# Regressive and progressive feline leukemia virus infections – clinical relevance and implications for prevention and treatment

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## *Introduction*

Pathogenesis of feline leukemia virus (FeLV) infection has always been subject of discussions and intensive research. Sensitive polymerase chain reaction (PCR) assays have provided new data on the courses of FeLV infection. In light of these new data, new aspects of FeLV pathogenesis have been detected. Tests that detect genome-integrated FeLV provirus, e.g. PCR, reveal a higher number of FeLV-infected cats than tests routinely used for detection of FeLV infection (i.e. ELISA detecting free FeLV p27 antigen). These FeLV provirus-positive, however FeLV antigen-negative cats are called "regressively infected cats". Although regressive FeLV infections are frequently detected, their role is still not well understood. It is currently subject of discussion, how common these regressive infections are, how often they are reactivated, and which clinical signs they can cause.

Today, FeLV infection is less commonly diagnosed than in the previous 2 decades. Prevalence of FeLV antigen-positive cats has decreased in most countries. Still, the importance of FeLV as a pathogen is likely underestimated as it has been shown that regressively infected cats (that are negative in routinely used antigen FeLV tests) can also develop clinical signs.

### What are the different courses of FeLV infection and their new definitions?

A new classification has been proposed, in which the courses of FeLV infection are defined as abortive infection (comparable to the former "regressor cats"), regressive infection (comparable to the former "transient viremia" followed by "latent infection"), and progressive infection (comparable to the former "persistent viremia") (see table 1).

### What is regressive FeLV infection?

PCR-positive (FeLV provirus-positive), but ELISA-negative (FeLV antigen-negative) cats are classified as regressively infected. The clinical relevance of regressive FeLV infection and the role in FeLV epidemiology is still not fully understood. Regressively infected cats are considered FeLV carriers. They do not shed FeLV, but reactivation with reoccurring virus shedding is possible. Following reactivation of FeLV, they can act as an infection source. As FeLV provirus is integrated into the cat's genome, it is unlikely to be fully cleared over time.

Regressive and progressive infections can be distinguished by repeated testing for viral antigen in peripheral blood; regressively infected cats will turn FeLV antigen-negative usually at latest 16 weeks after infection, while progressively infected cats will remain FeLV antigen-positive. Initially both, regressive and progressive infections, are accompanied by persistence of FeLV provirus in the blood detected by PCR, but later are associated with different FeLV loads when measured by quantitative PCR; regressive infection is characterized by with low, progressive infection by high virus load.

### How common is regressive FeLV infection?

FeLV infection shows a decreasing prevalence in many countries. With few exceptions, FeLV prevalence studies are, however, uniquely based on detection of FeLV antigen in blood using ELISA or similar immunochromatographic assays. Hence, regressively infected cats are negative in those tests. Therefore, antigen testing underestimates the true prevalence. A study in Switzerland showed that 6.9% of cats were antigen-positive, provirus-positive (progressively infected) cats, but additionally, 10.0% of the antigen-negative cat population were positive for proviral DNA in blood (regressively infected). In a German study, 9/495 (1.8%) cats were progressively infected, and in addition 6/495 (1.2 %) were regressively infected. The difference in prevalence could be due to the time gap between the Swiss and the German study. The Swiss study was performed in 1999 and 2000, the German study in 2011 and 2012. Hence, FeLV prevalence could have further decreased in between the 2 study time points. Alternatively, FeLV infection rate in Switzerland might be truly higher than that in Germany.

### What is the clinical relevance of regressive FeLV infection?

Regressive FeLV infection can be of clinical relevance. It can be transmitted to other cats under certain circumstances, it can be reactivated, and it can cause some clinical signs.

### Are regressively infected cats infectious to others?

Regressively infected cats do not shed virus with saliva, and therefore they do not transmit FeLV under natural circumstances. It has been shown recently, however, that when blood from a regressively infected cat is transmitted to another cat by blood transfusion, the recipient can become regressively infected or even progressively infected.

### How often is regressive FeLV infection reactivated?

In regressive infection, the information for producing complete viral particles prevails and can, therefore, be

potentially reused when antibody production decreases (e.g., after immunosuppression). This is why regressive FeLV infection can be reactivated. This usually occurs after stress. Reactivation can also be experimentally induced in cats by administration of high doses of glucocorticoids. The earlier the stress factor occurs after the viremic phase, the more likely is reactivation. During the first weeks following viremia, viral replication can be experimentally reactivated in most cats. The more time passes, the more difficult it becomes to reactivate virus replication. However, some cats can reverse to a viremic state even many years after infection in case of immunosuppression. It has been demonstrated that the proportion of experimentally infected cats with regressive FeLV infections decreased with time following disappearance of viremia. However, new experimental studies demonstrate that all cats in which regressive infection was detected by provirus PCR remained PCR-positive lifelong.

**How commonly does regressive FeLV infection cause clinical signs?**

Most cats with regressive infections are clinically healthy. The reason for this is that active virus replication is necessary to trigger the majority of pathogenic mechanisms that cause clinical signs associated with FeLV. This is, however, not the case in regressive FeLV infections, in which the virus is harbored in a "dormant" and non-productive form. Besides the potential risk of reactivation, FeLV provirus also can be inserted at many different sites in the host's genome, carrying potent regulatory signals. In the development of tumors or myelosuppressive disorders, integrated FeLV provirus can interrupt or inactivate cellular genes in the infected cells, or regulatory features of viral DNA can alter expression of neighboring genes. In addition, FeLV not only contributes its genes to the host, it also has been shown to appropriate cellular genes. Several such transduced genes that are also present in regressively infected cells have been implicated in viral oncogenesis. Some studies demonstrated involvement of regressive FeLV infection in tumors. Cats from FeLV cluster households had a 40-fold higher rate of development of FeLV antigen-negative lymphoma than did those from the general population. FeLV antigen-negative lymphomas have also occurred in laboratory cats infected previously with FeLV. It is still unclear, up to which extent regressive FeLV infection is responsible for FeLV-associated tumors in the field as study results have been controversial. In 7/11 FeLV antigen-negative cats with lymphoma, proviral DNA was detected in formalin-fixed, paraffin-embedded tumor tissue. However, other groups found evidence of provirus in only 1/2217 and in 0/50 FeLV antigen-negative lymphomas.

Since bone marrow microenvironment cells (e.g. myelomonocytic progenitor cells and stromal fibroblasts) provide a reservoir of regressive FeLV infections, it is possible that integrated provirus can alter cellular functions and hence contribute to the pathogenesis of myelosuppressive disorders. Hematologic disorders described in association with FeLV include anemia (non-regenerative or regenerative), persistent, transient, or cyclic neutropenia, panleukopenia-like syndrome, platelet abnormalities (thrombocytopenia and platelet function abnormalities), and aplastic anemia (pancytopenia). For the majority of pathogenic mechanisms in which FeLV is the causative agent for bone marrow suppression, active virus replication is required. However, it has been demonstrated that regressive FeLV infection can be responsible for bone marrow suppression in some FeLV antigen-negative cats. In one study including 37 FeLV antigen-negative cats with myelosuppression, 2/37 cats were found regressively infected with FeLV (both had non-regenerative anemia). As FeLV provirus can interrupt or inactivate cellular genes in the infected cells, or regulatory features of viral DNA can alter expression of neighboring genes, bone marrow suppression might develop in these cats. Additionally, cell function of provirus-containing myelomonocytic progenitor and stromal fibroblasts providing bone marrow microenvironment might be altered. Alternatively, FeLV provirus could cause bone marrow disorders by inducing expression of antigens on the cell surface, resulting in an immune-mediated destruction of cells.

**Can regressive FeLV infection be prevented by vaccination?**

Experimental studies demonstrate that vaccination does not prevent development of regressive infections. They only prevent progressive FeLV infection.

**Which cats should be vaccinated against FeLV?**

Only cats showing neither progressive, regressive, nor abortive FeLV infection should be vaccinated. In older cats that have natural resistance to progressive FeLV infection, vaccination should only be performed under certain circumstances.

**How should cats with regressive or progressive FeLV infection be treated?**

The most important life-prolonging advice for progressively infected cats is to keep the cats strictly indoors. This not only avoids spread to other cats in the neighborhood, it also prevents exposure of the immunosuppressed, FeLV-infected cat to infectious agents carried by other animals. Progressively FeLV-infected cats should receive routine vaccinations. Inactivated vaccines are recommended out of concern that modified-life virus (MLV) vaccines given to immunosuppressed animals might regain pathogenicity, in spite of the fact that there is no scientific proof that FeLV-infected cats are at increased risk from MLV vaccines. Studies investigating the immune response to

rabies vaccination demonstrated that progressively FeLV-infected cats might not be able to mount adequate immune responses. Therefore, post-vaccination protection in a progressively FeLV-infected cat is not comparable to that in a healthy cat, and more frequent vaccinations than recommended in general must be considered (e.g., every 6 months), particularly in cats allowed to go outside.

Early detection of changes in health status is important in FeLV-infected cats. Therefore, routine health care visits at least semiannually are highly recommended. A complete blood count, biochemistry profile, and urinalysis should be performed every 6 months to detect anemia or other cytopenias associated with progressive FeLV infection. Intact male and female FeLV-infected cats should be neutered to reduce stress associated with estrus and mating behavior and the desire to roam outside the house and to interact aggressively. Surgery is in general well tolerated by asymptomatic FeLV-infected cats, but perioperative antibiotic protection is necessary for all surgical and dental procedures. FeLV survives for only minutes outside of the host and is susceptible to all disinfectants (including common soap); therefore, simple precautions and routine cleaning procedures prevent transmission within the hospital. FeLV-infected cats (including progressively FeLV-infected cats) can be housed in the same ward as other hospitalized patients, however in individual cages. However, FeLV-infected cats can be immunosuppressed and should hence be kept away from cats with other infectious diseases, and they should never be placed in a "contagious disease ward" with cats suffering from infections, such as by respiratory viruses.

If FeLV-infected cats are sick, prompt and accurate identification of the secondary illness is essential. Often clinical signs of FeLV-infected cats are not caused by the retrovirus infection. This is why intensive diagnostic testing for secondary diseases should be performed early in the course of illness to enable appropriate therapeutic intervention. Many cats with FeLV infection respond just as well as uninfected cats to appropriate medications, although a longer or more aggressive course of therapy (e.g. antibiotics) might be necessary. Glucocorticoids or other immunosuppressive as well as bone marrow-suppressive drugs should be avoided.

Antiviral chemotherapy has been evaluated in many studies for their efficacy in FeLV-infected cats; however, if well designed placebo-controlled double-blinded studies are performed, most antivirals do not show efficacy or cannot be used because they are too toxic. Thus, antiviral treatment is only recommended in few FeLV-infected cats. *Zidovudin:* Zidovudin (3'-azido-2',3'-dideoxythymidine, AZT), is a nucleoside analogue (thymidine derivative) that blocks the reverse transcriptase of retroviruses. It is integrated in the developing DNA strand, and thus, inhibits new infection of cells. Zidovudine is active against FeLV *in vitro*. When treated less than one week after experimental challenge, cats were protected from FeLV bone marrow infection and persistent viremia. In a study with naturally FeLV-infected cats, however, six weeks of treatment with zidovudine did not lead to a significant improvement of clinical, laboratory, immunologic, or virologic parameters. Zidovudin should only be used at low dosage (5 mg/kg PO or SQ q 12 h) in FeLV-infected cats due to its bone marrow-suppressive effects. During treatment, a complete blood count should be performed regularly (weekly for the first month) because non-regenerative anemia is a common side effect, especially if the higher dosages are used.

*Human interferon-α:* Human interferon-α has antiviral properties through induction of a general antiviral state of cells that protects against virus replication. Two common treatment regimens exist for use of human interferon-α in cats, SQ injection of high-dose ($10^4$-$10^6$ IU/kg q 24 h) or PO application of low-dose (1-50 IU/kg q 24 h). When given SQ in high dosages, interferon-α leads to detectable serum levels. However, it becomes ineffective after 3 to 7 weeks due to development of neutralizing antibodies. If human interferon-α is given PO, is not absorbed but destroyed in the gastrointestinal tract, and no measurable serum levels develop. The only way oral interferon can have an effect is by stimulation of the local lymphoid tissue in the oral cavity. In mice studies, it was shown that subcutaneous administration of interferon-α had an antiviral effect, while oral administration only caused immunomodulation. Treatment of high dosages of human interferon-α subcutaneously ($1.6 \times 10^4$ and $1.6 \times 10^6$ IU/kg SQ) in experimentally FeLV-infected cats resulted in significant decreases in circulating FeLV p27 antigen. In a study of naturally FeLV-infected cats, high-dose subcutaneous treatment with human interferon-α ($10^5$ IU/kg SQ q 24 h for 6 weeks) did not lead to a significant improvement of clinical, laboratory, immunologic, or virologic parameters. In an experimental placebo-controlled study, low-dose oral interferon-α (0.5 IU/cat or 5 IU/cat PO) did not cause a difference in the development of viremia when treatment was started directly after challenge, but treated cats had significantly fewer clinical signs and longer survival times when compared to a placebo group. In a placebo-controlled study including ill client-owned FeLV-infected cats that were treated with low dose PO interferon-α (30 IU/cat q 24 h for 7 consecutive days on a 1-week-on/1-week-off schedule), treatment, however, did not result in a significant difference in FeLV status, survival time, clinical or hematologic parameters, or subjective improvement in the owners' impression.

*Feline interferon-ω:* Feline interferon-ω is licensed in Japan, Australia, and Europe. Interferons are species-

specific; therefore, feline interferon-ω can be used life-long without antibody development. No adverse effects have been reported in cats. In a placebo-controlled field study, 48 cats with FeLV infection were treated with interferon-ω at $10^6$ IU/kg SQ q 24 h on 5 consecutive days. This treatment was performed 3 times with several weeks between treatments. A statistically significant difference was noted in the survival time of treated *versus* untreated cats. No virologic parameters, however, were measured throughout the study to support the hypothesis that the interferon actually had an anti-FeLV effect rather than inhibited secondary infections, and further studies are needed.

*Immunomodulators:* Immunomodulators are widely used medications in FeLV-infected cats. Most of the reports, however, are difficult to interpret due to unclear diagnostic criteria, lack of clinical staging or follow-up, lack of placebo control groups, the natural variability of the course of disease, small numbers of cats used, and additional supportive treatments given. Although reports of uncontrolled studies frequently suggest dramatic clinical improvement, these effects are usually not observed when followed by controlled studies.

***Suggested Reading References***

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2. Levy J, Crawford C, Hartmann K, Hofmann-Lehmann R, Little S, Sundahl E, Thayer V. 2008. J Feline Med Surg. 10: 300-6.
3. Lutz H, Addie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T, Hartmann K, Hosie MJ, Lloret A, Marsilio F, Pennisi MG, Radford AD, Thiry E, Truyen U, Horzinek MC. 2009. J Feline Med Surg 2009; 11: 565-74.

***Other references can be provided by the author on request.***

| **Stages of FeLV infection** | **FeLV p27 antigen in blood** | **FeLV blood culture** | **FeLV RNA in blood** | **Amount of FeLV DNA in blood** | **FeLV anti-bodies** | **FeLV shedding** | **FeLV-associated disease** |
|---|---|---|---|---|---|---|---|
| **Abortive** | Negative | Negative | Negative | Negative | High | No | Not present |
| **Regressive** | Negative | Negative | Negative | Low | High | No | Possible |
| **Progressive** | Positive | Positive | Positive | High | Negative | Yes | Common |