Feline leukemia virus – past, present and perpetually perplexing

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Despite its discovery over 50 years ago, the feline leukemia virus (FeLV) continues to challenge our thinking about the nature of the disease and our ability to diagnose the infection. Testing, vaccination, and segregating progressively infected cats remain effective practices to help control the spread of the disease. However, we continue to refine our understanding of the different stages of the infection and how this relates to the results of available diagnostic assays. The complex viral behavior, which sometimes yields confusing test results, gives us a renewed appreciation for those cats who have this retroviral infection and are able to control it and go on to live a nearly normal life.

Introduction

There are a several of perplexing questions associated with FeLV infections. While many do not have definitive answers, we will discuss the available data and clinical observations for the following:

- Why do all domestic cats have genomic copies of the endogenous feline leukemia virus? Does this play a role in protecting them from the exogenous (infectious form) of the virus?
- Why do serologic surveys for FeLV identify older age is a risk factor for FeLV when experimental infection studies have shown that it is very difficult to infect adult cats?
- Why has the seroprevalence of FeLV remained unchanged in recent years despite regular testing, vaccination and segregation of infected cats?
- Why does a cat who tested negative for FeLV as a kitten and has lived indoors his whole life in an FeLV-negative household now test positive as a senior cat when evaluated for an illness?
- Why can a queen who tests negative for FeLV have kittens that are infected with FeLV? If only one kitten is infected, does that mean the entire litter should be considered infected?
- Why can a cat test positive for FeLV antigen (SNAP), be negative by IFA, and still be infected?
- Why can at cat test positive for FeLV antigen on SNAP with whole blood, be negative when tested with serum, and still be infected?
- Why can a cat test negative for FeLV antigen and still be infected?

Prevalence of Feline Leukemia Virus

The feline leukemia virus was first discovered by Dr. William Jarrett at the University of Glasgow in 1964. In collaboration with a local veterinarian, he identified the virus in a cluster of cats presenting with lymphoma (1). By the early 1970's, diagnostic tests were being developed and

screening for the infection in the feline population revealed that the prevalence of the infection was as high as 40% (2). In the last 40 years, efforts to control the spread of FeLV have reduced the prevalence ten-fold. However, the proportion of infected cats identified through testing appears to have reached a plateau with perhaps a slight increase in the number of positive test results in recent years (3-6).

Interesting observations:

- In 1975:
  - Cats over 3 years of age were more likely to test positive (74%) than cats at 5-6 months of age (29%)
  - Stray cats were twice as likely to test positive for FeLV as pet cats
- In 1989:
  - Prevalence was 5% in healthy cats and 18% in sick cats
- In 2010:
  - Overall seroprevalence for FeLV was 3.1%
  - Shelter cats had a lower prevalence of FeLV (2.6%) than cats presenting to a veterinary hospital (3.4%)
  - Retroviral infections (FeLV and FIV) were more common in sick cats (13.9%) compared to healthy cats (3.6%)
  - Significant risk factors associated with FeLV positive status included: intact male, outdoor access, adult age, and clinical illness (respiratory signs and oral disease)

FeLV related disease

While recognized for its ability to cause lymphoma and leukemia, the feline leukemia virus primarily targets the hematopoietic and lymphoid tissues where it can cause either cytoproliferative or cytosuppressive conditions (7). This creates the potential for immune dysregulation and increased susceptibility to opportunistic infections and co-infections. Conditions that are typically encountered include: 1.) bacterial enteritis, gingivitis, pneumonia or sepsis; 2.) viral infections like coronavirus (FECV and FIPV), herpesvirus, and calicivirus; 3.) hemotropic parasites like Mycoplasma hemofelis or M. turicate.

In a 1989 study of over 2000 cats in Germany (8), it was found that 16% of 'sick' cats had immunohistochemical evidence (spleen and/or bone marrow) of FeLV infection at necropsy while only 3% of the control population tested positive. Only 20% of these cats had FeLV infections diagnosed ante-mortem. Of the cats with FeLV infections, 77% died of non-neoplastic conditions. The most common findings in the FeLV infected cats were: anemia, FIP, liver degeneration and icterus, FeLV-specific enteritis, neurologic signs (based on case history), lymphatic hyperplasia, and bacterial infections.

FeLV – the virus

Feline leukemia virus is a Gammaretrovirus and contains an RNA genome, a reverse transcriptase and an integrase. As a retrovirus, the RNA genome must be converted to DNA by

the reverse transcriptase before any viral proteins can be synthesized. Once a copy of the viral DNA is made, it is able to integrate into the genomic DNA of that cell when the cell divides. All new cells arising from that cell will now carry copies of the 'proviral' DNA, thereby establishing the infection. New viral proteins and virions are constructed using the cell's own machinery and nutrients. These new virus particles bud from the surface of cell, leaving the cell intact to allow further propagation of the infection.

The FeLV virus is not very hardy in the environment – it does not survive for long outside of the host. It can be destroyed by disinfectants, soap, heat, or dessication and is unlikely to be spread via objects that an infected cat has come in contact with. It requires moisture and room temperature conditions for survival (9).

Diagnosing FeLV

There are 2 primary ways to diagnose FeLV infections. The first is to detect the proteins or antigens that make up the structure of the virus. The second is to detect the nucleic acid (proviral DNA or RNA) that provides the instructions for making the proteins.

- Protein/Antigen Tests
    - Soluble antigen tests detect a core protein of the virus called p27. It is produced in abundance as the virus is constructed. It forms a structure to encapsulate the RNA, reverse transcriptase and integrase that are needed when the virus infects the cell. Examples of antigen tests include:
        - Microtiter plate ELISA tests (reference lab)
        - In-clinic tests (SNAP)
    - Cellular antigen tests detect viral proteins associated with proliferation of the virus within an infected cell. Usually collected from the peripheral blood or bone marrow.
        - Immunofluorescent Assay (IFA)
- Nucleic Acid Tests
    - Polymerase Chain Reaction (PCR) is a method of exponentially amplifying minute quantities of nucleic acid to allow their detection. The system works like a copy machine – start with a single copy and through repeated cycles you end up with many copies.
        - Proviral DNA PCR (detects integrated FeLV genes in the feline genome)
        - Viral RNA PCR (detects circulating virus or replicating virus within a cell)

Pathogenesis of FeLV

Definitions:

- Abortive infection: cat eliminates the virus prior to proviral integration
- Progressive infection: cat is infected and capable of shedding virus from epithelial tissues (salivary gland). This cat is considered to be at high-risk of FeLV-related disease
- Regressive infection: infection that is controlled by the cat's immune system either prior to or shortly after bone marrow infection.

    - Latent infection: bone marrow infection present but no/minimal evidence of secondary viremia
    - Focal infection: control occurs prior to bone marrow infection thereby limiting infection to focal lymphoid or other organ systems (mammary gland)
- Reactivation: a cat with a previously controlled, regressive infection develops evidence of increased viral activity or a progressive infection
- Recovery: a cat with a known progressive or regressive infection that shows evidence of decreased viral activity or change to a more regressive state. Recovery does *not* mean abortive infection or elimination of the virus. It suggests a more latent state of infection.

An infected cat primarily sheds virus in saliva and respiratory secretions, but it can also be shed in milk, urine and feces. Young cats (<1yr of age) or older immunocompromised cats (those with other ongoing, chronic infections) are most susceptible to acquiring FeLV infections that become progressive. A cat typically comes in contact with the virus through an oral-nasal route with initial infection of cells within lymphoid tissues of the oropharynx. Integration of the viral genome as proviral DNA into a long-lived and dividing cell equates to infection even at this early stage.

An initial or primary viremia then occurs which allows the virus and infected cells to spread throughout the body primarily targeting lymphoid tissues like the spleen, lymph nodes, gut-associated lymphoid tissues, etc. If the infection is controlled at this stage, a regressive focal infection will occur. Otherwise, the infection continues to the bone marrow where progenitor cells for granulocytes, monocytes and platelets become infected. Some cats will manage to control the infection at this point and prevent further activity or replication of the virus thereby limiting further the spread. A latent, regressive infection is established in this cat.

Without immune control of the virus following bone marrow infection, a secondary viremia occurs and spreads infected cells and virus to other organs in the body including those epithelial tissues where the virus will be shed. This is a progressively infected cat that should be considered infectious and at higher risk of FeLV-associated disease (10).

Understanding FeLV Test Results

The presence of FeLV soluble antigen (p27 protein), especially when confirmed by the reference laboratory plate ELISA, indicates that an FeLV infection is present. It does not indicate the stage of the infection. Using a multi-modal testing approach, which includes PCR followed by IFA, is best for staging FeLV patients. **Any cat used as a blood donor or for breeding should be tested by both an ELISA for soluble p27 antigen and PCR for proviral DNA. Ideally, both tests should be performed twice and at least several (3-6 months) months apart.**

Stage	ELISA for p27 antigen	PCR for proviral DNA	IFA
Abortive	Negative	Negative	Negative
Regressive, focal	Positive or Negative	Negative	Negative
Regressive, latent	Positive or Negative	Positive	Negative
Progressive	Positive	Positive	Positive

Outcome of FeLV Infections

There are no current studies describing the expected survival times of FeLV infected cats stratified by stage of infection, lifestyle/husbandry, and health status. The early studies looked mostly at very large, multi-cat households where the risk of other chronic infections in the environment was high. In those studies, an infected cat typically did not survive 3 years beyond the time of diagnosis. Based on the experience of those who routinely work with FeLV infected cats today and adopt them into smaller, more stable home environments, the impression is that regressive cats can live a nearly normal life span. In fact, some cats with progressive infections may do so as well. Diagnosing a healthy cat with an FeLV infection should not be an indication for euthanasia.

Keys to Management

- AAFP Guidelines recommend vaccinating kittens – initial series and 1 yr booster minimum. Prevents progressive disease.
- Husbandry – prevent overcrowding, good quality diet, regular veterinary care, parasite prevention (fleas, ticks, worms), dentistry, reduce exposure to opportunistic infections, allow limited/controlled outdoor access
- Therapy – important to manage underlying conditions and infections aggressively in any cat with clinical signs. Anti-retroviral therapy is only indicated in exceptional cases due to the lack of proven efficacy and potential for toxicity. More studies are needed to demonstrate in vivo benefit (11). Likewise, the effectiveness of immune modulators is still under investigation.

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