In This Issue:
In the fall issue of the NVL Newsletter we will review the feline leukemia virus (FeLV). FeLV is one of the most important infectious agents of pet cats. We will discuss the biology of FeLV and the test methods for detection of infected cats. In 1972 we developed the first FeLV test, the IFA test, and subsequently used the test to define many of the FeLV-induced diseases.

Feline Leukemia Virus
William D. Hardy, Jr., V.M.D.

Three subfamilies of Retrovirinae exist in pet cats: 1) Oncovirinae, 2) Lentivirinae, and 3) Spumavirinae. Cats have more retroviruses than any other species.

FELINE RETROVIRUSES

1. Subfamily Oncovirinae:
A. Endogenous Viruses: Genetically transmitted
   1) FeLV related full length and shorter DNA sequences. Cannot be induced to replicate
   2) Recombines with exogenous FeLV-A DNA to form FeLV-B and FeLV-C

B. Exogenous Viruses: Spread contagiously
   Chronic transforming (leukemia) retroviruses:
   1) FeLVs:
      Subgroup A- Found in all infected cats.
      Only subgroup transmitted contagiously
      Subgroup B- Found in 50% of infected cats
      Subgroup C- Found less than 1%
   2) FeLV-A-FAIDS
      Experimentally induces FAIDS

Acute transforming retroviruses:
3) FeSVs: (Feline Sarcoma Viruses) 11 isolates.
   Recombinants- FeLV and cellular oncogenes

2. Subfamily Lentivirinae:
A. FIV (Feline Immunodeficiency Virus)
   Induces FAIDS

3. Subfamily Spumavirinae:
A. FeSFV (feline syncytium-forming virus)
   Causes no known disease

Background:
Domestic cats are infected with members of all 3 retrovirus subfamilies. The feline leukemia virus (FeLV) was first isolated from a cat in Scotland in 1964. At that time all retroviruses were thought to be endogenous viruses that were only transmitted genetically (vertically). However, using the FeLV IFA test in pet cats, we demonstrated that FeLV is an exogenous retrovirus that is transmitted contagiously amongst cats.1 This observation was the first conclusive proof that any retrovirus was transmissible by contagious means, and this finding changed the prevailing concepts on these viruses. Before the introduction of the FeLV vaccines, about 2%, or more than 1 million of the estimated 60 million pet cats in the United States, were infected with FeLV.2-8 The incidence of FeLV-infected cats has not been studied after the introduction of the FeLV vaccines.

Endogenous FeLV-Related Sequences:
Healthy FeLV-uninfected domestic cats possess cellular DNA sequences that are partially homologous to the RNA of exogenous contagiously transmitted FeLVs. Only the cellular DNA of rodents, and in particular rats, contains related retrovirus gene sequences. The presence of related sequences in rodents suggests that endogenous FeLVs were acquired by cats via trans-species infection with a rodent retrovirus.

Sequence analysis of the genomes of the three subgroups of exogenous infectious FeLVs (FeLV-A, -B and -C) has shown that FeLV-B and FeLV-C arise through recombination of contagiously transmitted FeLV-A with endogenous env –B and –C sequences to form envelope (env) recombinant FeLVs. These de novo generated subgroups are not transmitted contagiously and are far more pathogenic than the contagiously transmitted FeLV-A.

FeLV Proteins:
Nine proteins are encoded by the FeLV genome and include: 1) the gag gene internal viral structural proteins p15 (matrix protein, MA), p12 (unknown) function, p27 (capsid protein, CP) and p10 (nucleocapsid protein, NC); 2) the pol gene enzymes: p14 (protease, PR), p80 (reverse transcriptase, RT), p46 (integration protein, IN) and; 3) the env gene envelope proteins gp70 (surface protein, SU) and p15E (transmembrane protein, TM).

The FeLV structural proteins are produced in great excess in the cell membrane and the cytoplasm of infected cells and free viral proteins are released into the plasma and tissue fluids of infected cats after the cells die.

FeLV Tests:
The study of the occurrence and control of FeLV in pet cats has been accomplished by detection of FeLV antigens in the cytoplasm of peripheral blood leukocytes by indirect immunofluorescent antibody (IFA) tests or by detection of soluble antigens in the plasma by enzyme linked immunosorbent assays (ELISA). All of the FeLV biology and control
Preparation of Thin, Feathered-Edge, Blood Smears for the FeLeuk® IFA Test for FeLV

1. Clip the cat’s nail to obtain a drop of blood. Touch the drop of blood from the cat’s nail to the slide. If you obtain blood by syringe, place a SMALL drop of fresh blood or blood from an EDTA tube near one end of a slide. The drop should be no larger than the eraser on a pencil.

Blood drop size template= O

2. If you place an overly large drop of blood by mistake you can correct this by dipping one corner of a second slide (top slide) into the large drop of blood and placing the blood on the corner of the slide further up on the bottom slide. This small drop can then be spread properly

3. Take a second slide and hold the edge at a 45° angle on the slide containing the drop of blood. Pull the top slide back into the drop of blood and allow the blood to completely spread along its edge

4. While holding the top slide at the same angle, rapidly and smoothly push the slide forward to spread the blood into a “feathered-edge” smear. Wave the slide in the air, or blow on the slide, to ensure the smear dries rapidly in order to preserve the WBC morphology. DO NOT FIX THE BLOOD SMEARS.

Feathered-edge

5. Write the name of the owner on the slide with a marker pen and store at room temperature. The WBCs with FeLV antigens are stable at least 1 month at room temperature. DO NOT STORE SLIDES IN THE REFRIGERATOR as WBCs will lyse when removed from the refrigerator due to condensation.

SUBMIT 2 BLOOD SMEARS

There is no need to submit bone marrow smears for the FeLV IFA test since all cat peripheral blood WBCs are replaced twice daily from the bone marrow pool.

IFAX result: No evaluation due to non-specific reaction.

Non-specific reaction due to blood smears that were too thick. The leukocytes (WBCs) cannot be seen clearly and are non-specifically stained.

IFAX result: No evaluation due to lack of WBCs.

No WBCs were found in this smear due to a severe leukopenia or because the smears were made after the blood had coagulated on the slide.

was significant enough to convene an AVMA Expert Panel on FeLV in 1991 to address the problem. The panel met for 2 days and wrote a recommendation published in the JAVMA which recommended that all FeLV ELISA positive test results be immediately confirmed by an IFA test. The AVMA has not changed these recommendations but the American Association of Feline Practitioners (AAFP) has disregarded the Panel’s recommendations.

An excellent paper presented by Dr. Andrea N. Torres in Dr. Edward Hoover’s laboratory at Colorado State University presented evidence that some experimentally FeLV-infected cats will have circulating viral antigens in the blood for long periods after they clear the viral infection. These cats tested positive in the in-hospital ELISA test but had no infectious virus in the blood by isolation, were not shedding FeLV in their saliva, and remained healthy. Although IFA tests were not performed, these cats probably represent those pet cats that are ELISA positive but IFA confirmatory test negative. Antigen positive/virus-negative cats should not be managed as if they were infected. It has been our recommendation for more than 30 years that any FeLV test should only detect persistently infected cats that will be shedding virus in their saliva. It is these cats that should be isolated from all other cats in the Test and Removal Program that was developed 30 years ago. It is these cats that the AVMA panel, in their recommendations in 1991, wanted to ensure are the only cats that test positive.

FeLV Antigens:
FeLV tests detect viral antigens in the leukocytes in the blood (IFA Test) or soluble viral antigens (ELISA Tests) released from cells into the blood.

There were 527 in-hospital FeLV negative ELISA tests (most were Snap Tests, Idexx) by our IFA test. 4.2% were not confirmed by our IFA test and should have been managed as if they were infected.

### In Hospital ELISA (+) vs. FeLeuk® IFA Tests

<table>
<thead>
<tr>
<th>ELISA Positive</th>
<th># Tests</th>
<th>% Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA Positive</td>
<td>1,838</td>
<td>65%</td>
</tr>
<tr>
<td>IFA % Positive</td>
<td>51</td>
<td>2%</td>
</tr>
<tr>
<td>IFA Negative</td>
<td>898</td>
<td>32%</td>
</tr>
<tr>
<td>IFA Indeterm.</td>
<td>34</td>
<td>1%</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>2,821</td>
<td>100%</td>
</tr>
</tbody>
</table>

### In Hospital ELISA (-) vs. FeLeuk® IFA Tests

<table>
<thead>
<tr>
<th>ELISA Negative</th>
<th># Tests</th>
<th>% Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA Positive</td>
<td>21</td>
<td>4%</td>
</tr>
<tr>
<td>IFA % Positive</td>
<td>1</td>
<td>0.2%</td>
</tr>
<tr>
<td>IFA Negative</td>
<td>496</td>
<td>94%</td>
</tr>
<tr>
<td>IFA Indeterm.</td>
<td>9</td>
<td>1.8%</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>527</td>
<td>100%</td>
</tr>
</tbody>
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The finding of FeLV antigenemia in the absence of FeLV in the blood of some cats makes the recommendation to confirm all ELISA positive in-hospital FeLV tests more imperative.

### AVMA FeLV Expert Panel Recommendations:
In 1991 the AVMA Expert FeLV Panel recommended that all FeLV positive ELISA tests be immediately confirmed by an IFA test. There is no recommendation to repeat the ELISA test again. ELISA positive but IFA negative results indicate the cat is not infected with FeLV. We now know that most of these cats may be antigenemic but have no infectious FeLV in their blood or saliva and should not be managed as infected cats.

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**The Feline Retrovirus Symposium:**

By William D. Hardy, Jr., V.M.D.
The 8th International Feline Retrovirus Research Symposium was expanded to include genomics of the cat and other infectious disease microorganisms such as avian influenza, SARS, feline coronaviruses, Helicobacter spp., Bartonella, and feline retroviruses: FeLV, FIV and Foamy viruses. Dr. Albert Osterhaus gave the Keynote lecture entitled: Newly Emerging Infections, Dr. Oswald Jarrett from the University of Glasgow gave a wonderful review of the FeLV infections and Infectious Diseases in the 21st Century held in Washington DC. Dr. Hardy was honored to present the meeting’s Banquet Keynote Lecture on October 10, 2006. Of practical importance was the scientific evidence for some of the discrepancies (~32%) between in-hospital ELISA positive FeLV tests and negative confirmatory IFA tests that we have reported. These finding indicate that FeLV positive ELISA tests should be confirmed by an IFA test.

**Scientific Explanation for the Discrepancy Between In-Hospital FeLV ELISA Positive Tests and IFA Negative Confirmatory Tests:**
Ever since the introduction of the in-hospital FeLV ELISA tests, there have been reports of discrepancies between ELISA positive results and confirmatory IFA tests. The problem was significant enough to convene an AVMA Expert Panel on FeLV in 1991 to address the problem. The panel met for 2 days and wrote a recommendation published in the JAVMA which recommended that all FeLV ELISA positive test results be immediately confirmed by an IFA test. The AVMA has not changed these recommendations but the American Association of Feline Practitioners (AAFP) has disregarded the Panel’s recommendations.

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AAFP FeLV Recommendations:

In 2001 (reprinted in 2005) the American Association of Feline Practitioners (AAFP) produced a report on Feline Retrovirus Testing and Management. None of the panel members had any experience developing or evaluating FeLV tests. The AAFP panel did not mention the AVMA FeLV Panel’s recommendation, that all ELISA positive tests should be immediately confirmed by an IFA test, but rather modified the recommendations: “The work of the AAFP/AFM Advisory panel on Feline Retrovirus Testing and Management was made possible by an educational grant from IDEXX Laboratories, Inc.” Editor’s note: IDEXX Laboratories is the maker of the predominant in-hospital FeLV ELISA test kit.

The following statements were made:

1. “The preferred initial tests are soluble antigen tests, such as ELISA...” Editor’s note: The panel never mentioned that the IFA test was used for years as the sole FeLV test and all the biology of FeLV was elucidated using the IFA test.

2. “Indirect immunofluorescent antibody (IFA) tests detect cell-associated antigens.” Editor’s note: The IFA test detects FeLV antigens in cells that are replicating.

3. “In populations with a low prevalence of FeLV infection, more than half of cats for which test results are positive are likely to be uninfected.” Editor’s note: They neglected to state that the study refers to ELISA tests and not IFA tests. This degree of false positive test results did not occur in studies that used the IFA test.

4. “If results of a soluble antigen test are positive and results of an IFA test are negative, both tests should be performed again in 60 days and then annually until results of both tests are in agreement.” Editor’s note: This is the most unscientific recommendation in the report. There are no scientific publications that show the test results will eventually become concordant. In fact, the recent studies have elucidated the scientific explanation for ELISA positive but IFA negative discordant cats should be immediately confirmed by an IFA test.

5. “In-hospital positive ELISA tests should be immediately confirmed by an IFA test.” Editor’s note: The panel on feline retrovirus testing and management (AVMA) recommends that all in-hospital positive ELISA tests should be immediately confirmed by an IFA test. ELISA positive but IFA negative discordant cats should be considered FeLV uninfected.

Pathogenesis of FeLV Infection:

In order to understand the correct use of FeLV tests, it is important to understand the pathogenesis of FeLV infection in cats. The pathogenesis was elucidated by Dr. Jennifer Rojko, Dr. Edward Hoover and colleagues. After experimental infection of FeLV, which in nature usually occurs most often as oronasal exposure in the head area by mutual grooming, there are 6 stages in the pathogenesis leading to persistent infection. Stage 1: Days- 2-4 FeLV replicates in the tonsils and pharyngeal lymph nodes. Stage 2: Days 1-14 FeLV replicates in a small number of peripheral blood lymphocytes and monocytes. Stage 3: Days 3-12 FeLV replicates in spleen, lymph nodes, and gut associated lymphoid tissues. Stage 4: Days 7-21 FeLV replicates in bone marrow, neutrophils, platelet precursors and intestinal crypt epithelium. Stage 5: Days 14-28 FeLV infects bone marrow neutrophils and megakaryocytes-platelets and establishes a peripheral blood viremia. Stage 6: 28-56 days FeLV establishes epithelial infections in many tissues (nares, oropharynx and salivary glands) and is shed in the saliva and urine.

Consequences of Exposure to FeLV

Not all pet cats exposed to an FeLV infected cat will become infected. In fact, more exposed cats became immune (42%) to the virus than develop persistent, life-long, infection (30%).

References:

1. Hardy, WD, Jr. & Zuckerman, EE. Ten-year study comparing enzyme-linked immunosorbent assays with the immunofluorescent antibody test for detection of FeLV infection in cats. JAVMA, 199, 1365, 1991.