1978[173] Competition RIA for FeLV p30	1324 people exposed to FeLV or with hematological tumors	No antigen found

Seroepidemiological Studies of Humans Exposed to FeLV

It is difficult to evaluate the conflicting results of the epidemiological studies. In addition, epidemiological methods are often inadequate to detect the etiologic factors for diseases which have long latent periods. More recently, seroepidemiological methods have been employed to determine the likelihood of FeLV transmission to people. In most of the studies, attempts were made to detect antibodies to FeLV in human sera [Table 20], although in a few studies attempts were made to find FeLV antigens in human sera, leukocytes or tumor cells [Table 21].

Search for Antibody to FeLV in People

Fink and her coworkers reported hemagglutinating antibody to FeLV in three of 200 people with cancer and in six of 189 people without cancer.[168] The highest titer was 1:16 but most of the reactors had low titers of 1:4. Schneider and Riggs found one of 626 veterinarians to have antibody to an antigen found on feline LSA (FL-74) cells, but when that veterinarian was retested eight months later no antibody remained.[169] Using the FeLV serum neutralization tests, Sarma and his coworkers and the author's group did not find SN antibody in a total of 173 people, many of whom had been exposed to FeLV.[45,170] Olsen and his coworkers, using a complement fixation inhibition test (CFI) for antibody to FeLV, found antibody in 70% of people with lymphosarcoma, 41% of people with osteosarcomas, 57% of people with reticulum cell sarcoma, but in only 6% of healthy people.[171] The CFI test is a very sensitive test but one which is difficult to ensure the specificity of the reaction. Similarly, Caldwell and his coworkers, using a variety of tests, including the CFI test, found antibody to FeLV in 74 of 107 (69%) people exposed to FeLV infected cats.[172] Krakower and Aaronson have tested the greatest number of people (2,074) for antibody to FeLV using the very sensitive and specific radioimmunoprecipitation assay for FeLV p27 and gp70, but found no person to have antibody to FeLV.[173] However, Jacquemin, Saxinger and Gallo found antibody to the FeLV reverse transcriptase enzyme on the surface of peripheral blood chronic myelogenous leukemia cells in eight of nine patients but not in 24 patients with other leukemias, nor in 29 healthy blood bank donors [Table 20].[174]

Search for FeLV Antigens in People

There have been four studies in which attempts were made to detect FeLV antigens in people [Table 21]. Sutherland and Mardiney reported finding FeLV antigens in the glomeruli of two people with acute myelocytic leukemia.[175] Similarly, Metzger and coworkers, using the cytotoxic test, found FeLV related antigens on the leukemic cell surfaces of 35 of 36 (97%) patients with lymphoid leukemias and on the leukemic cell surfaces of 43 of 53 (81%) patients with myeloid leukemias, but not on the surfaces of normal lymphocytes from 12 healthy people.[176] In contrast, we have not found FeLV antigens, by the immunofluorescent antibody test, in peripheral blood leukocytes or tumor cells of 167 healthy FeLV exposed people (including four who were bitten by FeLV infected cats) in 67 healthy nonexposed people or in 259 nonexposed people with cancer (200 lymphoid tumors and 59 nonlymphoid tumors).[45] Similarly, in a very large study Krakower and Aaronson did not find FeLV antigen by competition radioimmunoassay in 1324 people who either had been exposed to FeLV or had hematological tumors.[173]

When interpreting these seroepidemiological data one must keep in mind that most of the studies for antibody to FeLV and for FeLV antigens in people were negative. However, similar testing of cats with LSA for antibody to FeLV would show similar negative results.[45] In addition, 30% of cats with LSA do not even have detectable FeLV antigens in their tumors.[37,55,177]

The observations that infectious FeLV is shed in large numbers in the saliva of infected cats, that FeLV replicates well in human cells, that oncoviruses have crossed species barriers under natural conditions, that FeLV can induce LSA in puppies, that FeSV induces tumors in many species with many of the tumors being virus nonproducer tumors and that FeLV infected cats have a poor prognosis whether healthy or sick, strongly indicate to this author that FeLV infected cats should not be kept as pets. McClelland in the author's laboratory has also found that 83% of infected healthy cats die within three and one-half years.[15] The infected cat should at least be isolated, in a cage, away from other cats, children, sick adults, and pregnant women.[178-180] However, since cage isolation is somewhat unpleasant for the cat and often not effectively carried out, the author feels that infected cats should be euthanized. The recommendation of euthanasia is made based only on a positive IFA test result for FeLV obtained from a reputable laboratory. The author does not recommend euthanasia of in-hospital, ELISA, test positive cats since there appears to be a significant (67%) difference in the results of comparative IFA and ELISA tests.[107] In addition, several veterinary laboratories that perform the IFA test for FeLV do not recommend euthanasia for their test positive cats so practitioners should acquaint themselves with the recommendations of the laboratories performing their FeLV tests. Finally, it should be noted that the National Cancer Institute has assigned the highest risk category, that of a "moderate risk agent" designation to FeLV and FeSV.[181] Infected pet cats contain as much or more FeLV in their saliva as most laboratory preparations of the virus. If it is prudent to accept the NCI guidelines for research workers one must also consider them prudent for people exposed to FeLV from cats living in their homes.[182]

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