

## ABCD GUIDELINES ON:

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The following recommendations have been formulated by the European Advisory Board on Cat Diseases.



The European Advisory Board on Cat Diseases is an independent panel of 17 veterinarians from ten European countries, with an expertise in immunology, vaccinology and/or feline medicine. The ABCD was set up to compile guidelines for the prevention and management of major feline infectious disease in Europe based on current scientific knowledge and available vaccines.

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## 4. Feline Leukaemia Virus

### 4.1 *Biology of the virus*

#### 4.1.1 **Virus properties**

Feline leukaemia virus (FeLV) is a gamma retrovirus affecting domestic cats worldwide; it was first detected in 1964 by electron microscopy after experimental transmission by cell-free material [Jarrett et al. 1964]. FeLV also infects small wild cats including *Felis silvestris* and European and Iberian lynxes.

All retroviruses, including FeLV, are enveloped RNA viruses and rely on a DNA intermediate for replication. The single-stranded RNA genome is reverse transcribed into DNA, which is usually integrated into the host's cell genome with an integrase [Temin & Mizutani 1970]. The integrated DNA is known as provirus. After reverse transcription, synthesis of viral proteins occurs according to the conventional mechanisms of transcription with assembly of the virions near the cell membrane and budding from the cell [Coffin 1979]. Infection of a cell by a retrovirus does not usually lead to cell death.

The FeLV genome contains three genes: envelope (*env*) coding for the glycoprotein gp70 (SU) and the transmembrane protein p15 (E) (TM), the polymerase (*pol*) gene coding for reverse transcriptase, protease and integrase, and the group specific antigen (*gag*) gene coding for the structural proteins of the virus including p27 [Coffin 1979]. Besides this so-called exogenous FeLV, in the domestic cat two forms of endogenous gamma retroviruses are known: the endogenous feline leukaemia virus (enFeLV) [Soe et al., 1983] and the RD114 virus [Sarma et al., 1973].

The enFeLV is thought to have originated hundred thousands of years ago from cats that had eaten mice viraemic with a murine leukaemia virus (MuLV) which was able to incorporate its genome into the germ line cells of the predator. This MuLV was then inherited by all the offspring. The amount of enFeLV varies between different breeds of cats including *Felis silvestris* suggesting that this exposure to MuLVs is a continuing phenomenon [Tandon et al., 2007]. The enFeLV genome is not complete and therefore it is not competent to replicate by its own [Soe et al., 1983].

The RD114 virus is of primate origin, replication competent, and thought to have originated hundred thousands of years ago from an ancestor cat that had preyed on an early primate

infected with this RD114 virus [Barbacid et al., 1977]. Feline cells are not susceptible to RD114 virus, and this virus has no pathogenic potential for cats.

FeLV exists in four subtypes: A, B, C, and T [Anderson et al., 2000; Russell & Jarrett 1978]. The subtypes are defined by host cell spectrum; they are immunologically closely related. The subtype A is ubiquitous and is involved in every infection. Subtype B originates from recombination of FeLV A with enFeLV. Subtype C is the result of mutations in the env gene, and subtype T has a tropism for T lymphocytes.

FeLV does not survive for long outside the host as it is destroyed readily by disinfectants, soap, heating and drying. Transmission via fomites is unlikely. The virus will survive, however, if it is kept moist at room temperature so that there is potential for iatrogenic transmission to occur via contaminated needles, surgical instruments or blood transfusions.

#### **4.1.2 Epidemiology**

FeLV occurs worldwide. Its prevalence may be influenced by the density of cats and there may be noticeable geographical and local variation. There is little reliable information on the current prevalence of FeLV in different countries. In some European countries, the USA and Canada, the prevalence of FeLV infection in individually kept cats seems to be very low, usually less than 1 % [Hosie et al., 1989; Levy et al., 2006; Lutz et al., 1990]. In large multi-cat households without specific preventive measures for introduction of FeLV, the prevalence may be greater than 20 %.

Over the last 25 years, the prevalence and importance of FeLV infection in Europe has greatly diminished due to the availability of reliable tests, the test and removal programmes initiated, improved understanding of the pathogenesis and the introduction of highly efficacious FeLV vaccines.

Cats with FeLV viraemia act as a source of infection. Virus is shed from an infected cat in saliva, nasal secretions, faeces, and milk [Hardy et al., 1976; Pacitti et al., 1986]. Risk factors for infection are young age, high population density and poor hygiene. FeLV infection is transmitted mainly by mutual grooming, but also through bites. In viraemic queens, pregnancy usually results in embryonic death, stillbirth or in viraemic kittens which fade away rapidly. In latently infected queens, usually transmission does not take place during pregnancy. However rarely, some (but not all) kittens may become viraemic after birth [Pacitti et al., 1986]. In these instances, transmission takes place from individual mammary glands where the virus can remain latent until the mammary gland develops during the last

period of pregnancy. Young kittens are especially susceptible to FeLV infection while with age, cats become increasingly resistant to infection [Hoover et al., 1976; Grant et al. 1980]. Although aged cats are generally accepted to be more resistant to infection, they can still be infected providing the challenge is sufficiently severe.

## **4.2 Pathogenesis**

In most cases, infection starts in the oropharynx where FeLV infects individual lymphocytes that are transported to the bone marrow. Once the rapidly dividing bone marrow cells become infected, large amounts of virions are produced and as a consequence viraemia develops within a few weeks of infection. Often, viraemia may develop several months after constant exposure to shedding cats [Lutz et al., 1983b]. Viraemia leads to the infection of salivary glands and intestinal linings, and virus is shed in large quantities in saliva and faeces [Rojko et al., 1979].

Frequently, the development of viraemia as well as established viraemia may be overcome by a functioning immune system (transient viraemia) [Lutz et al., 1980a]. Such cats (so-called “regressor” cats) are generally not at risk of developing disease. In a multicat household without control of FeLV infection, 30-40 % of the cats develop persistent viraemia, 30-40 % exhibit transient viraemia and 20-30 % seroconvert without ever being detectably viraemic. A smaller proportion (~5 %) exhibits an atypical course of infection showing antigenaemia but no viraemia [Hoover et al 1977]. A cat that has overcome viraemia remains latently infected, i.e. from some cells that remain provirus-positive infectious virus can be recovered when for instance bone marrow cells are kept in cell culture for several weeks [Rojko et al., 1982]. Reactivation may also take place in vivo when latently infected cats experience immune suppression or chronic severe stress [Boretti et al., 2004]. It is not clear how often this happens but it is generally believed to be a rare occurrence.

Generally, up to 10 % of all feline blood samples submitted to a laboratory prove to be provirus-positive and p27 negative, and as in some of these cats, FeLV may be reactivated, they should be considered latently infected [Boretti et al., 2004]. It appears likely that no cat can completely clear FeLV infection from all cells. This might explain why virus neutralising antibodies persist in recovered cats for many years in the absence of overt infection, or exposure to viraemic cats. If this is the case, the risk of such latent persistence leading to re-excretion of virus or the development of disease, must be extremely low since recovered cats appear to have the same life expectancy as cats that have never been exposed to FeLV. However, proviral DNA has been found in the tumours of ostensibly FeLV-free cats [Jackson

et al., 1993], suggesting that the virus might be involved in an early event in the pathogenesis of the tumour and then persist only as a provirus, possibly in a defective form. Local foci of infections or latent virus may also be the source of the FeLV p27 antigen that is sometimes found in the plasma of cats from which infectious virus cannot be isolated, the so-called ‘discordant’ cats.

The typical clinical signs of FeLV infection usually develop in viraemic cats, sometimes not until after several years of viraemia [Hardy et al., 1976].

### **4.3 Immunity**

#### **4.3.1 Passive immunity**

Experimentally, it has been demonstrated that susceptible kittens can be protected from FeLV infection following passive immunisation with high titred antisera against FeLV [Hoover et al., 1977]. Once persistent viraemia has become established, treatment with virus neutralizing monoclonal antibodies to FeLV is ineffective [Weijer et al., 1986].

#### **4.3.2 Active immune response to FeLV**

Most cats that overcome FeLV viraemia exhibit high antibody titres to the virus (ELISA or VN) [Lutz et al., 1980a; Russel & Jarrett, 1978]; antibodies are directed against all components of the virus [Lutz et al., 1980a]. In most – but not all – cats that overcame viraemia, virus neutralising antibodies can be detected [Flynn et al., 2002]. Since not all immune cats develop high antibody titres, it was concluded that cytotoxic T-lymphocytes (CTLs) are also important in FeLV immunity [Lutz et al., 1980a]. Indeed, it was demonstrated recently that CTLs specific for FeLV appear before virus neutralising antibodies and that following adoptive transfer of FeLV specific CTLs stimulated *in vitro*, the viral load in FeLV viraemic cats could be lowered, consistent with an important role for CTLs in FeLV immunity [Flynn et al., 2002].

### **4.4 Clinical signs**

FeLV infection can cause variable and multiple clinical signs. The most common disease consequences of persistent FeLV viraemia are: immune suppression, anaemia, and lymphoma [Hardy et al., 1976; Hardy et al., 1973].

The prognosis for persistently FeLV viraemic cats is poor and most will develop an FeLV related disease. 70-90 % of these cats will be dead within 18 months to three years [Hardy et

al., 1976]. Some persistently viraemic cats may remain healthy for a prolonged period (many years) before FeLV related disease develops and occasional cases remain healthy indefinitely [Hofmann-Lehmann et al., 1995].

Age of the cat at the time of the infection is the most important factor determining the clinical outcome [Hoover et al., 1976]. Viral and host factors, including the virus subgroup and cell-mediated immune response, influence the pathogenesis of infection within individual infected cats.

#### **4.4.1 Immune suppression**

Immune suppression in FeLV is more complex and severe than the more selective one caused by FIV infection. Several abnormalities have been reported including thymic atrophy, lymphopenia, neutropenia, neutrophil function abnormalities, loss of CD4+, and more importantly loss of CD8+ [Ogilvie et al., 1988].

Irrespective of whether recognisable clinical signs are present or not, every FeLV-viraemic cat is immune suppressed [Orosz et al., 1985a; Orosz et al., 1985b; Perryman et al., 1972], with delayed and decreased primary and secondary antibody responses. The immune suppression can have many clinical consequences and may lead to infection with other primary infectious agents to which cats would be normally resistant, such as *Salmonella* spp. In addition, there may be exacerbation of disease caused by other pathogens, such as pox virus, *Mycoplasma haemofelis* and *Cryptococcus*, and infections normally not pathogenic in cats, e.g. due to *Toxoplasma gondii*. Concurrent FeLV infection may also predispose to chronic refractory disease such as stomatitis and chronic rhinitis [Knowles et al., 1989; Tenorio et al., 1991]. Some clinical problems such as chronic rhinitis and subcutaneous abscesses may take much longer to resolve in FeLV-infected cats and unexpected recurrences may arise.

#### **4.4.2 Anaemia**

FeLV-infected cats may develop many different types of anaemia, which are mainly non-regenerative and rarely regenerative. Regenerative anaemias, associated with haemolysis may be related to secondary opportunistic infections, for example by *Mycoplasma haemofelis*, or to immune-mediated destruction [Scott et al., 1973; Kociba, 1986]. FeLV-C can interfere with a haem transport protein [Cotter, 1979; Quigley et al., 2000], which directly results in a non-regenerative anaemia. Non-regenerative anaemias may be caused by chronic inflammatory mechanisms, myelodestruction, myelosuppression (either pancytopenia or pure erythrocyte

aplasia) and myeloproliferative disease. Other cytopenias may be present, in particular thrombocytopenia and neutropenia, probably caused by virus-induced immune-mediated mechanisms and myelosuppression.

#### **4.4.3 Lymphoma**

FeLV may cause different tumours in cats, mainly lymphoma and leukaemia, but also other non-haematopoietic malignancies. FeLV-induced lymphomas are among the most frequent tumour forms of the cat; myeloproliferative disorders are less common and not always associated with FeLV infection [Francis et al., 1979a; Louwerens et al., 2005].

Different forms of lymphoma have been classified according to its most frequent anatomic location:

- The thymic or mediastinal form;
- The alimentary form, where tumour cells are associated with organs of the digestive tract;
- The multicentric or peripheral form, which affects lymph nodes;
- The atypical or extranodal form, presenting with solitary tumours in kidneys, CNS, or skin;

In some cases, lymphoma is disseminated with multiple organ and site involvement. [Hardy et al., 1970; Reinacher & Theilen 1987]. Liver, spleen, bone marrow, blood and/or non-lymphoid organ involvement are associated with a poor prognosis [Vail & Thamm, 2005]

It is also possible for cats to develop some forms of lymphoma with no known or detectable association with FeLV infection, which carries a better prognosis [Vail & Thamm, 2005].

Different types of acute leukaemia have been described depending on the neoplastic transformed cell type.

Multiple fibrosarcomas in young viraemic cats have occasionally been associated with infection with FeSV (feline sarcoma virus), a recombinant virus developing from recombination of the FeLV-A genome with cellular oncogenes [Hardy, 1981; Donner et al., 1982; Besmer, 1983]. However, solitary fibrosarcomas or feline injection site sarcomas are related to neither FeLV nor FeSV infection.

#### **4.4.4 Other diseases linked to FeLV**

Immune-mediated diseases associated to FeLV infection have been reported, including haemolytic anaemia, glomerulonephritis and polyarthritis. Antigen-antibody complex

deposition and loss of T-suppressor activity may be the main factors contributing to immune-mediated diseases.

Benign peripheral lymphadenopathy has been diagnosed in FeLV-infected cats [Moore et al., 1986]; a clinical picture with potential to be mistaken as peripheral lymphoma.

Chronic enteritis associated with degeneration of intestinal epithelial cells and crypt necrosis has been associated with FeLV-infection in cats in which virus is present in intestinal crypt cells [Reinacher, 1987]. Inflammatory and degenerative liver disease has also been described associated with FeLV infection [Reinacher, 1989].

Reproductive disorders and fading kitten syndrome have been also reported. Foetal resorption, abortion and neonatal death are the main manifestations [Hardy et al., 1981]. Fading kittens and other reproductive disorders are rarely observed these days, largely as a result of the very low prevalence of infection in pedigree breeding cats, achieved by routine testing.

Neurological disease not associated to CNS lymphoma or opportunistic CNS infections has been described, mainly peripheral neuropathies presenting as anisocoria, mydriasis, Horner's syndrome, urinary incontinence, abnormal vocalization, hyperesthesia, paresis and paralysis [Haffer et al., 1987]. Neuropathogenicity has been investigated as a possible direct effect of the virus [Dow & Hoover, 1992].

## **4.5 Diagnosis**

### **4.5.1 Direct detection methods**

#### **4.5.1.1 ELISA (p27)**

The first p27 ELISA tests were based on polyclonal antibodies; such tests had the advantage of allowing quantitation of p27 but had a tendency to produce false-positive results as the antibodies did not detect only viral proteins but occasionally also non-viral components [Lutz et al., 1980b; Lutz et al., 1980c]. Improved ELISA tests based on monoclonal antibodies to p27 were introduced later to detect p27 capsid protein of exogenous FeLV present in blood or serum [Lutz et al., 1983a; Lutz et al., 1983b]. This assay utilizes a single monoclonal antibody specific for an epitope (A) of p27 fixed to a solid phase. The serum sample to be tested is mixed with one or two additional monoclonal antibodies specific for epitopes B and C of p27, and the mixture is then added to the solid phase. Hence the presence of p27 leads to insolubilisation of the enzyme-conjugated antibodies and the resulting colour change is

indicative for the presence of p27, a marker of infection (but not always of viraemia as soluble p27 may be detected in the absence of infectious virus). ELISA procedures have the advantage of high diagnostic sensitivity and specificity – which, however, depends on the gold standard used for comparison.

In a field study in which the gold standard was proviral PCR, the diagnostic sensitivity was found to be 90 %, i.e. about 10 % of all 597 cats tested and found to be PCR positive were not recognized by p27 ELISA due to the fact that they are not antigenaemic; the specificity was very close to 100 % in that none of the p27 positive samples turned out to be PCR-negative (Hofmann et al. 2001). If the gold standard is virus isolation, the diagnostic sensitivity is in the range of 90 % and the diagnostic specificity of >98 % [Hartmann et al. 2001].

#### 4.5.1.2 Immune chromatography

These tests are based on the same principle as the ELISA but small beads less than one micron in size are coated to the revealing antibodies rather than enzymes. The diagnostic sensitivity and specificity of immune chromatography tests was shown to be comparable to those of the ELISA [Hartmann et al., 2007; Hartmann et al., 2001; Pinches et al., 2007; Robinson et al., 1998].

#### 4.5.1.3 Immunofluorescent assay (IFA)

The first method that allowed FeLV detection in viraemic cats under field conditions was the indirect immunofluorescent assay (IFA), introduced in 1973 [Hardy et al., 1973]. It was based on the observation that granulocytes, lymphocytes, and platelets in viraemic cats contain gag components, which may be detected by IFA in blood smears. The diagnostic sensitivity of IFA compared to virus isolation as the gold standard is significantly lower than 100 %; but positive cats are usually persistently viraemic [Hawks et al., 1991]. If a viraemic cat has leukopenia or if only a small percentage of peripheral leukocytes are infected, the presence of FeLV infection may be overlooked using IFA tests. Furthermore, all eosinophils have a tendency to bind the FITC conjugates used for IFA resulting in false positive tests if slides are not read carefully [Floyd et al., 1983].

#### 4.5.1.4 Virus isolation

Virus isolation in cell culture has been considered to be the ultimate criterion for FeLV infection. [Jarrett 1980; Jarrett et al., 1982]. Indeed, in the early phase of infection, detection of infectious FeLV is often the most sensitive parameter (Lehmann et al. 1991). In view of difficult logistics, this test is no longer considered for routine testing.

#### **4.5.1.5 PCR for the detections of provirus (DNA PCR)**

Since every cat cell carries between 12 and 15 copies of endogenous FeLV, it proved to be somewhat difficult to determine sequences that allowed only detection of exogenous provirus [Jackson et al., 1996]. The value of PCR techniques was greatly enhanced by the development of real-time PCR that not only allows detection but also quantitation of FeLV provirus [Hofmann-Lehmann et al., 2001]. PCR procedures have the highest analytical and diagnostic sensitivity and – provided the laboratory tests are run with all precautions of clean work and separated labs and with all necessary controls under conditions defined by good laboratory practice – also a very high specificity.

PCR for the detection of provirus may be useful for the clarification of inconclusive p27 antigen test (see chapter section 4.5.3).

#### **4.5.1.6 PCR for the detection of viral RNA**

The detection of viral RNA added a new aspect to the diagnosis of FeLV infection [Tandon et al., 2005]. Using this test, viral RNA present in whole blood, serum, plasma, saliva or faeces, is extracted, reverse transcribed into a cDNA, which is then amplified by real-time PCR. This technique permits the detection and quantitation of virus in the absence of cells. RNA PCR does not provide the same information as DNA provirus PCR. Many cats that have overcome FeLV viraemia remain provirus positive but do not produce detectable viral RNA in plasma, saliva or faeces [Gomes-Keller et al., 2006a]. However, detection of viral RNA is a reliable parameter of viraemia.

In most situations, cats are tested for FeLV individually. However, in circumstances where the cost of testing is a limitation, it is possible to use the RNA PCR test to screen pooled saliva samples, as the test is sufficiently sensitive to detect a single infected cat in up to 30 pooled samples. This approach may be advantageous when screening multicat households [Gomes-Keller et al., 2006b].

### **4.5.2 Indirect detection methods**

Although it is possible to measure antibodies against FeLV, the results are difficult to interpret because many cats develop antibodies to their own endogenous FeLV. Therefore such tests are currently of little clinical value. In some research laboratories, the so-called FOCMA (feline oncornavirus associated cell membrane antigen) test was used to detect antibodies to what was believed to be a tumour-associated antigen. It was later found that FOCMA was indeed a combination of several viral components; as this test is difficult to

establish and to standardise, is not considered to be of clinical value. There are tests for virus neutralising antibodies but these are not widely available (mainly restricted to the UK) and are used only infrequently.

### **4.5.3 Test interpretation**

The first test that becomes positive after FeLV infection is usually virus isolation, followed within a few days by DNA and RNA PCR, ELISA, and later by IFA [Hofmann-Lehmann et al 2006]. Persistently viraemic cats are usually positive by all tests.

The most widely used tests for the diagnosis of FeLV infection in practice are antigen ELISA and immunochromatography. As the prevalence of FeLV infection seems to be decreasing in many European countries, there is a tendency for increasing false positive results. Therefore a doubtful positive result in a healthy cat should always be confirmed, preferably using provirus PCR (DNA PCR) offered by a reliable laboratory. A positive test in a cat with clinical signs consistent with FeLV infection is more reliable as in such cats the prevalence of FeLV is likely to be considerably higher.

Cats testing positive may overcome viraemia after two to sixteen weeks or in rare cases even longer. Therefore every positive cat without clinical signs should be separated and retested after several weeks or months; depending on discussions with the owner, retesting can occur at later time points up to one year when there is only a very small possibility that the cat will clear viraemia.

Cats that clear infectious virus from the plasma will be negative by VI, ELISA, immunochromatography, IFA, and RNA PCR, but remain positive by DNA PCR [Gomes-Keller et al., 2006a]. These cats should be considered latently infected, although the clinical significance is low in most cats. However, in rare instances, chronic stress, immune suppression or co-infection with other viruses may lead to reactivation in these cats. The mean proviral load in cats that overcome viraemia is several hundred times lower than in cats with persistent viraemia. A small proportion (2-3 %) of cats remain positive by ELISA and immunochromatography although no infectious virus can be isolated from the plasma. These cats have foci of infection outside the bone marrow from which soluble p27 is released into the circulation and such cats should be considered as potential sources of infection [Lutz et al., 1980c].

In summary, cats can be initially tested for p27. If the result is inconclusive for any reason, the test should be repeated by a qualified laboratory, using an alternate format, preferably PCR for provirus.

## **4.6 FeLV infection management**

### **4.6.1 General management**

FeLV-infected cats should be confined strictly indoors to prevent spread to other cats in the neighbourhood. There may also be benefits in preventing exposure of the immune-suppressed retrovirus-infected cat to infectious agents carried by other animals. This is true in the home environment as well as in the veterinary hospital. Although they can be housed in the same ward as other hospitalized patients, they should be housed in individual cages. It should be considered that they may be immune-suppressed and should be kept away from cats with other infectious diseases. They should not be placed in a "contagious ward" with cats suffering from infections such as viral respiratory disease.

The management of the cat should be adjusted to minimise potential exposure to other infectious agents. As well as confining the cat indoors it may be prudent to avoid feeding uncooked meat, which may pose a risk of bacterial or parasitic infections to which FeLV-positive cats are more susceptible.

Asymptomatic FeLV-infected cats should receive clinical check-ups ideally at least every six months. A complete blood count (CBC), biochemistry profiles and urinalyses should be performed periodically, ideally every six to twelve months.

Intact male and female retrovirus-infected cats should be neutered to minimize the risk of virus transmission and for health benefits. Surgery is generally well tolerated by asymptomatic FeLV-infected cats. The virus is infectious only for a short while outside the host [Francis et al., 1979b], and is sensitive to all disinfectants including common soap; simple precautions and routine cleaning procedures will prevent transmission in the hospital.

Routine vaccination in FeLV-infected cats is subject of discussion. Vaccination programmes to prevent common infectious diseases should be maintained in FeLV-infected cats, although it has been demonstrated that FeLV-infected cats may not be able to mount adequate immune response to rabies vaccination [Franchini, 1990]. Therefore, protection in a FeLV-infected cat after vaccination may not be comparable to that in a healthy, uninfected cat and so if cats are allowed to go outside – which is not recommended, certainly never in rabies-endemic areas -

more frequent vaccination may need to be considered. Inactivated vaccines are recommended whenever available as in immune-suppressed cats, modified live virus vaccines may retain some pathogenic potential and cause clinical disease.

## **4.6.2 Treatment**

### **4.6.2.1 Supportive treatment**

If FeLV-infected cats are sick, prompt and accurate identification of specific diseases affecting the cat is important to allow early therapeutic intervention and a successful outcome of treatment. Therefore, more intensive diagnostic testing should proceed earlier in the course of illness than might be recommended for uninfected cats. Many cats with retrovirus infection respond well to appropriate medications although a longer or more aggressive course of therapy (e.g., antibiotics) may be needed than in retrovirus-negative cats. Corticosteroids, other immune-suppressive or bone marrow-suppressive drugs should generally be avoided, unless used as a treatment of FeLV-associated malignancies or immune-mediated disease.

Good veterinary care is important for FeLV viraemic cats. Many may need fluid therapy. Some specific disease complications of FeLV infection may respond to treatment, such as secondary bacterial infections, especially with *Mycoplasma haemofelis* which often responds to doxycycline. If stomatitis/gingivitis is present, corticosteroids should be considered to increase the food intake. Blood transfusions may be useful in anaemic cats and in the case of leukopenia, granulocyte colony-stimulating factor (G-CSF) can be considered [Fulton et al., 1991]. Treatment regimes for lymphomas, particularly based on chemotherapeutic drugs, are now well established. Some cases of lymphoma respond well to chemotherapy with remission expected in most cases and some cats showing no recurrence within two years. Chemotherapy of FeLV positive lymphomas will not resolve the persistent viraemia and the outlook for such cats is not good [Ettinger, 2003].

### **4.6.2.2 Immune-modulators**

There is little evidence from controlled studies to support the efficacy of immune modulators on the health or longevity of FeLV-infected cats.

Nevertheless, it has been suggested that some of these agents may benefit infected animals by restoring compromised immune function, thereby allowing the patient to control its viral burden and recover from the disease. Although reports of uncontrolled studies frequently suggest dramatic clinical improvement (e.g., when using so-called “paramunity inducers”); these effects were not observed, in subsequent controlled studies [Hartmann et al., 1998].

Staphylococcus Protein A, SPA, is a bacterial polypeptide purified from cell walls of *Staphylococcus aureus* Cowan I that acts as an immune modulator. In a placebo-controlled study, treatment of ill, client-owned FeLV-infected cats with Staphylococcus Protein A (10 µg/kg twice per week for up to ten weeks) did not cause a statistically significant difference in FeLV status. However, it did result in a significant improvement in the owners' subjective impression of the health of their pets [McCaw et al., 2001].

#### 4.6.2.3 Antivirals

Occasionally, antiviral drugs are used; however, their efficacy is limited and many of these compounds have severe side effects in cats [Hartmann, 2006]. There are only a few controlled studies that have demonstrated some effect of a few drugs in FeLV-infected cats.

Treatment of FeLV viraemia with feline interferon omega (interferon-ω) was shown to significantly improve clinical signs and to extend the survival time of FeLV viraemic cats, although it did not lead to reversion of viraemia [de Mari et al., 2004]. Feline interferon-ω inhibits FeLV replication *in vitro*. In a placebo-controlled field study, 48 cats with FeLV infection were treated with interferon-ω (10<sup>6</sup> IU/kg SQ q24h on five consecutive days repeated three times with several weeks between treatments) [de Mari et al., 2004]. A statistically significant difference was found in the survival time of treated versus untreated cats. No viral 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.

An antiviral compound routinely used is 3'-azido-2',3'-dideoxythymidine (AZT), a nucleoside analogue (thymidine derivative) that blocks the reverse transcriptase of retroviruses. It has been shown that AZT effectively inhibits FeLV replication *in vitro* and *in vivo* in experimental infections. It can reduce plasma virus load, improve the immunological and clinical status, increase quality of life, and prolong life expectancy in some FeLV-infected cats. It should be used at a dosage of 5 - 10 mg/kg q12h PO or SC. The higher dose should be used carefully in FeLV-infected cats as side effects (e.g., non-regenerative anaemia) can develop [Hartmann, 2005].

### 4.7 **Vaccination**

After several experimental vaccines had been described [Jarrett et al., 1975; Jarrett et al., 1974; Pedersen et al., 1979], the first FeLV vaccine in the field was introduced in the USA in

1984. This vaccine was based on conventionally prepared FeLV antigens, and it protected cats from FeLV viraemia [Lewis et al., 1981].

A number of FeLV vaccines are now available in Europe. Some of these are based on new developments in recombinant DNA technology. One such vaccine consists of the viral envelope glycoprotein as well as part of the transmembrane protein expressed in *E. coli* [Kensil et al., 1991], and this was the first genetically engineered small animal vaccine. The most recent FeLV vaccine uses a canarypox virus vector that carries the genes for the envelope glycoprotein and the capsid protein [Tartaglia et al., 1993]. There is a single round of replication by the vector virus following vaccination, resulting in the expression of the inserted FeLV genes. In contrast to other cat vaccines, neutralising antibodies do not develop following vaccination with this product. The protective effect is achieved by stimulating cellular immunity which leads to rapid development of neutralising antibodies if vaccinated cats encounter field virus [Hofmann-Lehmann et al., 2006; Lehmann et al., 1991].

The differences between the various brands of FeLV vaccines are considered to be more significant than those for other feline infectious diseases, and there is evidence that this is reflected in differences in performance, particularly related to efficacy of protection. Comparison of the results of vaccine efficacy studies can be misleading because of differences in the protocols used – such as the route of challenge, the challenge strain used and the criteria for defining protection [Sparkes, 2003]. Different studies on the same vaccine have sometimes led to contrasting results. The first FeLV vaccine and some other vaccines, which have now been withdrawn from the market, have performed very badly in some independent vaccine efficacy studies indicating poor protection.

The European Pharmacopoeia defines certain criteria for assessing the efficacy of protection achieved by FeLV vaccines. This takes into account the difficulty in infecting some healthy control cats with a single experimental challenge and the criteria include a minimum acceptable infectivity rate in controls to confirm that an acceptably strong challenge has been provided. The “natural resistance” of some of the control cats is taken into account in calculating the level of protection achieved by vaccination and this is expressed as the preventable fraction [Scarlett & Pollock, 1991].

Some protocols for studies for assessing vaccine efficacy have been developed based on a “natural” challenge of FeLV – by co-mingling viraemic “challenge” cats with trial cats. Although these protocols are not in agreement with the European Pharmacopoeia, they take account of the natural mode of transmission of FeLV which is generally based not on a single

large exposure but chronic exposure over a period of time, usually through cohabiting of infected viraemic cats with susceptible cats, and clinical experts regard this as providing a more realistic indication of the efficacy of protection vaccines are likely to provide in the field.

No FeLV vaccine is likely to provide 100 % efficacy of protection and none prevent infection [Hofmann-Lehmann et al., 2007]. Recent studies have demonstrated that cats that are able to overcome p27 antigenaemia without exception become provirus positive in the blood and also positive for viral RNA in plasma, although at very low levels compared with persistently viraemic cats [Hofmann-Lehmann et al., 2007]. These experiments confirm that FeLV vaccination neither induces sterilising immunity nor protects from infection.

Long term observation of vaccinated cats following experimental challenge indicates that low level RNA viraemia and persistence of low levels of proviral DNA can be considered as not clinically significant and these cats can be regarded as “protected”.

In most circumstances FeLV should be included in the routine vaccination programme for pet cats. It provides good protection against a potentially life-threatening infection and the benefit for most cats considerably outweigh any small risk of serious adverse effects. In situations where the possibility of future exposure to FeLV can be discounted, vaccination is not required. Geographical variations of the prevalence of FeLV may influence the decision whether or not to vaccinate against FeLV. In some European countries FeLV has largely been eradicated and there may be important local variations in the prevalence of FeLV within countries where the virus is still a significant health issue that may be taken into account. The circumstances of individual cats may also be a factor and if it can be assured that a cat will not be exposed to FeLV, vaccination is unnecessary.

However, owners’ circumstances may change which may influence their cats’ lifestyle and lead to potential exposure in a cat that was previously at no risk of encountering FeLV, particularly when moving house. This possibility should be considered especially in kittens presented for primary vaccination.

#### **4.7.1 Primary vaccination**

Vaccination should be carried out in all cats that have a potential risk of exposure. In such cases it is recommended that kittens be vaccinated at the age of 8 or 9 weeks and 12 weeks together with core vaccinations. [Brunner et al., 2006]. As the combination of different

immunogens within one syringe is only legal when the company has registered it for the country of interest; the local veterinary regulations should be carefully consulted.

If the FeLV status is unknown, any cat should be tested for presence of FeLV antigenaemia prior to vaccination in order to avoid "vaccine failures", which are likely when cats infected prior to vaccination develop FeLV-related clinical signs. If FeLV infection prior to vaccination is unlikely, testing may not be needed (e.g. kittens from a FeLV negative mother and father which had no contact with other cats).

#### **4.7.2 Booster vaccinations**

No data have been published to support a duration of immunity (DOI) longer than 1 year after primo-vaccination. Therefore, most vaccine producers recommend annual boosters. However, considering the significant lower susceptibility of adult cats to FeLV infection, the ABCD suggests that in cats older than 3-4 years, a booster every two to three years would be sufficient.

### **4.8 *FeLV control in specific situations***

#### **4.8.1 Multi-cat households**

If a cat is diagnosed with FeLV in a multi-cat household, all cats in that household should be tested to determine their status. If other positive cats are identified in the same household the test and removal system should be applied. That involves periodic testing of cats and removal of positive cats until all of the cats test negative. The best method of preventing spread of infection is to isolate the infected individuals and to prevent interaction with uninfected housemates. Although protection conferred by FeLV vaccines is very good in most situations, the ABCD panel does not recommend reliance solely on vaccination to protect negative cats living together with FeLV positive cats.

#### **4.8.2 Shelters**

There appears to be marked geographical differences in the prevalence of FeLV in rescue cats in Europe. The prevalence may influence policies on testing and vaccination. In some countries the prevalence is very low (e.g. the UK) whilst in other countries the prevalence is noticeably higher, although regional differences may exist within countries.

Sick shelter cats which are confirmed as FeLV positive should be euthanised. Some rescue shelters are successful in adopting confirmed FeLV-positive, healthy cats to selected

households, but there is a strong responsibility to ensure that such cats do not pose any risk as a potential source of infection to uninfected cats. This may require positive cats being rehomed to households where they will live in isolation or just with other infected cats.

The possibility of transmission of FeLV between cats within a shelter should be avoided. Ideally, cats are housed individually to avoid disease transmission. If cats are housed in groups, they should be tested and positive and negative cats should be segregated. Vaccination may be considered.

### **4.8.3 Breeding catteries**

The prevalence of FeLV infection is now very low in pedigree breeding catteries in some European countries, largely as a result of routine testing and removal of positive cats. It is recommended that routine testing is maintained once or twice a year in such catteries. Contact should be limited to other cats from establishments that implement a similar routine screening programme. If any cats are allowed access outside and the opportunity of contact with neighbouring cats of uncertain FeLV status, (discouraged for pedigree breeding cats) they should be vaccinated.

### **4.8.4 Vaccination of immunocompromised cats**

#### ***4.8.4.1 FeLV-infected cats***

The vaccination of FeLV-positive cats against FeLV is of no benefit whatsoever.

#### ***4.8.4.2 FIV-infected cats***

In a long-term study where FIV infected cats were experimentally vaccinated against FeLV infection, FeLV vaccinated cats benefited greatly from vaccination compared with the non-vaccinated cats [Hofmann-Lehmann et al., 1995]. From these observations it is concluded that also under field conditions immune compromised cats with FIV infection should be vaccinated against FeLV infection, but only if they are at risk (indoor-only FIV-positive cats should not be vaccinated against FeLV). As the immune response in immunocompromised cats is decreased, more frequent boosters may be considered (in asymptomatic cats).

#### ***4.8.4.3 Other immunocompromised cats, cats with chronic disease***

There is general agreement that cats with acute illness should not be vaccinated but cats with chronic illness such as renal disease, diabetes mellitus or hyperthyroidism should be vaccinated regularly if they are at risk of infection.

#### 4.8.4.4 Cats receiving corticosteroids or other immunosuppressive drugs

Vaccination should be considered carefully in these cats. Depending on the dosage and duration of treatment, corticosteroids may suppress the immune response, particularly the cell-mediated immune response. Concurrent use of corticosteroids at the time of vaccination should therefore be avoided.

## 4.9 References

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