## FELINE LEUKAEMIA VIRUS INFECTION – ABCD recommendations and review of the literature

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## SYNOPSIS

Feline leukaemia virus (FeLV), a gammaretrovirus of domestic cats, is a member of the Orthoretrovirinae subfamily of retroviruses. It contains a protein core with single-stranded RNA protected by an envelope. FeLV does not survive for long outside the host under dry conditions and is readily inactivated by disinfectants, soap, heating and drying. Although transmission via fomites is very unlikely, FeLV will retain infectivity and significant amounts of virus can survive for at least 48 hours if kept moist at room temperature.

Infections occur worldwide in domestic cats and some closely related wild felids. The prevalence of FeLV in Europe has decreased in many countries thanks to reliable tests, programmes to segregate progressively infected cats, understanding of FeLV pathogenesis and the introduction of effective vaccines. In some countries, however, FeLV prevalence rates up to 8.8% were reported in a pan-European FeLV study, particularly in southern European countries. In addition, stagnation of the decrease in prevalence has been recognized in some geographic areas and increased awareness will be necessary to further decrease the prevalence and impact of FeLV infection.

Progressively infected cats are the main source of infection; virus is shed in particularly high amounts in saliva, but also to some degree in nasal secretions, faeces, and milk. Risk factors for FeLV infection are mixed-breed, free ranging, male intact sex, living in multi-cat environments with five or more cats, originating from geographic areas with a high FeLV prevalence or from environments with progressively infected cats and lack of FeLV vaccination. Transmission occurs mainly via saliva through friendly contacts, like grooming, but also via aggressive interaction, i.e. biting, and less frequently by sharing food bowls or litter boxes. Transplacental transmission and transmission through milk can occur. Transmission through blood transfusion is also possible. Blood donors need to be tested for FeLV provirus; antigen testing is not sufficient. The cat's age at the time of the virus exposure is the most important determinant of the susceptibility for progressive infection and clinical outcome, with kittens being most susceptible.

Common FeLV-associated diseases associated with progressive infection are tumours (particularly lymphoma), bone marrow suppression (e.g. anaemia), and immunosuppression, leading to chronic or recurrent infections. Cats with progressive FeLV infection have a decreased life expectancy, but they can be asymptomatic and have a good quality of life for many years. Cats with progressive FeLV infection and FeLV-associated lymphoma or bone marrow suppression have a grave prognosis. Strict indoors-only lifestyle is the most important life-prolonging advice for progressively FeLV-infected cats to reduce exposure of the FeLV-infected cat to other infections. At the same time, it prevents contagion with FeLV to other cats. Cats with regressive FeLV infection should not be exposed to stress to avoid viral reactivation.

Identifying FeLV-infected cats is, together with vaccination, the mainstay of preventing further transmission. The FeLV status of every cat should be known because FeLV infection affects long-term management, which should differ from that of uninfected cats. All cats at risk of exposure should be vaccinated, kittens at the age of 8 or 9 weeks and again at 12 weeks. Prior to vaccination, cats should be tested for FeLV antigenemia and, preferably, also for FeLV provirus to avoid unnecessarily vaccinating FeLV-infected cats since vaccination affords no benefits to such cats.

## AGENT PROPERTIES

Feline leukaemia virus (FeLV) is gammaretrovirus of domestic cats that belongs to the Orthoretrovirinae subfamily of retroviruses. It was first described in 1964 by William Jarrett and co-workers, when virus particles were seen by electron microscopy budding from the membrane of malignant lymphoblasts (Fig. 1) from a cat with naturally occurring lymphoma (Jarrett et al., 1964; Jarrett and Russel 1978; Willett and Hosie 2013). FeLV also infects small wild cats including European wild cats (*Felis silvestris silvestris*) and European and Iberian lynxes (*Lynx pardinus*), Florida panthers (*Puma concolor coryi*), the Chilean wildcat (*Leopardus Guigna*) and Jaguarundis (*Puma yagouaroundi*) of Central and South America (Leutenegger et al., 1999; Cunningham et al., 2008; Meli et al., 2009; Filoni et al., 2012; Mora et al., 2015; Silva et al., 2016).

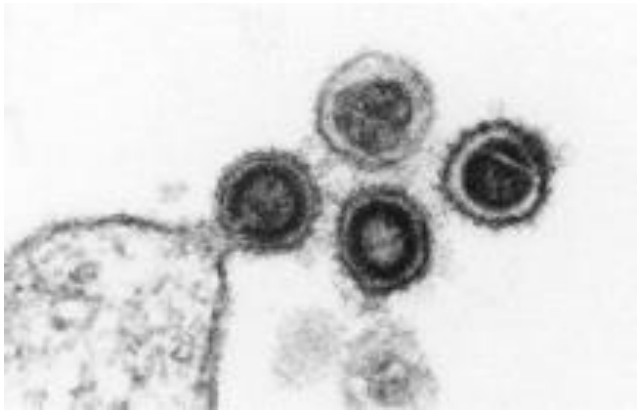

Fig. 1. Thin section electron micrograph of retrovirus particles (Courtesy of H. Lutz, Zurich, Switzerland; originally provided to him by Robert Munn, Davis, CA, USA.)

FeLV particles contains a protein core with single-stranded RNA protected by an envelope (Fig. 1 and Fig. 2). FeLV is an exogenous agent that replicates within many tissues, including bone marrow, salivary glands, and respiratory epithelium. If the immune response does not intervene after initial infection, FeLV spreads to the bone marrow and infects haematopoietic precursor cells.

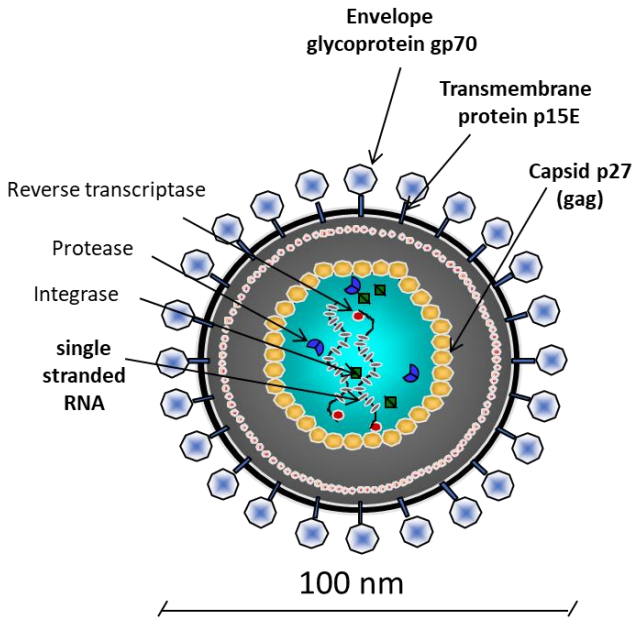


Fig. 2: FeLV particle. FeLV is an enveloped virus. The envelope lipid-bilayer makes it rather unstable in the environment. The envelope contains a gp70 glycoprotein and the transmembrane protein p15E. FeLV is a single-stranded RNA virus. Each viral particle contains two identical strains of RNA. To detect the viral RNA genome, a reverse transcriptase polymerase chain reaction (RT-PCR) is necessary. The virus carries enzymes, which include reverse transcriptase (RT), integrase and protease. The RT converts the viral RNA into DNA once the virus has entered the host cell. The virus contains a viral capsid containing a protein with a molecular weight of 27 kDa and called p27. This protein is detected in the virus antigen tests (point-of-care [POC] tests or laboratory plate-based enzyme-linked immunosorbent assay [ELISA]). ssRNA, single-stranded RNA. (Courtesy of R. Hofmann-Lehmann, Zurich, Switzerland)

All retroviruses, including FeLV, are RNA viruses and rely on a DNA intermediate for replication (Fig. 3). The single-stranded RNA genome is reversely transcribed into DNA, which is integrated into the host's cell genome through the viral integrase (Temin and Mizutani 1970). The integrated DNA is called "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 surface (Coffin 1979). Infection of a cell by a retrovirus does not usually lead to cell death. Once the provirus is integrated, cell division results in daughter cells that also contain FeLV provirus. The ability of the virus to become part of the host's genomic DNA is crucial for the lifelong persistence (Levy 2000; Cattori et al., 2008; Hofmann-Lehmann et al., 2008; Lutz et al., 2009).

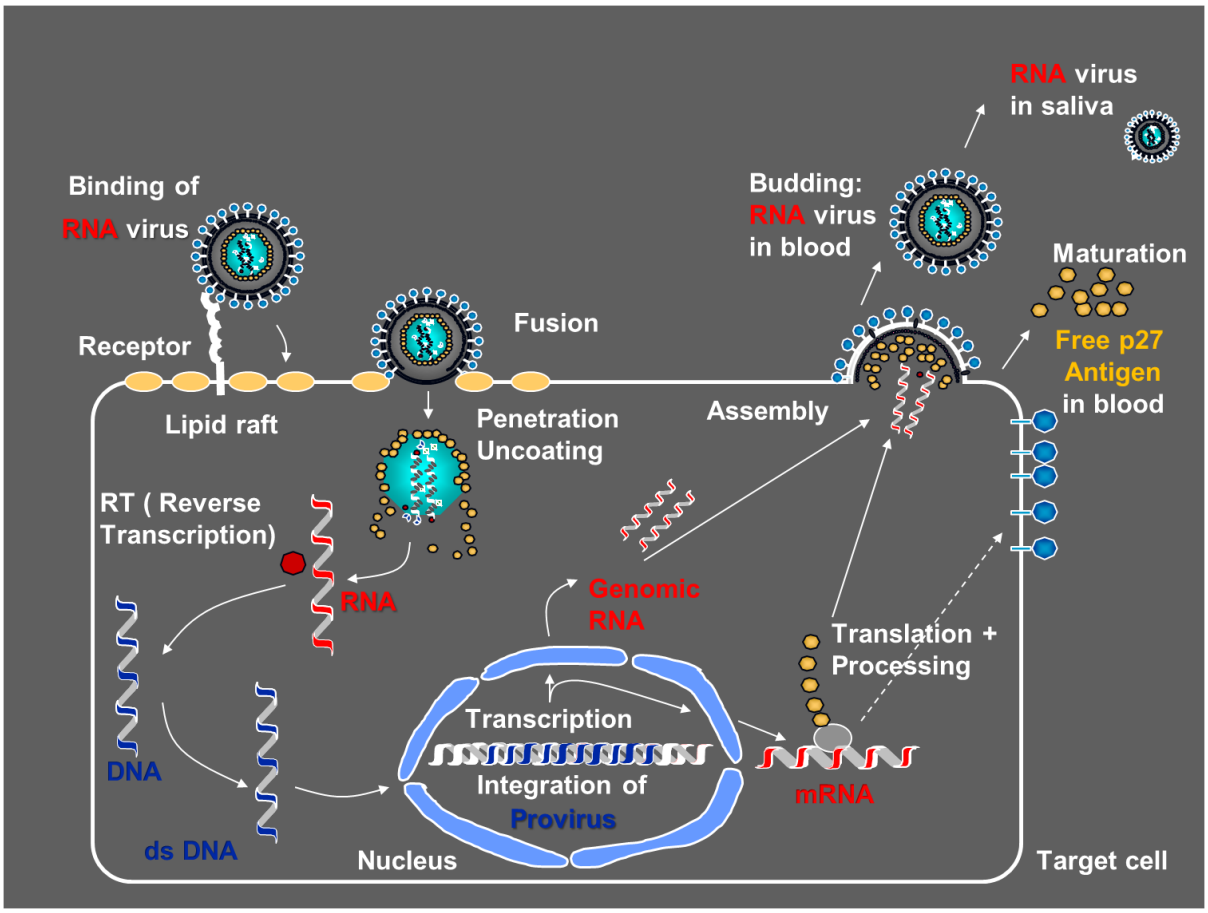


Fig. 3: FeLV replication. Once FeLV particles have attached to and fused with the host cell, the viral RNA is freed and converted into viral DNA (reverse transcription, RT). The viral DNA is transferred to the cell nucleus during cell division, where it is integrated into the host genomic DNA with the help of an integrase and then is called proviral DNA and can be detected using proviral DNA polymerase chain reaction (PCR). When the cell is activated, new viral genomic RNA and messenger RNA (mRNA) are generated (transcription) and proteins produced (translation and processing) and assembled at the host cell membrane to build new viral particles (assembly) that are shed into the blood and saliva (budding). In addition to viral particles, also free (soluble) FeLV p27 capsid antigen is shed into the blood by infected cells and can be detected by point-of-care (POC) tests and laboratory plate-based enzyme linked immunosorbent assays (ELISA). (Courtesy of R. Hofmann-Lehmann, Zurich, Switzerland).

Based on similarities in nucleotide sequences, it is likely that FeLV evolved from a virus in a rodent ancestor, most probably a rat. It is assumed that this event took place in the late Pleistocene, up to 10 million years ago in the North African desert. Ancestral rats and cats roamed freely, and the virus was likely transmitted to cats through ingestion or a rat bite. The initial spread of FeLV among cats might have been inhibited by the aridity of the North African desert (Benveniste et al., 1975). Besides this "exogenous" FeLV, in the domestic cat two forms of endogenous gamma retroviruses are known; the endogenous feline leukaemia virus, enFeLV (Benveniste et al., 1975; Soe et al., 1983; Polani et al., 2010), and the RD-114 (Sarma et al., 1973; Polani et al., 2010).

**Endogenous Gammaretroviruses**

The endogenous counterparts of exogenous FeLV, **enFeLV**, are endogenous DNA sequences that are integrated into the genome of domestic cats and closely related wild species of the genus *Felis* (Benveniste et al., 1975; Polani et al., 2010). The enFeLV is thought to have originated hundreds of thousands of years ago in cats that had eaten mice viraemic with a murine leukaemia virus (MuLV), which led to incorporation of the MuLV genome into the germ line DNA of the predator. This MuLV genome was then inherited by all offspring in a Mendelian manner. EnFeLV are present in every cat and in every feline cell, but are not identical in every cat; integrated endogenous elements can display polymorphisms between

individual cats (Polani et al., 2010). Additionally, the amount of enFeLV varies between different breeds and populations of cats, including European wildcats (*Felis silvestris silvestris*), (Tandon et al., 2007), suggesting that this recombination with MuLVs is a continuing phenomenon. The enFeLV loads are also influenced by the gender of the cat (Tandon et al., 2007). The enFeLV genome is incomplete and therefore does not replicate (Soe et al., 1983), but expression of short transcripts has been observed. Full length enFeLV elements with putative functional open reading frames have been characterized (Roca et al., 2004). These specific endogenous elements are present in only 9–15% of domestic cats, indicating that they are relatively recent additions to the feline genome (Roca et al., 2005).
Recombination can occur between enFeLV and exogenous FeLV-A following infection, which can give rise to new infectious agents that might be more pathogenic (see also FeLV Subgroups). Parts of, or the entire, group-specific antigen (*gag*) genes of enFeLV have been identified in FeLV isolates from naturally infected cats (Kawamura et al., 2015). Moreover, truncated envelope proteins encoded by enFeLV (FeLIX) can facilitate infection with fusion-defective exogenous FeLV by binding to the cellular receptor (Anderson et al., 2000). FeLIX activity was detected at a functional level in sera from domestic cats but no other feline species (Sakaguchi et al., 2015). An association between enFeLV loads and FeLV-A replication was demonstrated and only five of ten cats with high enFeLV loads but nine of ten cats with low enFeLV loads developed progressive FeLV infection (Tandon et al., 2008). The later observation was subsequently substantiated, when a significant association between enFeLV loads and the infection outcome was found in another experimental study: cats that developed regressive infection had significantly higher enFeLV loads than cats that developed progressive FeLV infection (Boesch et al., 2015). Similarly, also in naturally FeLV infected cats of a breeding colony with 65 cats, higher enFeLV loads were associated with better infection outcome (Powers et al., 2018). A strong association between domestic cat cell susceptibility tested in cell culture and FeLV LTR copy numbers was demonstrated, suggesting that the protective effect is specific to FeLV-LTR elements, either via gene regulation of antiviral or immune genes or via direct interference by encoding for, e.g. small interfering RNAs to specifically target FeLV transcripts (Chiu and VandeWoude 2020).

In addition to enFeLV, other endogenous retroviral elements have been found in the domestic cat, such as **RD-114**. The RD-114 virus is of primate origin and most closely related to an endogenous baboon retrovirus and only distantly related to FeLV. It is thought to have originated hundreds of thousands of years ago from an ancestor cat that had preyed on an early primate infected with this RD-114 virus (Barbacid et al., 1977). However, subsequently it was found that there were multiple invasions of RD-114 into the cat genome since most RD-114 related sequences (RDRSs) have not been fixed in domestic cats' genomes. In addition to the very old sequences, new RDRSs had entered the genome of the cat after domestication around 10 thousand years ago (Miyazawa et al., 2016). One of the new RDRSs might have integrated into migrating cats in Europe (Shimode et al., 2015). RD-114 is assumed to be replication-competent in the cat, and several feline cell lines produce infectious RD-114 viral particles. Two feline sodium-dependent neutral amino acid transporters (ASCTs), fASCT1 and fASCT2, have been found to function as RD-114 virus receptors (Shimode et al., 2013). However, no RD-114-related proviral loci found in the feline genome were capable of producing infectious virus; rather a virus nearly identical to RD-114 can be produced by recombination between two defective RD-114 virus-related proviruses (Beyer et al., 1987; Shimode et al., 2015). Although there is no evidence of pathogenicity of RD-114 (Barbacid et al., 1977) or any immune response to RD-114 virus in cats, RD-114 could play a role in foetal differentiation (Todaro et al., 1973; Cotter 1990; Cotter 1998). It also appears important to monitor RD-114 virus production in feline cell lines used for biological products as substrates. Some years ago, concern was raised when infectious RD-114 virus was detected in commercially available live attenuated vaccines for cats and dogs produced on certain feline cell lines, such as Crandell feline kidney (CRFK) cells (Miyazawa et al., 2010). In cats the risk of adverse effects after using a vaccine containing RD-114 is probably low, as endogenous retroviruses can be considered non-pathogenic for their original host (Miyazawa et al., 2010; Yoshikawa et al., 2014). The risk of RD-114 through off-label use of live attenuated cat vaccines in non-domestic felids, however, is unknown. Assays to screen for RD-114 infection in cell culture, and a method to establish cell lines with reduced risk of endogenous retroviruses have been developed (Narushima et al., 2012; Fukuma et al., 2013).

In early studies, a recombinant between RD-114 virus and FeLV-related sequences, named endogenous cat retrovirus element (ECE 1), has been partially characterized, as well as a *Felis catus* endogenous retrovirus (FcEV) being a recombinant of RD-114 *gag/pol* and baboon endogenous virus BaEV *env* (Beyer et al., 1987; van der Kuyl et al., 1999). This later class of endogenous retroviruses of domestic cats (now named **ERV-DCs**) was further investigated (Anai et al., 2012; Song et al., 2013; Ngo et al., 2020). Several proviral clones were identified that were numbered according to their genomic insertions, i.e. ERV-DC1 to ERV-DC18, and classified into genotypes I, II, and III by phylogenetic analysis. The proviruses were characterized according to their capacity to produce infectious viruses; their recombination with other retroviruses, such as FeLV or RD-114; and their biological functions as host antiviral factors (Kawasaki and Nishigaki 2018). Most ERV-DCs contain deletions and mutations that render them non-infectious, but there are several ERV-DCs that are infectious and mobile in the domestic cat, i.e. ERV-DC10 and ERV-DC18 are novel infectious gammaretroviruses that are inherited and can infect a broad range of mammalian cells, including humans. The replication-competent ERV-DC10 has not yet been fixed in the cat genome; it entered the genome of domestic cats more recently and appeared to translocate to or reintegrate at a distinct locus as infectious ERV-DC18 (Anai et al., 2012). Approximately one third of the cats tested in Japan carry ERV-DC10 loci (Anai et al., 2012). In another study, also approximately one third of 1646 cats harboured ERV-DC10, and ERV-DC10 was detectable in cats in Tanzania, Sri Lanka, Vietnam, South Korea and Spain (Ngo et al., 2020). Moreover, pedigree cats, including Singapura, Norwegian Forest, and Ragdoll cats, showed high frequencies of ERV-DC10 (60-100%) (Ngo et al., 2020). It is not known whether ERV-DC10 will eventually disappear or become fixed in cats. Another replication

competent endogenous retrovirus, ERV-DC14, was characterized that shows a different receptor usage and transcriptional activity (Kuse et al., 2016). ERV-DC14 was found in only 4% of the tested 1646 cats, and it was detectable only at a low frequency in pedigree cats (Ngo et al., 2020). It is unclear whether ERV-DCs are harmful or beneficial to the host (Kawasaki and Nishigaki 2018). Hosts tend to tightly control their ERVs, particularly those with high transcriptional activity, e.g. by promotor methylation. Replication-competent viruses with weak promoter activity, such as ERV-DC14, seem to escape strict control via promoter methylation and expand in the cat genome. It was shown that ERV-DCs behave as donors and/or acceptors in the generation of infectious, recombinant viruses, and a novel exogenous FeLV-D was described that contains ERV-DC *env* (Anai et al., 2012). In addition, a novel recombinant FeLV *gag* gene harbouring an insertion that derived from a replication incompetent feline ERV (FcERV-gamma4) has been described (Kawasaki et al., 2017). The insertion is an unrelated sequence that is commonly shared (CS) by ERVs of different species. FeLV carrying this insertion appeared to be replication defective; nonetheless, these FeLV variants were found in 6.4% of 265 Japanese domestic cats naturally infected with FeLV, some of them with lymphoma, leukaemia or suspicion of clonal lymphocyte proliferation (Kawasaki et al., 2017). ERV-DCs have also been characterized in European wild cats and four loci have been identified: ERV-DC6, ERV-DC7, ERV-DC14, and ERV-DC1 (Ngo et al., 2019). In contrast to the domestic cat, ERV-DC14 is not replication-competent in the European wild cat due to a single nucleotide substitution and it is present in a high frequency. Thus, ERVs have evolved species-specific phenotypes; moreover, there might be similar mechanisms of viral inactivation (Refrex-1, a restriction factor against feline retroviruses, was also present in European wildcats) irrespective of the evolutionary history of retroviruses (Ngo et al., 2019).

**FeLV Subgroups**

FeLV is divided into several subgroups based on interference testing, virus neutralisation, and their host cell spectrum. FeLV subgroups are immunologically closely related but use different cellular receptors (Bupp et al., 2006; Mendoza et al., 2006; Shojima et al., 2006; Rey et al., 2008; Miyazawa 2009). The three well and longest known FeLV subgroups are FeLV-A, FeLV-B, and FeLV-C (Table 1).
The cell surface entry receptor for **FeLV-A** is a putative thiamine transporter (THTR1), which is widely expressed in cat tissues (Mendoza et al., 2006; Helfer-Hungerbuehler et al., 2011). The wide expression corresponds to the broad target tissue range of FeLV. The lowest levels of feline THTR1 mRNA expression were found in the blood, whereas high levels were present at sites of virus entry and shedding, i.e. oral mucosa and salivary glands, but also in muscle tissue (Helfer-Hungerbuehler et al., 2011). In the peripheral blood, T lymphocytes showed significantly higher THTR1 mRNA expression levels than neutrophil granulocytes. It was speculated that FeLV-A infection could disrupt thiamine uptake with pathological consequences (Mendoza et al., 2013). Thiamine uptake was indeed blocked by FeLV-A infection, and in feline fibroblasts that naturally express feline THTR1, this blockade resulted in a growth arrest at physiological concentrations of extracellular thiamine; however, the biological impact of this observation in the field is unknown. Sequence analyses revealed that THTR1 was highly conserved among different feline species (domestic cat, Iberian lynx, Asiatic and Indian lion, European wildcat, jaguarundi) and therefore it was concluded that differential susceptibility of different feline species to FeLV cannot be attributed to variations in the THTR1 sequence (Helfer-Hungerbuehler et al., 2011).
**FeLV-B** uses Pit1 and Pit2 as a cellular receptor for virus entry; Pit1 and Pit2 are related transmembrane proteins that function as sodium-dependent phosphate transporters (Bupp et al., 2006; Shojima et al., 2006). A mutation in the first variable region of the envelope of the virus was shown to be important in determining whether a FeLV-B variant can infect cells as efficiently using feline Pit1 as feline Pit2 (Anderson et al., 2001). The complete genome sequences of two novel FeLV-B isolates, FeLV-4314 and FeLV-2518, revealed a recombination site within the *pol* gene of a FeLV-B genome (Stewart et al., 2013). Investigation of FeLV-B phylogeny in a natural FeLV epizootic outbreak in a closed private-owned colony of cats (Powers et al., 2018) revealed a high degree of genetic dissimilarity of FeLV-B sequences in the 22 out of 32 cats that harboured FeLV-B, while FeLV-A circulating in the population, as well as enFeLV env sequences, were highly conserved (Erbeck et al., 2021). The high diversity of FeLV-B and the detection of many recombination breakpoints supports de novo recombination as primary source of FeLV-B emergence, while the presence of more than one FeLV-B variant in half of the cats suggests multiple recombination events within the same host (Erbeck et al., 2021). The authors, however, state that while FeLV-B generation is assumed to be mainly de novo within a cat, they “could not definitively rule out horizontal transmission of FeLV-B, as nearly all cats harboured FeLV-B sequences that were genetically highly similar to those identified in other individual” (Erbeck et al., 2021).
The subgroup **FeLV-C** receptor FLVCR is a 12-transmembrane domain surface protein that exports haem from cells. FLVCR was shown to be required for erythroid maturation (Chiabrando et al., 2012; Fleming and Hamza 2012; Khan and Quigley 2013). Proerythroblasts derived from FLVCR-deleted cells undergo apoptosis, which can be due to a maturation arrest followed by senescence or haem excess and toxicity, while deletion of FLVCR can increase platelet production (Byon et al., 2013). Remarkably, FLVCR was also required for development and maturation of CD4+ and CD8+ T cells (Philip et al., 2015). Binding of FeLV-C to FLVCR seems to involve interaction of two domains of the virus envelope, the receptor-binding domain and the carboxy terminal C domain of the surface protein (Rey et al., 2008).

Replication of FeLV-B, FeLV-C and FeSV is usually only possible with the **help of FeLV-A** because important genomic sequences are replaced in these recombinant viruses. Proposed FeLV-A helper functions include enhanced replication efficiency, immune evasion, and replication rescue for defective virions. However, in some experiments, it was possible to induce replication of FeLV-B and FeLV-C without FeLV-A. In new-born specific-pathogen free kittens, experimental FeLV-B

or FeLV-C infection was established in the absence of FeLV-A (Sarma et al., 1978; Bechtel et al., 1999). Moreover, after screening 300 field isolates using interference assays, a FeLV isolate, designated FeLV-4314 was identified that displayed the FeLV-B phenotype alone, in the absence of a fully functional helper FeLV-A virus (Stewart et al., 2011). The authors speculated that whilst no helper subgroup A viral genome was detected in virions of FeLV-4314, "the acquisition of an exogenous LTR by the endogenous FeLV in 4314 may have allowed a recombinant FeLV variant to outgrow an exogenous FeLV-A virus that was presumably present during first infection" (Stewart et al., 2011). Nevertheless, naturally infected cats usually carry FeLV-A either alone or in combination with FeLV-B, FeLV-C, or both. In consequence, if immunity against FeLV subgroup A is present, the cat is protected against any FeLV infection.
A virus that is usually discussed in connection with FeLV is the feline sarcoma virus (**FeSV)**. FeSV is a recombination of the FeLV-A genome with tumour-associated cellular genes (proto-oncogenes) and likewise is generated *de novo* in a FeLV-A-infected cat. FeSV is replication defective and relies on FeLV-A as a helper virus. FeSV is acutely transforming causing multifocal fibrosarcomas after a short incubation period. FeSV has become uncommon due to the decrease in FeLV prevalence.
Only subgroup FeLV-A is infectious and **transmitted** from cat to cat in nature. FeLV-B is not transmitted from cat to cat under natural circumstances, unless it is co-transmitted with FeLV-A (Sarma and Log 1973), with rare exceptions, e.g. in new-born specified pathogen-free kittens (Stewart et al., 2013; Silva et al., 2016), in a case of a cross-species transmission of FeLV-B from domestic cats to a Florida panther (Chiu et al., 2019), or in a naturally infected cat infected with a specific FeLV isolate (FeLV-4314) (Stewart et al., 2011; Stewart et al., 2013). Subgroup B originates from recombination of FeLV-A with enFeLV *env* genes (Stewart et al., 1986; Overbaugh et al., 1988b) and was reported to be present in up to 40% of the FeLV-A infected cats (Jarrett et al., 1978; Donahue et al., 1988). Subgroup C is far less common; it was found in only about 1% of viraemic cats and only in cats with aplastic anaemia (Jarrett et al., 1978; Donahue et al., 1988). FeLV-C arises from *de novo* mutations in the *env* gene of FeLV-A (Riedel et al., 1986; Willett and Hosie 2013), possibly through intermediates that are multitropic in their receptor use (Shalev et al., 2009). Indeed, it was found that alterations in the amino acid sequence of the receptor binding domain of the envelope glycoprotein resulted in a shift in the receptor usage and the cell tropism of the virus. Within each isolate of FeLV-C, variants were identified that were ostensibly subgroup A by nucleic acid sequence comparisons, but which bore mutations in the receptor binding domain. Differences in the receptor binding domain of the envelope protein, thus, might predispose towards enhanced replication *in vivo* and subsequent conversion to FeLV-C, but the selection pressures that drive the emergence of FeLV-C in a proportion of infected cats remain to be established (Stewart et al., 2013). Remarkably, in one cat that was experimentally infected with FeLV-A and developed FeLV-associated disease and anaemia, both FeLV-B and FeLV-C could be detected additionally within four weeks after infection (Boesch et al., 2015).

Other FeLV subgroups have been described more recently. **FeLV-D** arose from recombination of FeLV-A and the *env* gene of a feline endogenous gammaretrovirus (ERV-DC), genotype I, but it belongs to a different receptor interference group from FeLV-A, FeLV-B, and FeLV-C (Anai et al., 2012; Ito et al., 2013) (Table 1). FeLV-D could be detected in the blood or tumour tissues of three of 283 FeLV-infected cats, but it is neither clear whether FeLV-D is infectious by itself nor whether it is pathogenic. Receptors for FeLV-D and ERV-DCs have not yet been identified. FeLV-D and ERV-DC genotype I are restricted by a feline soluble restriction factor termed Refrex-1, which is a truncated ERV-DC genotype II (ERV-DC7 and ERV-DC16) envelope protein and includes a signal peptide and the receptor binding domain (Ito et al., 2015).

In an experimental setting, **FeLV-T** was identified, which is highly cytolytic for T lymphocytes and causes severe immunosuppression (Lauring et al., 2001; Lauring et al., 2002; Barnett et al., 2003). FeLV-T also use Pit-1 as receptor, like FeLV-B, but the host ranges of FeLV-B and FeLV-T are not exactly the same, suggesting a different Pit-1 use at the post-binding level (Shojima et al., 2006). FeLV-T is fusion-defective and requires a cofactor, termed FeLIX. This cellular protein, a truncated envelope protein encoded by an endogenous FeLV corresponding to a receptor-binding domain, can function as either a transmembrane protein or a soluble component to facilitate infection (Anderson et al., 2000; Nakaya et al., 2010; Sakaguchi et al., 2015). Another naturally occurring powerfully immunosuppressive FeLV isolate was designated "FeLV feline acquired immunodeficiency syndrome" (**FeLV-FAIDS**) (Hoover et al., 1987). The virus was isolated from a pet cat with a thymic lymphoma that was referred to the Colorado State University Teaching Hospital (Mullins et al., 1989). When inoculated experimentally, the virus induced a fatal acquired immunodeficiency syndrome in persistently viraemic cats with survival times ranging from less than three months to a little over one year, depending on the age of the cat at time of infection (Hoover et al., 1987). Molecularly cloned viruses derived from the natural FeLV-FAIDS isolate composed of FeLV-A and a highly immunopathogenic variant that infects CD4+ and CD8+ T lymphocytes and B lymphocytes in blood, lymph nodes, as well as myeloid cells (Quackenbush et al., 1996). The widespread proliferation of FeLV-FAIDS greatly impaired the immune response of the host. Experimental infections of specified pathogen-free cats with the molecular virus clones that induce feline AIDS were suggested as a model to study different aspects of human immunodeficiency virus (HIV) infection in man (Mullins et al., 1989). Moreover, **FeLV-TG35-2** has been described containing a novel *env* gene deriving from FeLV-A after subtle changes (Miyake et al., 2016). The virus sequence originated from a one-year old male castrated FeLV-infected cat, TG35, which had presented with a bite injury, stomatitis, and loss of appetite at a private veterinary hospital in Japan (Watanabe et al., 2013; Miyake et al., 2016). The TG35-2 envelope protein displayed strong sequence identity to FeLV-A envelope protein, suggesting that selection pressure in cats can cause novel FeLV subgroups to emerge. TG35-2 does not interfere with any so far known FeLV subgroup (Miyake et al., 2016). A folate transporter (reduced folate carrier, RFC) was recognized as potential receptor for TG35-2 (Miyake et al., 2019).

Table 1: Host cellular receptors involved in infection of the most common FeLV subgroups

| FeLV Subgroup | Receptor | Receptor Function | Comments |
|---|---|---|---|
| FeLV-A | THTR1 (Mendoza et al., 2006) | Thiamine transporter protein | Present in all cats with FeLV; transmitted exogenously |
| FeLV-B | Pit1 or Pit2 (Anderson et al., 2001) | Inorganic phosphate transporter protein | Results from recombination between FeLV-A and feline endogenous FeLV-related retrovirus sequences (enFeLV); can facilitate development of lymphoma or enhance neuropathogenicity |
| FeLV-C | FLVCR (Keel et al., 2008) | Haem transporter protein | Arises from point mutations in FeLV-A *env* gene; associated with nonregenerative anaemia by interfering with erythrocyte precursor maturation |
| FeLV-D | unknown | | Recombination of FeLV-A with ERV-DC14 genotype I (unclear whether infectious and/or pathogenic) |
| FeLV-T | Pit1, fusion-defective and requires cofactor (FeLIX) (Lauring et al., 2002) | Inorganic phosphate transporter protein | Arises from point mutations in FeLV-A *env* gene; cytolytic for T lymphocytes and can cause severe immunosuppression |
| FeLV-FAIDS | unknown | | Highly immunosuppressive; composed of FeLV-A and FeLV-B subgroup viruses originating from a cat with thymic lymphoma from Colorado, USA |
| FeLV-TG35-2 | FRC (Miyake et al., 2019) | Folate transporter | Arises from point mutations in FeLV-A *env* gene; isolated from a cat with stomatitis from Japan |
| FeSV | unknown | | Recombination of FeLV-A with cellular proto-oncogenes); needs FeLV-A as a helper virus; leads to multifocal fibrosarcomas |

**Virus Genome and Proteins**

FeLV is a single-stranded RNA virus (Fig. 2). The viral genome contains three genes coding for the structural proteins of the virus: the *gag* gene, including p27; the polymerase (*pol*) gene coding for the reverse transcriptase, protease, and integrase enzymes; and the envelope (*env*) gene coding for the glycoprotein gp70 and the transmembrane protein p15E (Coffin 1979) (Fig. 2 and Fig. 4). The gene sequence also contains long terminal repeats (LTRs), which are repeated sequences that have regulatory function and control expression of the other viral genes but generally do not code for a protein product. From the 5' to the 3' end, the gene order is LTR-*gag*-*pol*-*env*-LTR (Fig. 4 and Table 2). LTR regions play a critical role in tissue tropism and pathogenic potential of the viruses. Within the LTRs, recurrent enhancer sequences in the upstream region of the enhancer (URE) are frequently found in cats with myeloid leukaemias and thought to play a role in oncogenesis (Matsumoto et al., 1992; Nishigaki et al., 1997). Of the URE, the U3-LTR of FeLV upregulates specific cellular genes in an integration-independent way. The U3-LTR region does not encode a protein but instead makes a specific RNA transcript. It was demonstrated that FeLV U3-LTR upregulates the NFκB signalling pathway via activation of Ras-Raf-IκB kinase and degradation of IκB, providing new explanations of LTR-mediated cellular gene transactivation that might play a role in oncogenesis (Abujamra et al., 2006). The *gag* (group-associated antigen) gene encodes the internal structural proteins, including p15, p12, p27, and p10 (Table 2). The Gag protein p27, which is routinely used for diagnosis of FeLV infection, is produced in virus-infected cells in amounts exceeding what is necessary for assembly of new virus particles. Thus, p27 is abundant in the cytoplasm of infected cells and also in the blood of infected cats, which is why most available antigen detection tests, such as the enzyme-linked immunosorbent assay (ELISA) or immunomigration tests and immunofluorescence assays (IFA), are designed to detect this protein, as free protein in blood or intracellularly in blood cells, respectively. Free p27 not only circulates in blood but is shed in tears and saliva, where it also can sometimes be detected, but shedding is often intermittently. The *pol* (polymerase) gene specifies the viral enzymes RT, polymerase and integrase; they are responsible for synthesis of proviral DNA on the viral RNA template and integration of the provirus into the host's genome. The *env* (envelope) gene encodes the envelope components gp70 and p15E (Fig. 2). The envelope protein gp70 defines the virus subgroup and appears to be important for inducing immunity. Antibodies to gp70 are subgroup-specific and result in neutralisation of the virus and immunity to reinfection. Thus, gp70 is important in immunity and, therefore, as a target for vaccine production. The transmembrane protein p15E is thought to interfere with host cell immune responses, thus facilitating viral persistence (Mathes et al., 1979; Lafrado and Olsen 1986). Phylogenetic analysis of FeLV *env* genes from naturally infected cats revealed the existence three distinct genetic clusters, termed Genotypes I, II, and III (Watanabe et al., 2013). The genotypes correlated with the geographic origin; mainly Genotype I but also II are found in Japan, while isolates from Europe and the Americas belonged to Genotype III (Watanabe et al., 2013; Makundi et al., 2017). Genotype I was further classified into Clades 1–7 in Japan. The observed diversity of the FeLV *env* gene appears to be caused primarily by mutation, deletion, insertion and recombination within individual cats. The genotypes do not correlate with the known FeLV subgroups (Watanabe et al., 2013).

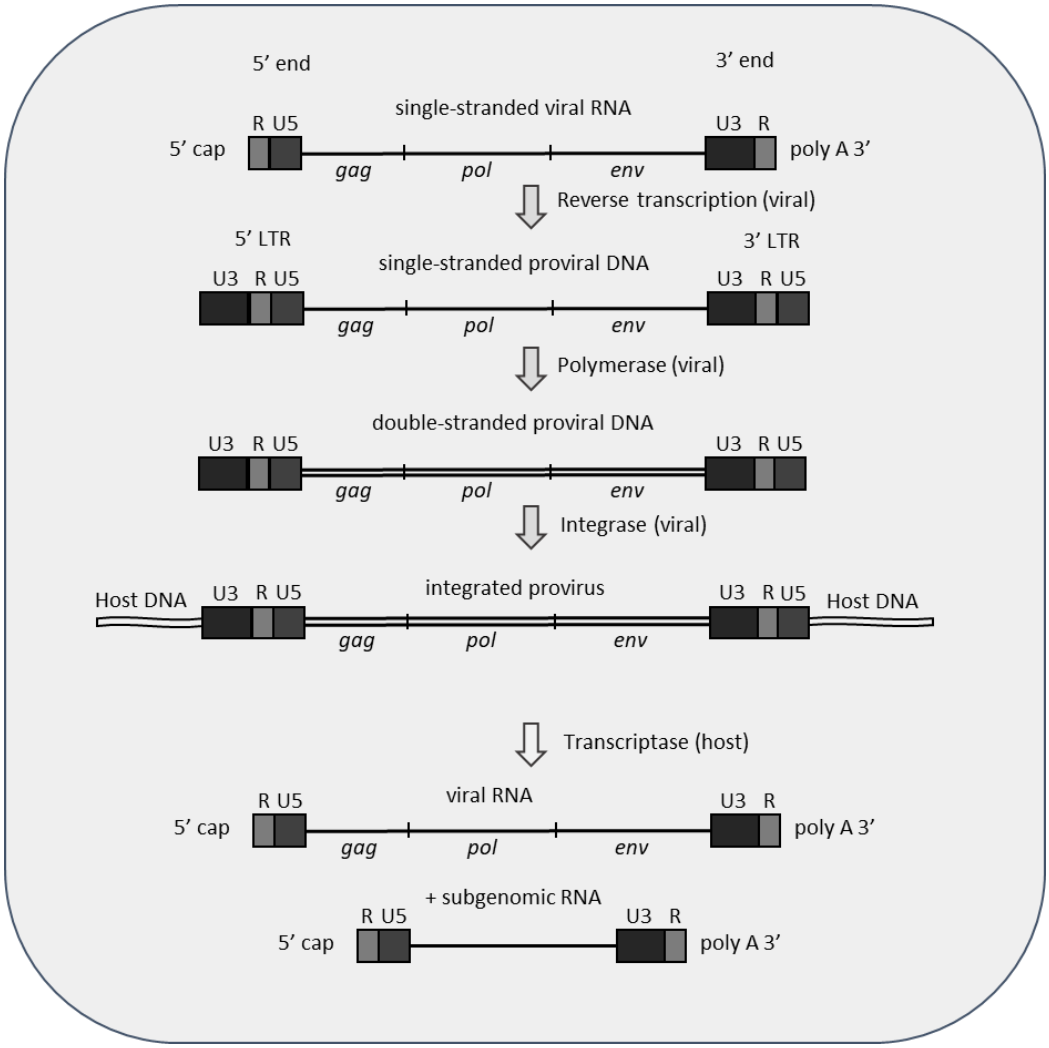


Fig. 4: The retroviral genome, its reverse transcription into proviral DNA and transcription into mRNA. The retroviral genome (single-stranded RNA) is reverse transcribed into proviral DNA and subsequently double stranded provirus, which is integrated into the host genomic DNA with the help of an integrase. Cellular transcription leads to synthesis of new viral RNA and subgenomic mRNA, from which viral proteins are produced. The LTR contains enhancer and promotor elements; *gag* encodes capsid structural proteins; *pol* encodes viral replicative enzymes; and *env* encodes envelope proteins, SU and TM (see also Table 2). (Courtesy of R. Hofmann-Lehmann, Zurich, Switzerland)

Table 2: Summary of the genetic map and function of feline leukaemia virus proteins

| Gene | Location | Proteins | Function |
|---|---|---|---|
| *gag* | Core | p15 | Matrix protein; structural protein |
| | | p12 | Probably involved in the integration process |
| | | p27/CA | Capsid protein; used for antigen testing |
| | | p10 | Nucleocapsid protein; structural protein |
| *pol* | Core | p14/PR | Protease; enzyme splitting proteins to their final form |
| | | p80/RT | Reverse transcriptase; enzyme copying viral RNA into complementary DNA strand ("reverse transcription") |
| | | p46/IN | Integrase; enzyme integrating viral DNA into the host's genomic DNA |
| *env* | Envelope | gp70/SU | External surface unit; primary target for protective humoral immune response; FeLV subgroup-specific antigens FeLV-A, FeLV-B, FeLV-C |
| | | p15E/TM | Transmembrane protein; role in immunosuppression |

**Host Range**

In nature, FeLV has been reported to mainly infect domestic cats. There is evidence, however, that some closely related wild felids are susceptible, and many studies have focused on the presence of FeLV in wildlife species.
***In vitro***, FeLV can replicate also in non-felid cell lines. FeLV-B replicates in cells derived from cats, dogs, cows, pigs, hamsters, monkeys, and humans; FeLV-C replicates in cells of cats, dogs, guinea pigs, and humans (Jarrett et al., 1969; Sarma et al., 1975; Jarrett and Russel 1978). It was expected that FeLV-A replicates only in cells of domestic cats *in vitro*, and that FeLV infection *in vivo* always requires FeLV-A and that, therefore, it cannot occur in non-felids. However, it has been found that two independent FeLV-A isolates from United Kingdom and United States also could infect various non-feline cell lines, including cells from rabbits, pigs, minks, and humans (Nakata et al., 2003). Although certain human cells can be infected *in vitro* by FeLV-A, FeLV does not pose a risk for humans; FeLV-A shed by cats carries carbohydrates on the virus envelope (α-Gal epitope) that are readily recognized by natural anti-αGal antibodies in humans, and the virus is neutralised by complement (Takeuchi et al., 1996). Although malignant transformations do not occur in non-felid cell cultures (Loar 1987), experimental FeLV infection with development of lymphomas could be induced in young dogs and marmosets (Rickard et al., 1973). In experimental infections with FeSV, fibrosarcomas also could be produced in non-felids *in vivo* (Anderson and Theilen 1980). However, no reports exist on natural transmission of FeLV to non-felids.
Documentation of FeLV in non-domestic felids, however, are more and more frequently reported, and FeLV appears to be enzootic in some wild felid populations, such as the European wildcat. Introduction of FeLV into free-living and captive non-domestic felid populations has serious consequences for their health and survival, such as in the Florida panthers or Iberian lynx. In several instances of FeLV infection in wild felids, contact with domestic cats could be demonstrated as the source of infection.

The following paragraph gives an overview on the importance of **FeLV in wild felid** populations and lists the wild felid species reported to be affected by FeLV thus far (Table 3). The felid species are listed according to their phylogenetic lineage (Johnson et al., 2006), starting with lineage 8 to which the domestic cat (*Felis silvestris catus*) belongs, since the genetic background of the host might have an influence on the susceptibility to FeLV and the course of the infection.
Within felids from lineage 8, FeLV was isolated from the European wildcat (*Felis silvestris silvestris*) (Boid et al., 1991), which is very closely relate to the domestic cat. FeLV-infected free-ranging European wildcats were reported in Great Britain, Switzerland, France, Germany, Luxembourg, Portugal, and Spain (McOrist et al., 1991; McOrist 1992; Daniels et al., 1999; Leutenegger et al., 1999; Fromont et al., 2000; Duarte et al., 2012; Leon et al., 2017; Heddergott et al., 2018); up to 53% of the investigated wildcats were FeLV antigen-positive, but no evidence of FeLV-associated disease was found, although in one study the FeLV antigen-positive wildcats showed poorer body condition than the FeLV antigen-negative wildcats (Fromont et al., 2000). In a study that detected FeLV infection by provirus polymerase chain reaction (PCR), European wildcats that tested provirus-positive were significantly more often co-infected with feline haemoplasmas than FeLV provirus-negative wildcats (Willi et al., 2007). Presence of FeLV antibodies was reported in a study investigating a Natural Park (Serranía de Cuenca) in Central Spain and four of nine wildcats tested positive; no further details about the test were reported (Candela et al., 2019). FeLV antigen was also found in a wildcat (*Felis silvestris silvestris*) from Saudi Arabia using a commercial ELISA (Ostrowski et al., 2003). The same study demonstrated the first detection of FeLV antigen in an adult Sand cat (*Felis margarita*) that tested positive twice 20 months apart; the Sand cat also belongs to the genus *Felis* and to the domestic cat lineage 8.

In the phylogenetic **felid lineage 7**, the leopard cat lineage (genera *Otocolobus* and *Prionailurus*), FeLV antigen was detected using a commercial immunochromatography test in wild Pallas' cats (*Otocolobus* [*Felis*] *manul*) of the Daurian Steppe in Russia (Naidenko et al., 2014). FeLV antigen or provirus, however, were not detectable in the 90 leopard cats (*Prionailurus bengalensis euptilurus*) living on Tsushima Island, Nagasaki Prefecture, Japan, and sampled between 1999 and 2014, while 6.4% of the domestic cats from this island were FeLV antigen positive (Makundi et al., 2017). Moreover, FeLV provirus was not detected in samples from 23 live-trapped leopard cats and 29 that were found dead in northwestern Taiwan between 2015 and 2019 (Chen et al., 2021).

Several studies documented FeLV also in wild felid species of **lineage 6**, the puma lineage (genera *Puma* and *Acinonyx*). A severe FeLV outbreak occurred in a previously naïve population of Florida panthers (*Puma concolor coryi*) from 2002 to 2005 (Brown et al., 2008; Cunningham et al., 2008) in which five FeLV antigen-positive panthers died. The FeLV-infected animals suffered from lymphadenopathy, anaemia, and weight loss, and likely died from septicaemia. Due to the severity of the outbreak, a FeLV vaccination program for the Florida panthers was instituted from 2003-2007; no additional FeLV cases were of observed until 2010. However, thereafter (until 2016), six additional cases were documented (Chiu et al., 2019). Genetic analysis of the panther FeLV, designated FeLV-Pco, revealed that the outbreak likely originated from cross-species transmission, and FeLV-Pco closely resembled a highly virulent FeLV strain of domestic cats. After the introduction of FeLV into the Florida panther population, at least two different circulating FeLV strains were found, which were separately introduced in the Florida panther population from domestic cats (Chiu et al., 2019). Moreover, a case of a cross-species transmission event of an oncogenic FeLV recombinant (FeLV-B) from domestic cats to a Florida panther was suspected (Chiu et al., 2019). Outcomes of FeLV infections in the Florida panthers resembled those of domestic cats with

progressive (persistently antigen-positive and developing disease), regressive (only PCR-positive) and abortive infection (only antibody-positive) (Cunningham et al., 2008). However, puma fibroblasts, lacking enFeLV, are more permissive to FeLV than domestic cat cells (Chiu and VandeWoude 2020). Terminal tissues from FeLV-infected Florida panthers and domestic cats had similar FeLV proviral loads, but Florida panther tissues have higher FeLV antigen loads (Chiu and VandeWoude 2020).
FeLV was also detected in pumas (*Puma concolor*, also called mountain lion or cougar) in other regions of the United States and South America; antibodies to FeLV were confirmed in two free-ranging Brazilian pumas (Filoni et al., 2006). FeLV antigen was detected by commercial ELISA in 5.4% of 490 California mountain lions sampled between 1990 and 2008 (Foley et al., 2013). It was speculated that the latter mountain lions acquired the infection via ingestion of FeLV-infected domestic cats, although direct transmission among mountain lions could not be excluded. Evidence of FeLV infection was also found in small species of the genus *Puma*; two free-ranging Brazilian jaguarundis (*Puma yagouaroundi*) tested positive for antibodies to FeLV by western blot (Filoni et al., 2012). A subsequent investigation of the population of jaguarundis at Fundacao Parque Zoologico de Sao Paulo for natural FeLV infection using antibody and antigen tests and molecular methods and monitoring for FeLV-related diseases for five years revealed evidence of FeLV infection in four of 23 animals (Filoni et al., 2017). An intestinal B-cell lymphoma in one jaguarundi was not associated with FeLV. Two jaguarundis had FeLV test results consistent with an abortive FeLV infection (presence of antibodies as the only sign of virus exposure), and two jaguarundis had results consistent with a progressive FeLV infection and potentially FeLV-associated clinical disorders and postmortem changes (Filoni et al., 2017). It was concluded that FeLV infection in jaguarundis mimics the outcomes observed for the domestic cat.
In the genus *Acinonyx* (also lineage 6), FeLV was demonstrated in a captive cheetah (*Acinonyx jubats*) in an early study (Briggs and Ott 1986). A second animal, a Namibian wild-born captive cheetah developed a multicentric FeLV-associated T-cell lymphoma (Marker et al., 2003). Thus, the FeLV infection resembled that of a domestic cat. The animal had acquired the infection after being housed in an enclosure adjacent to a FeLV-infected cheetah that had previously been in contact with domestic cats. The transmission across an enclosure fence was unusual, and the authors concluded that this might be indicative of a high susceptibility to FeLV in cheetahs. However, surveys in free-ranging cheetahs inhabiting Namibian farmlands demonstrated no presence of FeLV antigen in any of the 146 tested samples collected from 1992 to 1998 and from 2002 to 2004 (Munson et al., 2004). A more recent study in Namibian cheetahs found gammaretrovirus-specific antibodies in up to 19% of the free-ranging animals and 27% of the captive non-vaccinated animals (Krengel et al., 2015). The trigger of this immune response could not be determined; all animals tested negative for FeLV proviral DNA. A strong immune response was found in captive cheetahs vaccinated against FeLV (Briggs and Ott 1986); FeLV vaccination, therefore, can be beneficial should FeLV infection become a threat to cheetahs.

In the lynx **lineage 5**, (genus *Lynx*), FeLV was detected in an 11-month-old captive-bred bobcat (*Lynx rufus*) showing signs of lethargy, anorexia, neutropenia, lymphopenia, and nonregenerative anaemia (Sleeman et al., 2001). The bobcat had been in contact with a domestic cat (surrogate mother) and had not been vaccinated against FeLV; the cat was suspected to be the source of the infection. Based on sequencing analysis, FeLV-A from domestic cats was also assumed to be the source of FeLV infection in critically endangered Iberian lynxes (*Lynx pardinus*) inhabiting the Donana and Sierre Morena areas in southern Spain (Luaces et al., 2008). FeLV provirus was detected in six of 21 lynx samples collected between 1993 and 2003, and the infection resembled regressive FeLV infection of the domestic cat as no FeLV antigen was detectable. A subsequent study on 77 of the approximately 200 remaining free-ranging Iberian lynxes in the same area between 2003 and 2007, revealed 14 FeLV provirus-positive animals (18%), but this time, most of the animals were also antigen-positive (Meli et al., 2009). During a six month period in 2007, about two thirds of the FeLV-infected lynxes died (Meli et al., 2010). High-throughput sequencing of the virus revealed a common origin for most samples, and that the virus most likely originated from domestic cats (Geret et al., 2011). A subsequent study with transmission of blood from lynxes infected with FeLV (and *Candidatus* Mycoplasma haemominutum) to specified pathogen-free domestic cats did not lead to a particularly severe outcome of infection in the cats, and it was concluded that the fatal outcome of the FeLV outbreak in Iberian lynxes in 2007 was rather due to a particular susceptibility of this species to infectious pathogens (Geret et al., 2011). Many domestic cats and European wildcats were found to be FeLV antigen-positive in the same area (Millan et al., 2009; Leon et al., 2017). Preventative measures, including surveillance and vaccination programs for the Iberian lynx were therefore introduced. Moreover, domestic cat populations should be controlled and vaccinated to reduce the infectious pressure also in wildlife. Carcases of domestic cats have been found in Iberian lynx territories in Andalusia with signs of predation by the lynxes, and in one case killing of the domestic cat and consumption by the Iberian lynx has been documented (Najera et al., 2019). The domestic cat incidentally was FeLV antigen-positive (viraemic) but the lynx had been vaccinated against FeLV, although with the last booster more than 2 years back; the lynx tested FeLV-negative three weeks after predation (Najera et al., 2019). From 2015 to 2019, a low FeLV infection rate was found in 40 re-introduced and 29 wild-born Iberian lynxes in southwestern Spain: only one animal (1/67; 1.5%) was provirus positive (Najera et al., 2021).

FeLV was also confirmed in several species of the ocelot **lineage 4** (genus *Leopardus*). FeLV proviral DNA was detected in three out of 14 (21%) free-ranging kodkods, also known as guignas (*Leopardus guigna*) and in 33% of sympatric domestic cats sampled between 2008 and 2010 on a Chilean Island (Mora et al., 2015), as well as in 20% of 102 free-ranging kodkods sampled between 2008 and 2018 in central and southern Chile (Sacristan et al., 2021). A similarly high prevalence of provirus positivity was detected in domestic cats in the same areas (20% of 262 cats positive). Sequencing analysis placed guigna FeLV within worldwide domestic cat virus clades with high nucleotide similarity, and guigna FeLV infection was

significantly associated with fragmented landscapes with resident domestic cats (Sacristan et al., 2021). FeLV proviral DNA was further detected in a captive ocelot (*Leopardus pardalis*) and a captive oncilla (*Leopardus tigrinus*) in Brazil (Guimaraes et al., 2009). Moreover, three captive neotropic felids, one margay (*Leopardus wiedii*) and two pampas cats (*Leopardus pajeros*), were found to be FeLV antigen-positive in a survey conducted in United States zoos (Kennedy-Stoskopf 1999). The pathogenic potential of FeLV in these wild felid species is unknown. There was no evidence of FeLV infection in Geoffroy's cats (*Leopardus geoffroyi*) in Argentina (Uhart et al., 2012).

One study investigated FeLV in felids in **lineage 3 (**caracal lineage): 55 servals (*Lepteilurus serval*) live-trapped in Secunda, Mpumalanga Providence, South Africa, between 2015 and 2018 were tested for FeLV p27 antigen using a plate-based ELISA assay (Loock et al., 2021). All animals were FeLV antigen-negative.

No studies are available on FeLV infections of felids in **lineage 2** (bay cat lineage)

There is ample evidence of absence of FeLV infections in wild felids of the *panthera* **lineage 1** (genus *panthera*). The genus *panthera* belongs to another subfamily of Felidae in the traditional morphology-based taxonomy, subfamiliy Pantherinae, in contrast to lineages 2 to 8, which belong to the subfamily Felinae. FeLV infection was not found in many studies on African lions (*Panthera leo*), Asian lions (*Panthera leo persica*), jaguars (*Panthera onca*), Amur tigers (*Panthera tigris altaica*) and Bengal tigers (*Panthera tigris tigris)* (Hofmann-Lehmann et al., 1996; Endo et al., 2004; Driciru et al., 2006; Ramanathan et al., 2007; Ramsauer et al., 2007; Harrison et al., 2010; Goodrich et al., 2012; Furtado et al., 2013; McCauley et al., 2021). There is one short report describing the detection of a short FeLV-like DNA sequence in blood from a free-ranging jaguar in Brazil (Silva et al., 2016). The 232 bp sequence deposited in GenBank (KU288756) showed 98% identity with the U3 LTR of FeLV-A Glasgow 1. Furthermore, an investigation in African lions with lymphomas mentioned that the zoo records of one of the lions reported antibodies to FeLV, but no information was available on the antibody test used (Harrison et al., 2010). Further investigations are necessary to corroborate those two observations on FeLV in felids of the *panthera* lineage. Apart from these two reports, FeLV was found only in felid species within the lineages 4 to 8, thus, in wild felids phylogenetically more closely related to the domestic cat.

Table 3: Overview of FeLV in wild felid species

| Felid lineage | Feline Species | Observation | Reference |
|---|---|---|---|
| lineage 8<br>domestic cat lineage | European wildcat<br>(*Felis silvestris silvestris*) | detected in several European countries; prevalence up to 53%; also, in Saudi Arabia | (Boid et al., 1991; McOrist et al., 1991; McOrist 1992; Daniels et al., 1999; Leutenegger et al., 1999; Fromont et al., 2000; Ostrowski et al., 2003; Willi et al., 2007; Duarte et al., 2012; Leon et al., 2017; Heddergott et al., 2018; Candela et al., 2019) |
| | Sand cat<br>(*Felis margarita*) | Central West Saudi Arabia | (Ostrowski et al., 2003) |
| lineage 7<br>leopard cat lineage | Pallas' cats<br>(*Otocolobus* [*Felis*] *manul*) | Daurian Steppe in Russia | (Naidenko et al., 2014) |
| | leopard cats<br>(*Prionailurus bengalensis euptilurus*) | FeLV **not detected** in Tsushima Island, Nagasaki Prefecture, Japan | (Makundi et al., 2017) |
| lineage 6<br>puma lineage | Florida panther<br>(*Puma concolor coryi*) | severe outbreak in Florida (FeLV-Pco); progressive, regressive and abortive infections | (Brown et al., 2008; Cunningham et al., 2008; Chiu et al., 2019) |
| | puma<br>(*Puma concolor*) | 5% of California mountain lions antigen-positive; antibody-positive free ranging pumas in Brazil | (Filoni et al., 2006; Foley et al., 2013) |
| | jaguarundi<br>(*Puma yagouaroundi*) | free-ranging and zoo-kept animals in Brazil; progressive and abortive infection | (Filoni et al., 2012; Filoni et al., 2017) |

| | | | |
|---|---|---|---|
| | cheetah<br>(*Acinonyx jubats*) | captive animals;<br>wild-born captive animal in Namibia;<br>animals with multicentric T-cell lymphoma;<br>19% of the free-ranging animals and 27% of captive non-vaccinated animals antibodies-positive | (Briggs and Ott 1986; Marker et al., 2003)<br><br>(Krengel et al., 2015) |
| lineage 5<br>lynx lineage | bobcat<br>(*Lynx rufus*) | sick FeLV virus isolation-positive animal after contact with domestic cat | (Sleeman et al., 2001) |
| | Iberian lynx<br>(*Lynx* pardinus) | severe outbreak with death of about two thirds of the FeLV antigen-positive animals in Spain;<br>one provirus-positive animal in Switzerland;<br>one antigen-positive animal in Germany | (Luaces et al., 2008; Meli et al., 2009; Meli et al., 2010; Geret et al., 2011);<br><br>unpublished observations (Meli, Hofmann-Lehmann et al) |
| lineage 4<br>ocelot lineage | kodkod<br>(*Leopardus guigna*) | provirus-positive free-ranging animals on Chilean Island | (Mora et al., 2015) |
| | ocelot<br>(*Leopardus pardalis*) | provirus-positive captive animal in Brazil | (Guimaraes et al., 2009) |
| | oncilla<br>(*Leopardus tigrinus*) | provirus-positive captive animal in Brazil | (Guimaraes et al., 2009) |
| | margay<br>(*Leopardus wiedii*) | antigen-positive animal, in United State zoo | (Kennedy-Stoskopf 1999) |
| | pampas cat<br>(*Leopardus pajeros*) | antigen-positive animals in United State zoo | (Kennedy-Stoskopf 1999) |
| lineage 3<br>caracal lineage | Serval<br>(*Lepteilurus serval*) | FeLV **not detected** in free-ranging animals in South Africa | (Loock et al., 2021) |
| lineage 2<br>bay cat lineage | no studies available | | |
| lineage 1<br>panthera lineage | - African lions<br>(*Panthera leo)*<br>- Asian lions<br>(*Panthera leo persica*)<br>- jaguars<br>(*Panthera onca*)<br>- Amur tigers<br>(*Panthera tigris altaica*)<br>- Bengal tigers<br>(*Panthera tigris tigris*) | **not detected** | (Hofmann-Lehmann et al., 1996; Endo et al., 2004; Driciru et al., 2006; Ramanathan et al., 2007; Ramsauer et al., 2007; Harrison et al., 2010; Goodrich et al., 2012; Furtado et al., 2013; McCauley et al., 2021) |

## EPIDEMIOLOGY

### Prevalence

FeLV occurs worldwide. Its prevalence is influenced by the density of cat populations, and there is noticeable geographical and local variation. FeLV prevalence studies that usually determine FeLV antigenemia (or viral RNA in saliva as a measure for antigenemia, see below) report a prevalence of 2.3% to 3.3% in the United States, 0% to 15.6% in Europe, 3.0% to 28.4% in South America, and 0% to 24.5% in Asia and Australia/New Zealand (Arjona et al., 2000; Yilmaz et al., 2000; Dorny et al., 2002; Muirden 2002; Bandecchi et al., 2006; Levy et al., 2006b; Gleich and Hartmann 2009; Hellard et al., 2011;

Bande et al., 2012; Englert et al., 2012; Spada et al., 2012; Sukhumavasi et al., 2012; Bande et al., 2014; Najafi et al., 2014; Rypula et al., 2014; Cong et al., 2016; Garigliany et al., 2016; Hwang et al., 2016; Gates et al., 2017; Lacerda et al., 2017; Luckman and Gates 2017; Hofmann-Lehmann et al., 2018; Sivagurunathan et al., 2018; Biezus et al., 2019; Sacristan et al., 2019; Studer et al., 2019; Szilasi et al., 2019; Westman et al., 2019a; Westman et al., 2019b; Latrofa et al., 2020; Alcover et al., 2021; Capozza et al., 2021; Kokkinaki et al., 2021; Mendes-de-Almeida et al., 2021; Szilasi et al., 2021; Villanueva-Saz et al., 2021).

More recent prevalence studies have mainly focused on the detection of FeLV (antigenemia) in third-world countries or on remote islands, where the prevalence of virus infections in cats was previously unknown. Also in these studies, FeLV has been detected almost everywhere (Danner et al., 2007; Mendes-de-Almeida et al., 2007; Coelho et al., 2008; Macieira et al., 2008; Blanco et al., 2009; Akhtardanesh et al., 2010; Al-Kappany et al., 2011; Stojanovic and Foley 2011; de Almeida et al., 2012; Filoni et al., 2012; Abdou et al., 2013; Muchaamba et al., 2014; Munro et al., 2014; Ortega-Pacheco et al., 2014; Attipa et al., 2017; Biezus et al., 2019). Only cats on some remote islands, such as Grenada Island and St. Kitts Island, West Indies, and Isabela Island, Galapagos, were free of FeLV infection (Levy et al., 2008b; Dubey et al., 2009; Kelly et al., 2010; Chi et al., 2021).
Interestingly, in some areas FeLV infection still appears to be quite common, for example in northwestern China with a prevalence of over 11% of the tested cat population (Cong et al., 2016) and in Thailand with a prevalence of almost 25% in the cats tested (Sukhumavasi et al., 2012). A first study investigating FeLV in a limited number of samples (n = 39) from cats originating from an Indian Reservation (Pine Ridge Reservation) revealed 10% FeLV antigen-positive cats and it was suggested to develop a preventive medicine plan for cats in this and similar reservations (Scorza and Lappin 2017).

When comparing FeLV prevalence in different geographic regions, it is important to realize which parameter is measured and with what method; moreover any preselection of the cat population under investigation needs to be taken into account. One Chinese study that determined viral RNA in blood samples using molecular methods reported a FeLV prevalence of 60% viral RNA positive cats (Liu et al., 2020); however, the primers used for reverse transcriptase (RT-)PCR in this particular study seem to have amplified endogenous FeLV-like or feline genomic sequences also. Similarly, a study from Mexico reported 54% and 76% of cats being positive for FeLV using a *pol* and *env* PCR, respectively (Ramirez et al., 2016); but the specificity for exogenous FeLV or FeLV-related endogenous sequences is unclear. The same FeLV provirus PCR was used in a study investigating 1008 female stray cats undergoing neutering in western Turkey and a very high percentage of 70% of the cats (703/1008) was reported to be provirus-positive (Muz et al., 2021); however, the authors admitted that some of the amplified sequences were endogenous FeLV sequences. The same study also used a commercial assay to detect antibodies to FeLV gp70 and listed the specificity of this assay to be 100%; the authors found 45% of the cats (442/977) to be antibody-positive using this assay, while with a well-established ELISA-based POC test for FeLV p27 antigen detection, only 3% of the cats (32/977) tested positive (Muz et al., 2021). A study using a commercial FeLV provirus PCR amplifying the *env* gene of FeLV also found a high prevalence with 23 of 91 samples (25%) from sick cats being positive; however, the cats had been presented to veterinarians with clinical signs, such as reduced appetite, lethargy, weight loss, pallor, tachycardia, dyspnea, hepatosplenomegaly, lymphadenopathy, or fever (Demkin and Kazakov 2021). Among 80 cats that veterinarians referred to a specific Infectious Diseases Isolation Unit (IDIU) in Lisbon, Portugal, between 2013 and 2018, feline upper respiratory tract infection (38.2%) and FeLV infection (36.4%) were the most common diagnoses. For FeLV this is a very high percentage; however, the article does not state, what kind of test was used (Paulo et al., 2021).

A first pan-European prevalence study used the same method and sampling procedure in 32 different countries to be able to compare prevalence results Europe-wide. The prospective study investigated FeLV RNA in saliva samples as a measure for FeLV antigenemia (see below) in cats taken to veterinary practices in 32 countries in 2016/2017; an overall FeLV prevalence of 2.3% in 6,005 tested cats was reported (Studer et al., 2019). There was a high variability among different countries, with a very low prevalence in northern European countries, such as Denmark, Finland, Norway, and Sweden (all 0%) and the UK (1%) and in western European countries including the Netherlands (0%) and Germany, Austria, France, Belgium, and Luxemburg (0.3% - 1.3%) and a higher prevalence in southern European countries including Croatia (4.5%), Italy and Malta (5.5%) and Portugal (8.8%) (Fig. 5). A protective factor, apart from geographic origin from northern and western European countries was being a pedigree cat; pedigree cats were less frequently FeLV-infected than non-pedigree cats, and FeLV prevalence was higher in sick cats compared to healthy cats (Studer et al., 2019). Further risk factors for FeLV infection included male intact sex, 1-6 years of age, indoor and outdoor or outdoor living only, and living in groups of ≥ 5 cats (Studer et al., 2019). FeLV prevalence in individually kept cats is low, often – but not everywhere - less than 1% (Hosie et al., 1989; Lutz et al., 1990; Levy et al., 2006b; Little et al., 2011; Englert et al., 2012). In large multi-cat households without specific preventive measures for introduction of FeLV, the prevalence can be higher than 20%.

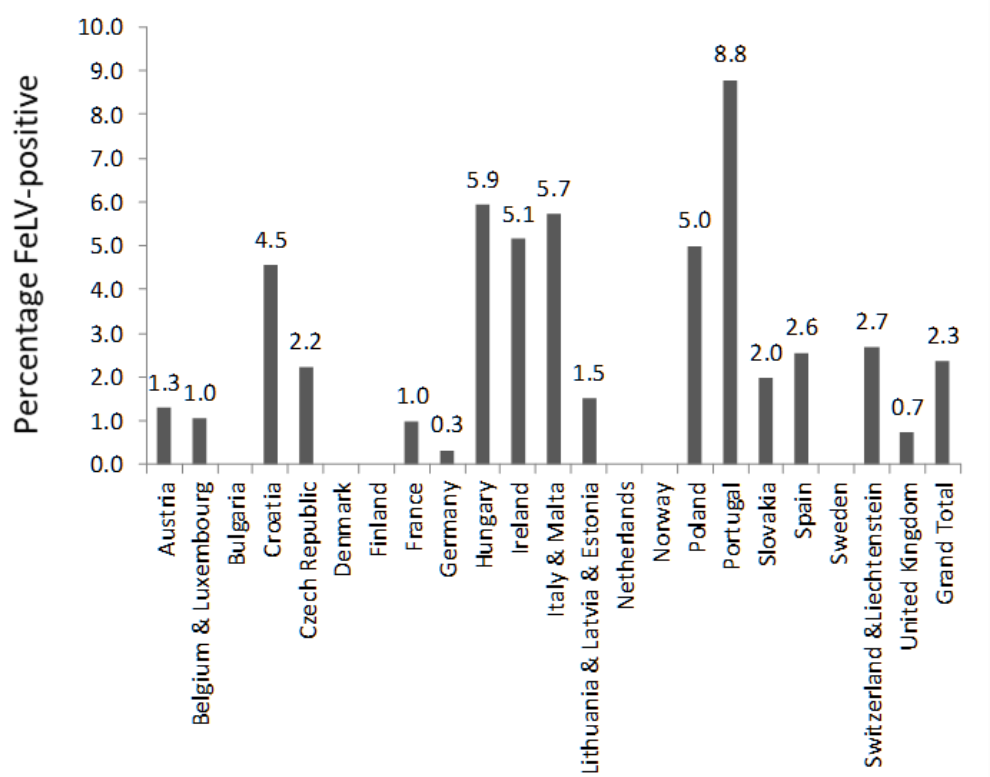


Fig. 5: FeLV prevalence in cats visiting veterinarians in the different European countries and country groups (determined as viral RNA in saliva using RT-PCR as a measure for FeLV antigenemia) (Studer et al., 2019)

FeLV prevalence (antigenemia) had been decreasing over the last decades in many countries (Arjona et al., 2000; Levy 2000; Yilmaz et al., 2000; Dorny et al., 2002; Muirden 2002; Bandecchi et al., 2006; Levy et al., 2006b; Englert 2009; Gleich and Hartmann 2009; Hofmann-Lehmann et al., 2018). The Tufts Veterinary Diagnostic Laboratory, where approximately 2000 serum samples are tested yearly for FeLV antigen, already reported a decrease from 8% in 1989 to 4% in 1995 (Cotter 1997). In Germany, a steady decrease in FeLV prevalence from 6% to 1% was observed when investigating the FeLV infection rate from 1993 to 2002 (Gleich et al., 2009) and in Switzerland, a major decrease was found in sick cats from 13% to 3% in 1990 to 2003 (Hofmann-Lehmann et al., 2018). The decrease in prevalence was most likely the result of test and removal programs and the widespread use of vaccination. The first vaccine was introduced in 1985; however, the observed decline in the overall infection rate in some areas already began before this time (Romatowski 1997). Thus, test and removal programs at breeding catteries and the practice of testing cats at animal shelters before adoption play a very important role, in addition to vaccination of cats at risk. Despite the documented decline, more recently studies indicate that the decrease in prevalence has stagnated in many countries (Gleich et al., 2009; Firth and Mostl 2015; Burling et al., 2017; Hofmann-Lehmann et al., 2018) and thus, awareness about this important infection and its prevention should not be neglected.

Deterministic models have been constructed to predict the dynamics of FeLV in cat populations. These models indicated that FeLV dynamics depend on the size of the host population and the relationship between host density and the pattern of contacts of individual cats. They found no threshold population size for virus persistence in large populations, but the possibility of FeLV extinction in small populations (Fromont et al., 1998). Models take into account that cat populations can be connected to each other by dispersal of individuals, which favours roaming of cats and spread of disease (Fromont et al., 2003). These models explain the geographic discrepancies of FeLV prevalence, e.g., the higher number of free-roaming cats in southern Europe increases the contact rate in these countries, which, as a consequence, increases the overall prevalence of FeLV infection (Fromont et al., 2003). A study investigating a large number of cats in United States revealed distinct spatial distribution patterns in the proportional prevalence of FeLV versus feline immunodeficiency virus (FIV) suggesting the presence of an additional or unique, but yet unknown, spatial risk factor, such as geographic variations in specific virus strains and rate of vaccination. There was a higher prevalence of FIV infections in the southern and eastern United States compared to FeLV. In contrast, FeLV infections were observed to be more frequent in the western United States compared to FIV (Chhetri et al., 2015).

**Predisposing Factors**

There are numerous factors that can influence the prevalence of FeLV in a population and factors that are associated with the outcome of a prevailing infection. The latter are discussed below in the pathogenesis section. Risk factors for FeLV

infection influencing FeLV prevalence have been relatively well characterized (Table 4), but the exact mechanisms for the different clinical responses are poorly understood.

***Cat population.*** First, the population of cats that is investigated has a significant influence on the prevalence (Little et al., 2009). When only sick cats are examined, prevalence of FeLV infection is much higher than if healthy cats are examined (Hosie et al., 1989; Lutz et al., 1990; Ueland and Lutz 1992; Arjona et al., 2000; Biezus et al., 2019; Westman et al., 2019b) as FeLV predisposes cats to certain diseases and secondary infections, and this is especially the case when cats are examined with clinical problems that are associated with FeLV, such as bite wounds and abscesses, because behavioural properties favour both, the FeLV infection and the clinical problem (Goldkamp et al., 2008). In healthy cats, FeLV infection rates from 1% to 8% in Europe and the United States (Bandecchi et al., 2006; Levy et al., 2006b; Solano-Gallego et al., 2006; Gleich and Hartmann 2009; Little et al., 2009; Studer et al., 2019) have been reported. If only sick cats are included in the surveys, FeLV prevalence was as high as 38% (Arjona et al., 2000; Levy 2000; Harrus et al., 2002). In a more recent study in Brazil, prevalence in health cats was 10% versus 28% in sick cats in a hospital population (Biezus et al., 2019); in a large pan-European study investigating 6,005 cats, sick animals were 2.43-times more likely to be FeLV-positive than healthy cats (Studer et al., 2019). Originally, certain diseases, such as lymphoma, were associated with very high FeLV infection rates (up to 75%). However, FeLV prevalence in cats with these diseases has decreased as FeLV prevalence in general has decreased (Stützer et al., 2011; Meichner et al., 2012).

***Signalment of the cat.*** In earlier studies, the FeLV infection rate similar in male and female cats (Lee et al., 2002). However, more contemporary studies, e.g., in the United States, Brazil, and Germany, found a significantly higher risk of FeLV infection among male cats (Levy et al., 2006b; Gleich et al., 2009; Luckman and Gates 2017; Biezus et al., 2019; Studer et al., 2019). Although FeLV transmission commonly occurs between infected queens and kittens and among cats living in prolonged close contact, it seems that aggressive behaviour, a common male attitude, plays a greater role than previously reported (Goldkamp et al., 2008). This is also supported by the findings that sexually intact cats are more frequently infected (Hofmann-Lehmann et al., 2018; Studer et al., 2019), that cats exhibiting aggressive behaviour have a higher risk of FeLV infection (Gleich et al., 2009; Biezus et al., 2019), and that more than 8% of cats examined by veterinarians for fighting injuries were FeLV antigen-positive, a prevalence considerably higher than in the healthy cat population (Goldkamp et al., 2008).
In older studies, young age also was associated with a higher prevalence of FeLV, but this statement must be revised. In a study in the United States, including 18,038 cats tested, adult cats were more likely to be progressively FeLV-infected than juveniles (Levy et al., 2006b). In a study investigating 6,005 cats from 32 European countries, adult cats (age 1-6 years) were more frequently infected than younger cats (Studer et al., 2019). Similarly, in a study in a shelter in UK (Stavisky et al., 2017) and in a study in Germany, the median age of FeLV-infected cats was not significantly lower than that of non-FeLV-infected cats (Gleich et al., 2009), which seems to be a consistent finding, at least in countries with good veterinary care. This is unexpected because the susceptibility of cats to progressive FeLV infection is age-dependent (Hoover et al., 1976; Hosie et al., 1989). However, likely because of the increasing awareness, more cats are tested for FeLV, and medical care is provided earlier leading to a longer life span.
Although no breeds are predisposed to being infected with FeLV, infection is less commonly found in purebred cats, probably because they are more commonly kept indoors and the awareness in the cat-breeder community leads to more frequent testing and vaccination.

***Behaviour.*** Prevalence is also higher in cats that are allowed to roam outside (Levy et al., 2006b; Gleich and Hartmann 2009; Studer et al., 2019), because direct contact is required for transmission. Although fighting, free-roaming, intact male cats are still considered mainly at risk for acquiring FIV infection, the same factors also facilitate FeLV infection (Gleich et al., 2009; Galdo Novo et al., 2016). However, it was shown that being male (neutered or not) and having access to outdoors had a stronger impact on FIV prevalence than on FeLV prevalence, while the presence of clinical illness was a stronger predictor for FeLV infection (Chhetri et al., 2015). Still, FeLV can no longer be considered primarily an infection of “social cats”.

***Environment.*** Generally, environmental factors play an important role. As an example, it has been reported that prevalence can significantly vary among shelters, depending on their testing and hygiene strategies (Stavisky et al., 2017). Living in households of animal hoarders was also detected as a risk factor for FeLV (among many other infectious agents). Animal hoarders accumulate animals in over-crowded conditions without adequate nutrition, sanitation, and veterinary care (Polak et al., 2014). Being a stray cat was identified as a risk factor. In some mostly southern European countries, cats are regarded as natural co-habitants in settlements; they are considered useful for their hunting activity and are not viewed as a domestic but a synanthropic species. These cats are left unneutered to breed naturally and with limited veterinary support, with serious consequences for the animals and often high mortality of kittens from infectious diseases (Natoli 1985). However, trap and neuter programs in stray cats have a positive influence on prevalence; FeLV prevalence decreased significantly by 0.18% per year in a trap-neuter-return program operating for over two decades (Kreisler et al., 2019). Interestingly, supplemental feeding and cat caretaker activity in stray cats was recently detected as being associated with a higher FeLV prevalence, likely through increased aggregation of cats and/or altered foraging strategies (Hwang et al., 2018). An alternative explanation might be the following. In Italy, free-living outdoor cats (*gatti liberi*) have been protected by law by a no-kill no-moving policy (Natoli et al., 2019). There are registered cat caretakers and compulsory

neutering of the cats, which has led to stable cat numbers, e.g. in Rome (Natoli et al., 2019). While the no-kill and no-moving restriction in place has many benefits for the cats, it can at the same time be a disadvantage in terms of FeLV infection. Healthy FeLV-positive shedders might stay unrecognized, and so pose an infection risk to uninfected cats. Moreover, if cats are identified as FeLV-infected, they cannot be removed from the cat population (Studer et al., 2019). One study looked into the risk of disasters on FeLV infection rates among cats exported from the 2005 Gulf Coast hurricane disaster area, but observed no increase in infection rates in this situation (Levy et al., 2007).

***Power parity per capita***. Interestingly, a comparative meta-analysis evaluated the association of FeLV prevalence with the purchasing power parity per capita (PPP). Information on FeLV prevalence in specific locations around the world was analysed from 47 published articles and showed that the highest percentage of FeLV infection was found in cats living in areas of lower PPP with a decreasing rate of FeLV infection with increasing income. Reasons for this could be that the lower PPP locations are also areas of higher stray cat populations with less emphasis on animal control programs and vaccination (Ludwick and Clymer 2019). In a prospective study including cats from 30 European countries, the hypothesis of a direct relationship of FeLV prevalence with gross domestic product per capita was not confirmed (Studer et al., 2019). The study used the same FeLV detection and cat recruitment method in all countries (Studer et al., 2019), while only limited and/or older data were available from European countries for the previous meta-analysis study, and FeLV prevalence was determined using different methods and heterogeneous study populations (Ludwick and Clymer 2019). However, the number of countries included in the prospective study and the range in PPP were somewhat smaller than in the meta-analysis study. Interestingly, there was a significant correlation between purchasing power per capita and the FeLV vaccination rate in the investigated European countries; vaccination rates were higher in areas with higher income and lower in countries with lower income. (Studer et al., 2019). This observation can easily be explained since vaccines are more affordable in countries with a higher PPP. The FeLV vaccination rate might have an indirect effect on the FeLV prevalence.

Table 4: Risk factors influencing prevalence of feline leukaemia virus (FeLV) infection (FeLV antigenemia; modified from (Hartmann and Hofmann-Lehmann 2020b)). For risk factors influencing the infection outcome see Table 6.

| | |
|---|---|
| Signalment | • Gender: male cats<br>• Reproductive status: intact cats<br>• Age: adult cats |
| Health status | • Presence of clinical signs<br>e. g. bite wounds, abscesses, secondary infections, lymphoma, anaemia, neuropathies, reproductive disorders, fading kitten syndrome |
| Behaviour | • Aggressive (cat fights, bites) or social (e. g., grooming, sharing food bowls) behaviour |
| Vaccination status | • FeLV-unvaccinated cats |
| Lifestyle | • Outdoor access<br>• Direct cat contact |
| Housing conditions | • Multicat household |
| Background | • Cats in households with FeLV Infection<br>• Stray cats (especially cats from regions without trap-neuter-return program)<br>• Cats from shelters (especially from shelters with inadequate testing programs and hygiene strategies)<br>• Cats from animal hoarders<br>• Cats with inadequate nutrition, sanitation, medical care |
| Origin | • Cats from countries with higher prevalence<br>• Cats from areas of a low purchasing power parity per capita and income |

**Transmission**

FeLV is contagious and spreads through close contact between virus-shedding cats and susceptible cats. Transmission of FeLV occurs primarily via saliva. Progressively infected cats constantly shed millions of virus particles in saliva, and shedding through saliva occurs relatively consistently in these cats (Francis et al., 1977; Gomes-Keller et al., 2006a; Gomes-Keller et al., 2006b). The concentration of virus in saliva and blood of healthy progressively infected cats is as high as it is in those with signs of illness (Helfer-Hungerbuehler et al., 2015b). FeLV is passed effectively horizontally among communal cats that have prolonged close contact. Fighting and biting behaviour, (Goldkamp et al., 2008; Gleich et al., 2009) as well as social behaviour, such as sharing food and water dishes, mutual grooming, and using common litter areas, are the most effective means of transmission. Although the virus can enter many tissues, body fluids, and secretions, it is less likely to spread via urine and faeces, and urine and faeces were not considered an important source of infection. However, it was shown that progressively infected cats shed FeLV RNA, DNA and infectious virus in faeces and urine (Cattori et al., 2009; Gomes-Keller et al., 2009). It was also demonstrated that naïve cats exposed to virus-containing faeces can develop anti-FeLV antibodies, demonstrating that infection through faeces without direct cat-to-cat contact can take place, but these cats remain

negative for FeLV antigen and provirus in blood and only develop abortive infection (Gomes-Keller et al., 2009). These results suggest that faecal shedding of FeLV might play a role in transmission but is probably of minor importance under natural circumstances. Nevertheless, sharing of litter pans by susceptible and progressively infected cats could increase the environmental infectious pressure (Gomes-Keller et al., 2009). Fleas have been considered a potential source of transmission because FeLV RNA has been detected in fleas and their faeces (Vobis et al., 2003b; Vobis et al., 2003a), but flea transmission does not seem to play a major role in nature. Iatrogenic transmission can occur via contaminated needles, instruments, fomites, or blood transfusions (Lutz et al., 2009; Nesina et al., 2015).
The viral envelope is lipid-soluble and readily susceptible to disinfectants, soaps, heating, and drying. The infectiveness of salivary FeLV declines relative rapidly on dry surfaces; however, during the first 30 to 60 minutes still significant amounts of FeLV survived under dry conditions at room temperature, which might be sufficient to transfer the agent, e.g. by sharing of food containers by several cats (Francis et al., 1979b). Under moist conditions FeLV survives even longer; after 48 hours only a minimal decrease of infectivity was found at room temperature (Francis et al., 1979b). Nonetheless close contact among cats is usually required for spread of infection, and indirect transmission (e.g., via saliva-contaminated humans) is very unlikely. Single cats kept strictly indoors are not at risk for acquiring infection. It is only because of latency (in regressively infected cats) and potential reactivation that viraemia is occasionally detected in middle-aged to old cats that have lived alone indoors since they were adopted as kittens. Due to the viral lability, a waiting period is not needed before introducing a new cat into a household after removal of an infected cat; nonetheless, thorough cleaning of all cat contact surfaces is recommended. FeLV is not a hazard in a veterinary hospital or boarding kennel if cats are housed in separate cages (Francis et al., 1977; Francis et al., 1979b), and routine cage disinfection and hand washing are performed between handling cats (Addie et al., 2015). FeLV is maintained in nature because infected cats can live and shed virus for many years.
Vertical transmission from mother to kittens occurs commonly in FeLV antigen-positive cats. Neonatal kittens can be infected transplacentally or when the queen licks and nurses them or through milk. Transmission also can occur in queens that are regressively infected (and therefore, have a negative result on routine tests). FeLV transmission via milk to offspring has even been described from in a queen with FeLV antigen-negative test results (Pacitti et al., 1986). If in utero infection occurs, reproductive failure in the form of foetal resorption, abortion, and neonatal death is common, although up to 20% of vertically infected kittens can survive the neonatal period and become progressively infected adults (Levy 2000). It is possible that new-born kittens from infected queens have negative FeLV antigen test results at the time of birth but can develop positive test results over the following weeks. Thus, if the queen or any kitten in her litter is infected, the entire family should be treated as if infected and should be isolated from uninfected cats. Moreover, not all kittens from an infected queen are necessarily infected; therefore, testing of all kittens individually is recommended.

## PATHOGENESIS

### Virus-Host Interaction: A(n) (un)balanced System

The outcome of FeLV infection (Table 5) is determined by a fight between the cat's immune system and the virus; this is true particularly in the early phase of infection, usually over the first 12 weeks after exposure, when in most cats the course the infection will take is determined (Hofmann-Lehmann et al., 2008). Therefore, it can be helpful to think of the outcomes of FeLV infection in terms of a set of balance scales, with the cat's immune response on one side and the virus on the other (Fig. 6). Depending on who wins the fight, FeLV or the cat's immune system, the balance can tip to one or the other side and the outcome can either be beneficial for the cat or not. Like a balance, the outcome is not set in stone for life, but it can tip to one or the other side also at a later timepoint, e.g. should the immune system be compromised. Indeed, the balance between host and virus can be altered by different factors, such as immunosuppression, coinfections, or change in environment, leading to a change in the outcome of FeLV infection.

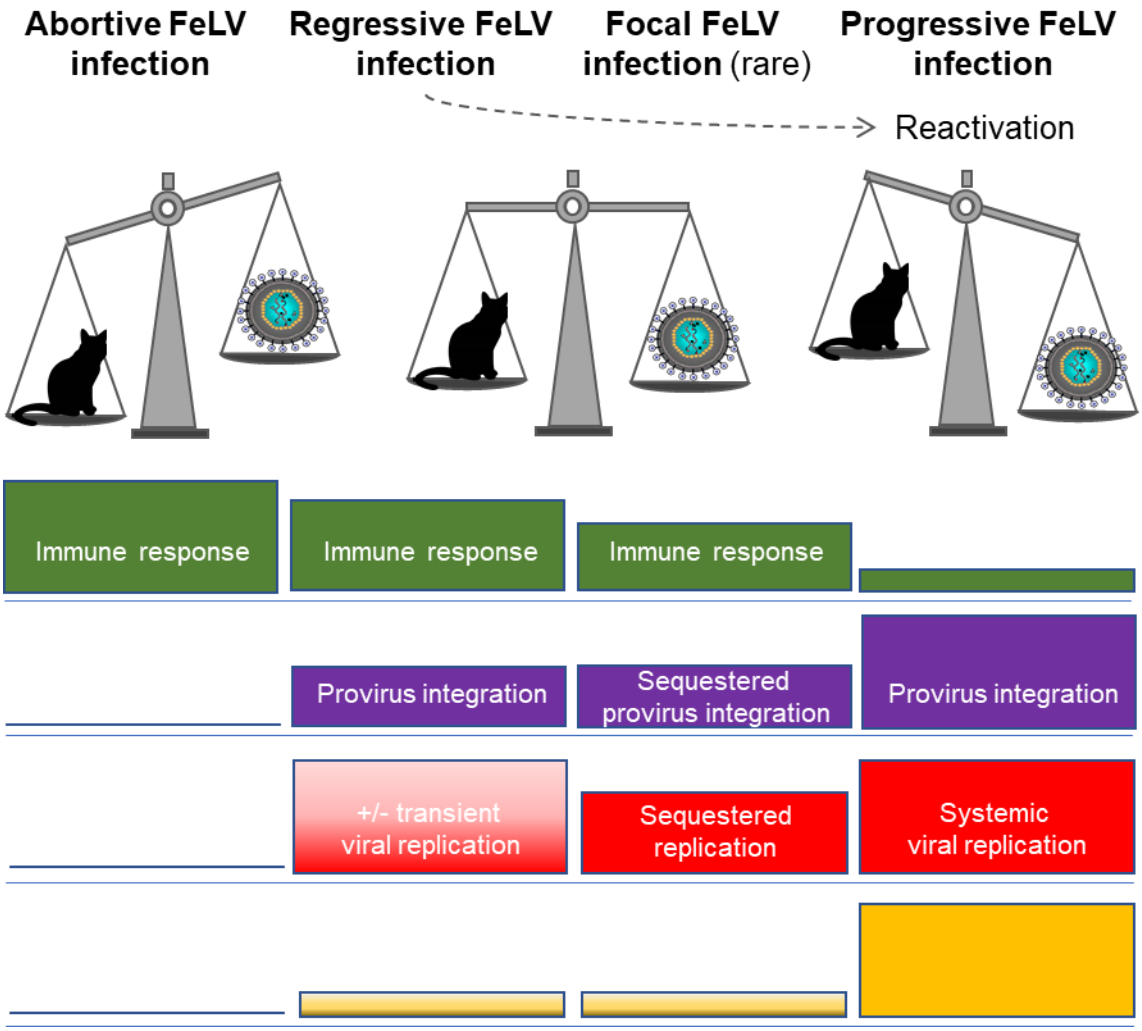


Fig. 6: Balance between the cat's immune system and FeLV. The outcome is not always a fixed situation; under certain circumstances the balance can tip to one side or the other over time depending on who has the upper hand, the virus, or the immune system of the cat. Immunosuppression for any reason can lead to shift in the balance, and the virus can gain the upper hand (balance on the far right; shift towards progressive infection). Protective immunity, e.g. vaccine-induced immune response, suppresses virus replication, and spread and leads to a situation that is more beneficial for the cat (situations on the left; regressive or ideally abortive infection). In abortive FeLV infection, the cat has the upper hand (more weight on the balance scales); in progressive FeLV infection, the virus has the upper hand. In regressive infection, the cat's immune system can keep the virus in check so that no, or only very limited, viral replication takes place, although reactivation of the infection (the recurrence of viraemia and virus shedding) can occur. In focal infection (rare), the cat's immune system keeps viral replication sequestered in certain tissues. For each infection outcome (abortive, regressive, focal, and progressive) the magnitude of the anti-FeLV immunity (green), provirus integration (violet), virus replication (red) and the potential to induce FeLV-associated disease (yellow) is shown. The three boxes with graduated colour indicate the possibility of either positive or negative status. Adopted from (Hofmann-Lehmann and Hartmann 2020).

**Virus Spread within the Cat**

In most cats exposed to FeLV, infection starts in the mucosa of the oropharynx where FeLV infects lymphocytes (Fig. 7). Subsequently, viral replication takes place in the adjacent tonsils and local lymph nodes (Rojko et al., 1979; Rojko and Kociba 1991). The virus can spread throughout the body via infected lymphocytes and monocytes in the lymphoid tissue (primary viraemia, mainly in lymphocytes and some monocytes) (Cattori et al., 2008). The virus can be transported to the bone marrow and infect rapidly dividing precursor cells. Once these cells become infected (usually in cats with progressive infection), large amounts of virions are produced. Replication in the bone marrow involves infection of neutrophil and platelet precursors and leads to the initiation of secondary viraemia and systemic infection (Rojko et al., 1979; Rojko and Kociba 1991). Thus, early in the infection, mainly lymphocytes (and to a lesser extend monocytes), later during viraemia mostly neutrophils become infected (Cattori et al., 2008). Often viraemia can develop several months after continuous

exposure of naïve cats to FeLV 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 (Francis et al., 1977; Rojko et al., 1979).

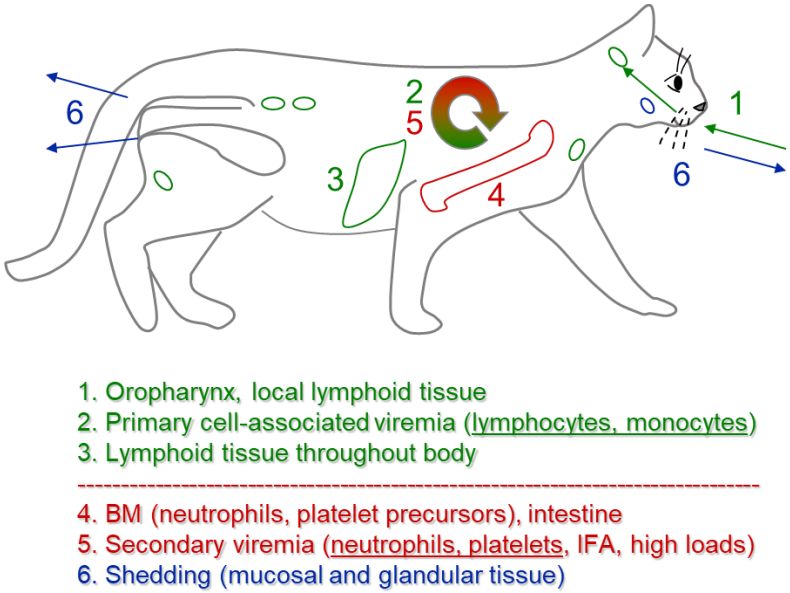


Fig. 7: Pathogenesis of FeLV infection. Cats are mostly infected via the oral-nasal route. The virus reaches the local lymphoid tissue and infects lymphocytes and monocytes spread the infection throughout the body (primary viraemia). In some of the cats, those with weak immunity to FeLV, the virus can reach the bone marrow and infect precursor cells, which results in the release of FeLV-infected neutrophils and platelets into the bloodstream (secondary viraemia). Subsequently, mucosal and glandular tissues are infected (gastrointestinal tract, salivary glands), and the virus is shed mainly by saliva, but also by faeces, urine, and milk. (Courtesy of R. Hofmann-Lehmann, Zurich, Switzerland)

**Outcomes of FeLV Infection**

The outcome of FeLV infection is different in each cat (Table 5). Although the primary outcome mainly depends on immune status and age of the cat, it is also affected by pathogenicity of the virus, and infection pressure (Hartmann 2005b). Outcome of FeLV infection also reflects genetic variation both in the virus and the naturally outbreeding host population. Mutational changes identified in FeLV strains were shown not to alter receptor usage, but to significantly increase the efficiency of receptor binding. Longitudinal studies of infected animals showed that certain mutations resulted in a significantly more rapid disease onset, whereas other substitutions in certain genes changed the disease outcome entirely, suggesting that the distinctive LTR and surface unit (SU) genes mediate a rapid pathogenesis with distinctive clinical features and oncogenic mechanisms (Levy 2000).
The outcome of FeLV infection can be characterized using different FeLV diagnostic tests for the detection of viraemia, integrated provirus, and the immune response directed to FeLV; in addition, it can be associated with potential virus shedding, development of FeLV-associated disease and in turn the prognosis (Table 5). Diagnostic tools, including very sensitive PCR methods, have created data that questioned the traditional understanding of FeLV pathogenesis (Torres et al., 2005; Hofmann-Lehmann et al., 2007; Hofmann-Lehmann et al., 2008). Previously, most FeLV pathogenesis studies were conducted using virus isolation and antigen detection. Accordingly, infection was characterized by undetectable, transient, or persistent viraemia. Using real-time PCR, the spectrum of host response categories to FeLV infection was refined by investigating proviral DNA and viral RNA loads. Cats believed to be immune to FeLV infection were identified to have positive provirus test results (Hofmann-Lehmann et al., 2001). FeLV provirus was found to persist for years, and recurrence of viraemia and disease development was observed in some cats (Helfer-Hungerbuehler et al., 2015b). Thus, cats with negative antigen and positive provirus test results are FeLV carriers and, after reactivation, can act as an infection source. However, integrated proviral DNA might also lead to solid protection and long-lasting maintenance of protective immunity (Hofmann-Lehmann et al., 2008). Therefore, the potential courses of FeLV infection have been reclassified (Torres et al., 2005; Hofmann-Lehmann et al., 2007; Hofmann-Lehmann et al., 2008), and the outcomes of FeLV infection are described as

- (1) abortive infection,
- (2) regressive infection (with or without “transient viraemia” followed by “latent infection”),
- (3) progressive infection (with “persistent viraemia”), and

- (4) focal infection.

In an early study in a multi-cat household without FeLV control measures, 30-40% of the cats developed persistent viraemia (progressive infection), 30-40% exhibited transient viraemia (regressive infection) and 20-30% developed antibodies without ever being detectably viraemic (abortive infection) (Hoover and Mullins 1991) (Fig. 8). A smaller proportion (approximately 5%) exhibited an atypical course of infection, displaying antigenemia but no or only low-level viraemia (Hoover et al., 1977; Lutz et al., 1983b). Newer research suggests that all infected cats remain infected for life after exposure but can revert to an aviraemic state in which no antigen or culturable virus is present in the blood but in which FeLV proviral DNA can be detected in the blood by PCR (regressive infection) or in which neither antigen or culturable virus nor proviral DNA, but only antibodies (abortive infection) are detected indication previous exposure (Hofmann-Lehmann et al., 2001; Torres et al., 2005; Pepin et al., 2007).

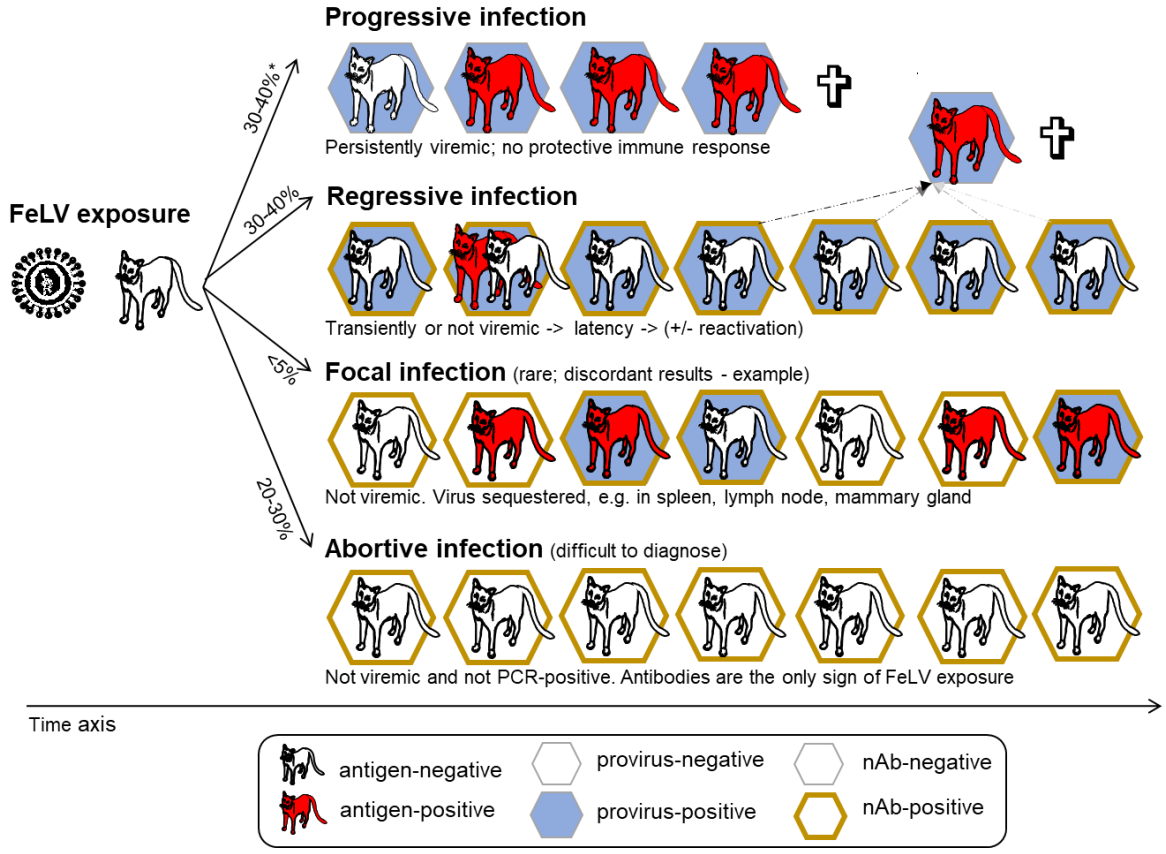


Fig. 8. Outcomes of an FeLV infection. Time course after FeLV exposure of a cat and the four potential FeLV infection outcomes (progressive, regressive, focal [rare] and abortive infection). *The percentages correspond to a multi-cat household with high infectious pressure with young cats (Hoover and Mullins 1991). In other situations, the rate of progressive infection will be much lower. Cats are depicted according to their FeLV p27 antigen (red), provirus (light blue) and neutralising antibodies (nAb; yellow) status. For regressive infection, the potential for reactivation in previously p27 antigen-negative cats decreases with time. ✞ = death. Adapted from (Hofmann-Lehmann and Hartmann 2020).

Before the development of PCR, a status of “latent infection” was described in which the absence of antigenemia was accompanied by persistence of culturable virus in bone marrow or other tissues but not in blood. “Latent infection” is now considered a phase through which cats pass during regressive infection. FeLV provirus and plasma viral RNA are usually detectable by PCR within one week of FeLV exposure, even if FeLV antigen is not yet detectable. All cats with progressive and regressive infection seem to undergo this phase and to develop similar proviral and plasma viral RNA loads in the peripheral blood during early infection (Hofmann-Lehmann et al., 2008). However, later in the infection, quantitative real-time PCR will show different proviral loads, which stays high in progressively infected cats but will decrease in regressively infected cats (Hofmann-Lehmann et al., 2001; Helfer-Hungerbuehler et al., 2015b; Duda et al., 2020).

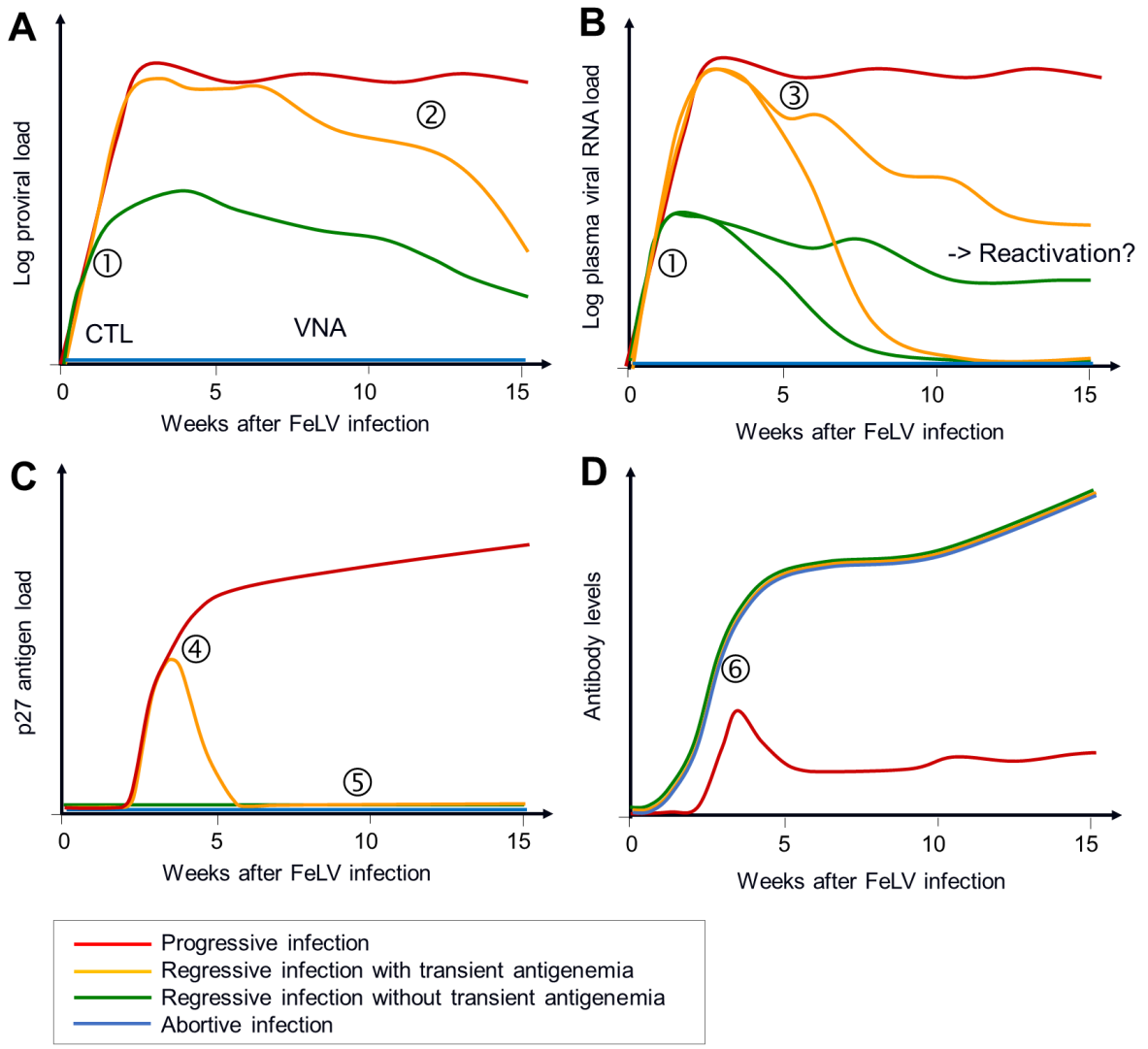


Fig. 9: Scheme of proviral (A), plasma viral RNA loads (B), FeLV p27 antigen loads (C) and anti-FeLV antibody levels (D) in cats with different FeLV infection outcomes (adapted from (Hofmann-Lehmann et al., 2008); data from cats with experimental infections). The outcome of FeLV infection (Table 5) is determined by a contest between the cat's immune system and the virus particularly in the early phase of infection (usually within 12 weeks). The proviral and plasma viral RNA loads during this period mirror this situation. Loads in cats with progressive infection are shown in red; those of cats with regressive infection with transient antigenemia in orange, without antigenemia in green. Cats with regressive infection can remain either plasma viral RNA positive or negative (B). Regressively infected cats with persistent viral plasma RNA might have a higher risk of reactivation (Hofmann-Lehmann et al., 2007). Cats with abortive infection are shown by blue lines; no provirus and plasma viral RNA are detectable in these cats. CTL: approximately time point when FeLV-specific cytotoxic T lymphocytes can be expected, as reported in an earlier study (Flynn et al., 2002). VNA: approximately time point when FeLV-specific virus neutralising antibodies become detectable (Hofmann-Lehmann et al., 2006). ① Proviral and plasma viral RNA loads are not significantly different between regressively and progressively infected cats in the first week after infection (primary viremia). ② Provirus loads are not significantly different between cats with progressive infection and cats with regressive infection and transient antigenemia prior to week 12 after infection (secondary viremia). ③ Plasma viral RNA loads are not significantly different between cats with progressive infection and cats with regressive infection with transient antigenemia prior to week 5 after infection. ④ FeLV p27 antigen loads are not significantly different between cats with progressive infection and cats with regressive infection and transient antigenemia in the first weeks after infection (primary viremia). ⑤ Thereafter, cats with regressive infection have very low FeLV p27 antigen loads. ⑥ Regressively infected cats have higher antibody levels as early as 3 weeks after virus exposure compared to progressively infected cats (Hofmann-Lehmann et al., 2008).

Table 5: Characterization of possible outcomes of FeLV infection and test results defining them. Adapted from (Hofmann-Lehmann and Hartmann 2020).

| **Parameter** | **Progressive infection** (formerly "persistent viraemia") | **Regressive infection** (with or without "transient viraemia") | **Focal infection** (rare) | **Abortive infection** (formerly "regressor cats") | **No infection** |
|---|---|---|---|---|---|
| **Viraemia** | Persistent viraemia | Undetectable or transient viraemia | No viraemia | No viraemia | No viraemia |
| **Replicating virus blood** (virus isolation from blood samples) | Positive | Negative (only positive during transient viraemia or after reactivation) | Negative | Negative | Negative |
| **Viral RNA blood** (RT-PCR of blood samples) | Positive | Positive or negative | Negative | Negative | Negative |
| **Free FeLV p27 antigen in blood** (POC tests or plate-based ELISA) | Positive (~ 3-6 weeks after infection) | Negative (only positive during transient viraemia or after reactivation) | Alternating or low positive | Negative | Negative |
| **Intracellular FeLV p27 antigen blood** (IFA on blood smear) | Positive (~ 3 weeks after FeLV p27 antigen tests) | Negative (only positive during transient viraemia or after reactivation) | Negative or alternating | Negative | Negative |
| **Provirus integration into host's genome** | Yes | Yes | Yes (localised) | No | No |
| **Proviral FeLV DNA blood** (PCR on whole blood) | Positive | Positive | Negative or low positive | Negative | Negative |
| **Immune response** | Poor | Good | Good | Very good | No |
| **Anti-FeLV antibodies serum** (tests on serum/plasma to detect different antibodies; e.g. against p15E or SU) | Negative (or low titres) | Positive (high titres) | Positive (high titres) | Positive (variable titres) | Negative |
| **Viral shedding** | Yes (continuously); major source of FeLV infection | No (only during transient viraemia or after reactivation) | Usually no (one report on shedding in milk) | No | No |
| **Viral RNA saliva** (RT-PCR of saliva samples) | Positive | Negative (only during transient viraemia or after reactivation) | Negative | Negative | Negative |
| **Transmission of infection via blood transfusion** | Yes | Yes | Potentially | No | No |
| **Reactivation of FeLV infection** | No (infection already continuously active) | Possible, but seldom (decreasing probability with increasing timespan after exposure) | No (infection sequestered continuously active) | No | No |
| **FeLV-associated disease** | Common | Uncommon (lymphoma or bone marrow suppression) common after reactivation | Unlikely | None | None |
| **Prognosis** (in respect to FeLV infection) | Poor | Good; poor after reactivation | Variable | Good | Good |

Once infection is established (compare also above – factors that influence the prevalence of FeLV), the clinical outcome in each individual cat is determined by a combination of viral and host factors (Table 6). Infectious pressure is very important to determine the course of infection, but outcome also reflects genetic variation both in the virus and the naturally outbreeding host population. Properties of the virus include the subgroup that determines differences in the clinical picture. Also, mutational changes identified in FeLV strains were shown to significantly increase efficiency of receptor binding. Probably the most important host factor that determines the outcome is the age of the cat at the time of infection (Hoover et al., 1976).
To make the picture and understanding of FeLV even more complicated it was shown that although the majority of cats after a few weeks to months into infection follow one of the four described courses, during the early phase of FeLV infection, during which the interplay of the cat's immune system and the virus is at its peak and the host-virus balance has not yet been definitively set, varying and discrepant FeLV test results can be observed (Westman et al., 2019a). However, even later on during the infection, the host-virus balance is not fixed and might tip to the advantage of either party (Fig. 6) and, in turn, change the outcome/course of FeLV infection (Hofmann-Lehmann et al., 1995). These exceptions include cats that have been positive for free FeLV p27 antigen for a long period of time (which would be classified as progressive infection) and then become antigen-negative (Hofmann-Lehmann et al., 1995). On the other hand, in some regressively infected cats, FeLV infection might be reactivated (Helfer-Hungerbuehler et al., 2015b). This shows that FeLV kinetics seems to be an instable balance, and not only during the initial infection; the outcome is determined by host and pathogen factors, but in some cats there can be a life-long battle between the immune system and the virus.

***Infection pressure.*** Outcome of FeLV infection is different based on the infectious pressure, e. g. a cat that has a one-time short contact to a FeLV-shedding cat will have a different infection course than in a cat living together with many FeLV-shedding cats in the same household over months to years sharing the same food and water bowls and litter boxes. It was suggested, that in a cat with a first-time single contact with a FeLV-shedding cat, the risk of developing progressive infection is only about 3%. However, if an FeLV-shedding cat is introduced into a naïve group of cats, and the cats are housed together for an extended period, the risk for a cat to develop progressive infection increases to an average of 30% (Hoover and Mullins 1991) The importance of infectious pressure on outcome was also demonstrated in an experiment, in which naïve cats were exposed to faeces of progressively FeLV-infected cats, which can be considered a low-grade challenge. These exposed cats only developed abortive FeLV infection (only antibodies were present, while all direct virus detection methods remained negative) (Gomes-Keller et al., 2009).

***FeLV Subgroups.*** FeLV isolates are divided in different subgroups that determine the outcome of FeLV infection and prognosis (see also above). Today, FeLV classification, transmission, and disease-inducing potential of different subgroups have been defined sequentially by viral interference assays, Sanger sequencing, PCR, and next-generation sequencing (Chiu et al., 2018). FeLV subgroups are immunologically closely related but use different cellular receptors (Table 1) (Bupp et al., 2006; Mendoza et al., 2006; Shojima et al., 2006; Rey et al., 2008; Miyazawa 2009). The three best and longest known FeLV subgroups are FeLV-A, FeLV-B, and FeLV-C. Only FeLV-A is contagious and passed horizontally from cat to cat in nature. The other subgroups evolve de novo in a FeLV-A-infected cat by mutation and recombination between FeLV-A and cellular or endogenous retroviral sequences contained in feline genomic DNA.
Pathogenicity of FeLV-B and FeLV-C, in combination with FeLV-A, is higher than that of FeLV-A alone (Rojko et al., 1988). Different properties of the envelope proteins of the subgroups have been shown to be the major pathogenic determinant, but the mechanisms by which envelope differences influence pathogenesis are not well understood (Moser et al., 1998). FeLV-B is commonly associated with malignancies; it is particularly frequently associated with mediastinal lymphoma but FeLV-B recombinants also occur infrequently and at low levels also in non-T-cell diseases in multicentric lymphoma (Ahmad and Levy 2010). In experimental infections, a FeLV-B strain caused lymphoma in nearly 100% of kittens by one year of infection (Pedersen 1998). FeLV-C is mainly associated with aplastic anaemia (Hoover et al., 1974; Mackey et al., 1975; Abkowitz 1991). Genetic characterisation of FeLV-C linked changes in the *env* gene to the disease phenotype (Riedel et al., 1986; Riedel et al., 1988); indeed precise deletions/mutations might be sufficient to confer the disease phenotype (Brojatsch et al., 1992; Rigby et al., 1992). The FeLV-C FLVCR receptor interaction blocks the differentiation of erythroid progenitors between burst-forming units and colony-forming units by interfering with signal transduction pathways essential for erythropoiesis (Quackenbush et al., 1990; Shelton and Linenberger 1995; Young et al., 2000; Philip et al., 2015). Bone marrow examination of FeLV-C infected cats shows an almost complete lack of erythroid precursors (at least of the late forms) with normal myeloid and megakaryocytic precursors and an increased myeloid-erythroid ratio. These cats typically have macrocytosis without reticulocytes (Cotter 1998; Levy 2000).
Other FeLV subgroups have been described more recently. FeLV-D arose from recombination of FeLV-A and the env gen of a feline endogenous gammaretrovirus (Anai et al., 2012; Ito et al., 2013), and was detected in the blood or tumour tissues of 1.1% of FeLV-infected cats but it is not clear whether FeLV-D is infectious or pathogenic. FeLV-T was identified in an experimental setting and is highly cytolytic for T lymphocytes and causes severe immunosuppression (Lauring et al., 2001; Lauring et al., 2002; Barnett et al., 2003; Shojima et al., 2006), but is likely not important in nature. FeLV-FAIDS was isolated from a cat with thymic lymphoma; when inoculated experimentally, it induced fatal acquired immunodeficiency syndrome in persistently viraemic cats (Hoover et al., 1987). In addition, it was suggested that selection pressure in cats can cause novel FeLV subgroups to emerge, and further new strains, not belonging to a known subgroup are detected (Miyake et al., 2016; Miyake et al., 2019).

A study examined the interplay between endogenous and exogenous FeLV in a large domestic cat breeding colony naturally infected with FeLV by investigating point-in-time enFeLV and FeLV viral loads, as well as occurrence of FeLV/enFeLV recombinants (FeLV-B) and factors relating to clinical disease in a closed breeding colony of cats during a natural infection of FeLV (Powers et al., 2018). Progressive FeLV disease and FeLV-B presence were associated with higher FeLV proviral and plasma viral loads. Female cats were more likely to have progressive disease and FeLV-B. Conversely, enFeLV copy number was higher in male cats and negatively associated with progressive FeLV infection. Males were more likely to have abortive FeLV infection (Powers et al., 2018).

***Mutations in the FeLV genome.*** Besides the influence of the different subgroups, outcome of FeLV infection is also determined by genetic variation within subgroups. Although not altering receptor usage, mutational changes in FeLV strains were identified that significantly increased efficiency of receptor binding. Longitudinal studies of infected cats showed that certain mutations resulted in a significantly more rapid disease onset (Levy 2000). Oncogene-containing retroviruses are generated by "oncogene capture", recombination events between viral and cellular sequences, e.g., a novel FeLV variant that has captured a feline oncogene was described (Kawamura et al., 2016).

***Age of the cat.*** One important host factor that determines the courses of infection and the clinical outcome is the age of the cat at the time of infection (Hoover et al., 1976) as susceptibility of cats to FeLV is age-dependent (Hoover et al., 1976; Hosie et al., 1989). As cats mature, they acquire an increasing resistance. Age resistance is independent of immunity from previous contact or vaccination. When older cats become infected, they tend to have abortive or regressive infections or, if developing progressive infection, at least to have milder signs and a more protracted period of apparent good health (Levy 2000). Thus, likelihood of becoming progressively infected is highest in young kittens. A study in a household with FeLV-infected cats showed that 70% of kittens placed there at three months of age became antigen-positive within five months (Cotter 1991). Neonatal kittens develop marked thymic atrophy after infection (“fading kitten syndrome”), resulting in severe immunosuppression, wasting, and early death. Experimental infection gets more difficult when cats become adult. However, this depends on the FeLV strains used. In one study, experimental infection was even difficult to achieve already in kittens older than 16 weeks of age (Hoover et al., 1976), while in another study, cats were experimentally infected with FeLV at the age of 14 months, and 83% of naïve cats developed progressive infection. The virus challenge was performed intraperitoneally but in the absence of any immunosuppressive drugs (Lehmann et al, 1991). Thus, the age resistance in adult cats might not be absolute and be overcome if the virus is transmitted directly into the blood stream, e.g. by biting of an infected cat. A potential explanation for the age resistance might be that the number of cellular receptors (THTR1) necessary for FeLV-A to enter target cells might decrease in older cats, and thus, establishment of infection might become more difficult. THTR1 protein and mRNA levels are higher in intestine and kidney of juvenile compared to adult mice (Reidling et al., 2006). However, a study on THTR1 mRNA transcription levels in oral mucosa scrapings and blood samples from cats found only a decrease of THRR1 transcription in blood but not in oral mucosa scrapings with increasing age of the cats and thus, the FeLV susceptibility is probably not primarily or solely related to the FeLV receptor levels at the site of virus entry or in the blood (Helfer-Hungerbuehler et al., 2011; Mendoza et al., 2013; Miyake et al., 2019). Another potential explanation for age resistance was related to maturation of macrophage function (Hoover and Mullins 1991). Age-related resistance is not absolute; however, the risk of an adult cat to become progressively infected after one short contact with a FeLV-shedding cat is certainly very low.

***Genetic host factors.*** It has been proposed that a genetic predisposition might contribute to a susceptibility of a cat for FeLV. Analysis of single-nucleotide polymorphisms (SNPs) in the APOBEC3H gene of domestic cats showed that one of the SNPs, A65S (A65I), was significantly correlated with the susceptibility to FeLV infections. On the other hand, haplotype analysis demonstrated that the combination "GGGGCC" was positively correlated with lack of FeLV infections. Thus, variability of restriction factors might contribute to susceptibility of cats to FeLV infection (de Castro et al., 2014). One study tried to define dominant host immune effector mechanisms responsible for the outcome of infection by using longitudinal changes in FeLV-specific cytotoxic T-lymphocytes (CTL). High levels of circulating FeLV-specific effector CTLs appeared before virus-neutralising antibodies in cats that have recovered from exposure to FeLV. In contrast, progressive infection with persistent viraemia was associated with a silencing of virus-specific humoral and cell-mediated immunity host effector mechanisms (Flynn et al., 2002).

Table 6: Risk factors influencing courses of feline leukaemia virus (FeLV) infection (modified from (Hartmann and Hofmann-Lehmann 2020b)). For risk factors influencing FeLV prevalence see Table 4.

| | |
|---|---|
| Signalment | • Young age at time of infection |
| Immune system | • Insufficient virus-specific humoral and cell-mediated immunity<br>• Absence of FeLV antibodies<br>• Lack of FeLV vaccination |
| Infectious pressure | • High FeLV viral load in the environment<br>• Long-term contact with FeLV-shedding cats (multicat households with FeLV) |
| Virus virulence | • Emergence of FeLV subgroups within the cat (e. g., FeLV-B, FeLV-C)<br>• Mutations in the FeLV genome or integration of the FeLV genome near proto-oncogenes, both leading to activation of proto-oncogenes |

The outcomes of FeLV infection are classified as (1) abortive infection, (2) regressive infection (with or without "transient viraemia" followed by "latent infection"), (3) progressive infection (with "persistent viraemia"), and (4) focal (or sometimes called atypical) infection.

**Abortive Infection**

There is a group of cats that is able to confine FeLV infection prior to provirus integration into the host's cells genome. The virus never spreads systemically, and infection usually remains undetected. All diagnostic assays that detect the virus or parts of it (FeLV p27 antigen test, virus isolation, RT-PCR, provirus PCR) test negative at any stage, and antibody responses are the only sign of previous FeLV exposure (Fig. 6 and Table 5). Of the various infection outcomes, this is the most favourable for the cat – the balance is tilted in favour of the cat (Fig. 6). Abortively infected cats have strong anti-FeLV immunity. Abortive infection outcome has been observed infrequently after experimental FeLV inoculation. It can occur particularly after low-dose infections (Torres et al., 2005; Gomes-Keller et al., 2009; Major et al., 2010). It is possible that abortive infections are quite common in nature; cats that are antigen- and provirus- negative and antibody-positive but have never been vaccinated were relatively common in the field in one study (Englert et al., 2012). Usually only specialized laboratories offer quantification of anti-FeLV antibodies. Only recently, the first routine anti-FeLV antibody POC test became commercially available in Europe; however, there is not yet sufficient data on the diagnostic efficiency of this test under field conditions.

**Regressive Infection**

A regressive infection outcome is a unique feature for a retroviral infection. It is the second-best outcome for the cat (Fig. 6). Regressive infection can occur with or without a transient antigenemia (Fig. 9) and is accompanied by a partially effective antiviral immune response, which contains virus replication and viraemia before or shortly after the time of bone marrow infection (Hoover et al., 1975; Rojko et al., 1979; Flynn et al., 2000; Torres et al., 2005; Hofmann-Lehmann et al., 2006). Regressively infected cats can be recognized in the field by antigen-negative and provirus-positive FeLV tests and a strong antiviral immune response (Fig. 6 and Table 5).
Regressive infection was first demonstrated in a study in Switzerland, where 597 cats were sampled in 1996/1997; among these, 7% had both positive antigen and proviral test results, while an additional 10% of the cat population tested negative for antigen and positive for proviral DNA using PCR, characteristic for regressive infection (Hofmann-Lehmann et al., 2001). Similarly, but somewhat lower percentages were subsequently found in a follow-up study in Switzerland, when in 2004/2005 still 5.4% of the tested 445 tested cats were provirus carriers in the absence of antigenaemia (Gomes-Keller et al., 2006a), and in a study in Germany in 2007/2008, where only 1.2% of the 495 tested cats had regressive infection (antigen-negative, provirus-positive) using the same methods (Englert et al., 2012). In Ireland, 7.8% (16/183 cats) of cats presented to veterinarians were provirus-positive and antigen-negative (Szilasi et al., 2021). So, between 1 and 11% of cats seem to be regressively infected, although there seem to be large geographic differences in the prevalence of regressively infected cats similar as for progressively infected cats.

After infection, FeLV spreads within lymphocytes and monocytes (Cattori et al., 2008) and during this phase (primary viraemia), FeLV antigen can be detectable also in regressively infected cats. These cats can thus temporarily test positive for antigen in (e.g., by POC test or ELISA-based laboratory tests). The initial viraemia can be characterized by lethargy, fever, or lymphadenomegaly resulting from lymphocytic hyperplasia, clinical signs, that are usually only observed during experimental infections and not after natural infection. The virus spreads to target tissue, including thymus, spleen, lymph nodes, and salivary glands. In most regressively infected cats, viraemia lasts 1-12 weeks; in rare cases, cats can overcome viraemia even after many months (Hofmann-Lehmann et al., 1995), although the likelihood of clearance of viraemia decreases with time. This phase of infection was formerly called "transient viremia" (Lutz et al., 1980a) and cats can shed

virus during this period and are infectious. After virus replication is contained, viral shedding seizes (Lutz et al., 1983a; Flynn et al., 2000; Flynn et al., 2002).
Whether a viremia occurs and can be cleared depends on the balance between the cat's immune system and the virus (Fig. 6) and can be influenced by many factors, such as the age and immune status of the cat, concurrent stressors, coinfections, the specific virus isolate and the exposure level (Table 6). In some cats, viraemia can persist longer than three weeks. After about this time period, bone marrow cells become infected (Fig. 7), and affected haematopoietic precursor cells produce infected granulocytes and platelets that circulate in the body (Pepin et al., 2007; Cattori et al., 2008). In this circumstance, a high level viraemia is present, and lymphoid organs and salivary glands become infected leading to shedding of up to 1 x $10^6$ virus particles/ml of saliva. From this time point on, viral antigen is also detectable intracellularly in platelets and granulocytes by tests that detect large quantities of intracellular antigen, such as IFA. In contrast to antigen tests (e.g., ELISA) that become positive during the first viraemia, IFA results become positive later and only after infection is established in the bone marrow. This explains discordant ELISA-positive and IFA-negative results. Even if bone marrow becomes infected, a certain percentage of cats can clear viraemia and develop regressive infection. To determine whether a cat that initially tests positive for FeLV antigen undergoes regressive or progressive FeLV infection, repeated testing for FeLV antigen is necessary. Regressively infected cats will eventually test antigen negative. Occasionally, outcomes can be observed with cats not following the defined FeLV infection courses. Some cats can test transiently antigen-negative after being positive during the initial viraemia and can later become persistently positive (developing progressive infection), while some cats show alternating test results in the early phase of infection but subsequently overcome viraemia and develop regressive infection (Hofmann-Lehmann et al., 1997; Hofmann-Lehmann et al., 2006).

Following recovery from potential viraemia, regressive infection can only be diagnosed by PCR to detect provirus (Hofmann-Lehmann et al., 2001; Gomes-Keller et al., 2006a; Hofmann-Lehmann et al., 2008; Helfer-Hungerbuehler et al., 2015b) or, as done before the invention of PCR, by in vitro culture of bone marrow samples in the presence of high doses of glucocorticoids for several weeks to recover infectious virus (Rojko et al., 1982). When dormant virus could be reactivated in the cell culture assay, the cat was thought to have "latent infection" and the virus would be kept in check by the cat's immune system and not eliminated. Using cell culture assays, latency was found to last up to many months (Pacitti and Jarrett 1985). Using molecular assays, it was subsequently shown that cats with experimentally regressive infection stayed provirus-positive life-long indicating that none of the regressively infected cats really cleared the infection (Helfer-Hungerbuehler et al., 2015b). The mean proviral load in cats with regressive infection is several hundred times lower than in cats with progressive infection once the infection has fully established several weeks after infection (Hofmann-Lehmann et al., 2001).
Importantly, reactivation can occur in any regressively infected cat, spontaneously or in response to immune suppression, and the cat can become viraemic and show positive results in antigen testing and shedding. Reactivation usually occurs after stress and can be experimentally induced in cats by administration of high doses of glucocorticoids (Rojko et al., 1982). Regressive infections can also reactivate in pregnancy due to immunosuppressive effects of endogenous progesterone, which also can explain the re-emergence of FeLV infection in kittens from regressively infected cats. Mammary glands of regressively infected queens can begin to produce infectious viral particles during the induction of lactation (Pacitti et al., 1986). Reactivation is more likely the earlier the stress factor occurs after the viraemic phase but becomes less likely over time (Rojko et al., 1982; Madewell and Jarrett 1983; Pedersen et al., 1984; Pacitti and Jarrett 1985; Hayes et al., 1992; Hofmann-Lehmann et al., 2001). Nonetheless, FeLV infection can be reactivated even many years later, if the immune system is severely depressed (Helfer-Hungerbuehler et al., 2010). This was demonstrated in an experimentally FeLV infected cat that was coinfected with FIV; FeLV infection was reactivated after 8.5 years, when FIV had depleted the immune system of this cat. A genetically altered FeLV variant reappeared in the blood of this cat, in conjunction with the development of multicentric lymphoma (Helfer-Hungerbuehler et al., 2010). Thus, at least part of the proviral DNA remained replication-competent in this regressively infected cat for many years. Regressively infected cats might or might not be viral plasma RNA-positive as detectable by RT-PCR (Tandon et al., 2005; Hofmann-Lehmann et al., 2008; Torres et al., 2008), and there is some evidence that persistence of plasma viral RNA in regressively infected cats might be a prognostic marker for subsequent reactivation (Hofmann-Lehmann et al., 2007; Helfer-Hungerbuehler et al., 2015b). Regressively infected cats probably never clear FeLV infection completely (Helfer-Hungerbuehler et al., 2015b); however, the proviral loads might be very low and might at least temporarily drop under the detection limit, depending on the sensitivity of the FeLV proviral PCR used.

The clinical relevance of regressive infection has not been fully established; in some instances, immunosuppression or infection with other viruses can lead to reactivation. However, many regressively infected cats might not be of great clinical and epidemiological importance. If the infection does not reactivate, the cat is not shedding FeLV and does not pose an infection risk for naïve cats. It needs, however, to be recognized that regressively infected cats will transmit FeLV infection via transfusion to blood recipients; thus, all blood donors must also be screened for regressive FeLV infection using PCR (Nesina et al., 2015). On the positive side, cats with regressive infection develop a very effective immunity characterized by virus neutralizing antibodies, which are a measure for protection against new infections with FeLV in these cats. Moreover, regressively infected cats usually have a low risk of developing FeLV-associated clinical signs, although lymphoma or bone marrow suppression have been described in some cats with regressive infection (Stützer et al., 2010; Stützer et al., 2011). For most pathogenic mechanisms by which FeLV causes clinical signs, active virus replication is necessary, and this is not the case in regressive FeLV infections. One study found a high prevalence of regressive infection in older cats at necropsy

and suggested an association with haematologic changes and inflammatory processes (Suntz et al., 2010). Moreover, regressive infections can explain how myelosuppression or haematopoietic malignancy could be FeLV-related in cats with negative FeLV antigen test results. In one study, two of 37 cats (5%) with nonregenerative cytopenias and negative FeLV antigen test had positive results with bone marrow PCR, suggesting that regressive FeLV infection can cause myelosuppression (Stützer et al., 2010). Some studies also detected FeLV provirus in lymphomas of cats that had negative FeLV antigen results (Gabor 1991; Jackson et al., 1993; Wang et al., 2001), although regressive infection does not seem to be common in cats with lymphoma in a more recent study (Stützer et al., 2011). FeLV provirus can be inserted at many different sites in the host's genome, carrying potent regulatory signals. In the development of myelosuppressive disorders or tumours, integrated FeLV provirus can interrupt or inactivate cellular genes in the infected cells, or regulatory features of viral DNA might alter expression of neighbouring genes. In addition, because bone marrow microenvironment cells (e.g., myelomonocytic progenitor cells and stromal fibroblasts) provide a reservoir for regressive FeLV infections, it seems possible that the integrated provirus can alter cellular functions and contribute to the pathogenesis of myelosuppressive disorders. Finally, FeLV not only contributes its genes to the host, it also has been shown to appropriate cellular genes. Several such transducted genes that are also present in regressively infected cells have been implicated in viral oncogenesis (Rezanka et al., 1992; Sheets et al., 1993; Rohn et al., 1994). It was also discussed whether endogenous retroviruses could recombine with FeLV and thus, produce infectious oncogenic particles. However, using next-generation RNA-sequencing this could not be confirmed and no expression of FeLV was shown in the investigated tumours. There was even a decreased expression of endogenous retrovirus genes in lymphoma versus control samples (Krunic et al., 2015).

**Progressive Infection**

Progressive infection is the worst outcome for cats with FeLV infection (Fig. 6). Cats with progressive infection have an insufficient FeLV-specific immune response; they are lacking neutralising antibodies and FeLV specific cellular immune response. The virus gains the upper hand. It is not contained early in the infection, and extensive replication occurs, first in the lymphoid tissues and then in the bone marrow and in mucosal and glandular epithelial tissues (Rojko et al., 1979). Mucosal and glandular infection is associated with excretion of infectious virus. Progressive infection is characterized by persistent viraemia. Bone marrow infection is associated with infection of neutrophils and platelets in addition to lymphocytes and monocytes (Pepin et al., 2007; Cattori and Hofmann-Lehmann 2008); these cats thus also test positive for intracellular FeLV p27 antigen as determined by IFA after bone marrow infection is established (Table 5).
Cats with progressive FeLV infection are clinically and epidemiologically the most important ones to identify. Progressively infected cats pose an infection risk to other cats for the remainder of their lives; they continuous shedding of high numbers of FeLV particles. They should be kept separated from FeLV-naive companions, regardless of the health status of the FeLV-infected cat. Moreover, progressively infected cats have a poorer prognosis than cats with regressive FeLV infection (Table 5). They are at high risk of succumbing to potentially fatal FeLV-associated diseases, sometimes within just a few months (Hofmann-Lehmann et al., 1997; McCaw et al., 2001; Hofmann-Lehmann et al., 2007; Gleich et al., 2009; Helfer-Hungerbuehler et al., 2015b; Little et al., 2020). Nonetheless, many progressively infected cats can continue to live a healthy and happy life for many years, if well cared for.
The risk for the development of a progressive infection primarily depends on immune status and age of the cat, but also on the infection pressure. Young and immunosuppressed cats are at higher risk for developing progressive infection. In a cat with a first-time single contact with an FeLV-shedding cat, the risk of developing progressive infection averages only 3%. However, if an FeLV-shedding cat is introduced into a naïve group of cats, and the cats are housed together for an extended period, the risk for a cat to develop progressive infection increases to an average of 30% (Hoover and Mullins 1991).

Regressive and progressive infections can be distinguished by repeated testing (Torres et al., 2005). Progressively infected cats usually are repeatedly positive for FeLV p27 antigen, when tested several weeks or months apart (Little et al., 2020). In most cats with progressive infection, FeLV p27 antigen testing is positive within a few weeks after virus exposure. However, in a few cats, the progressive FeLV infection status can take several weeks to develop after initial FeLV contact and/or cats can have FeLV p27 antigen test results that alternate between negative and positive, particularly during early infection before the host–virus balance finds a steady-state Fig. 6) (Hofmann-Lehmann and Hartmann 2020).
Early after FeLV infection, no difference is present in proviral and plasma viral RNA loads between cats with progressive and regressive infection (Hofmann-Lehmann et al., 2001; Hofmann-Lehmann et al., 2007). Thus, at very early time points after FeLV exposure, FeLV proviral or plasma viral RNA loads cannot be used to differentiate these two infection outcomes. However, a few weeks later, cats with progressive infection have higher proviral blood and plasma viral RNA loads than regressively infected cats (Hofmann-Lehmann et al., 2001; Tandon et al., 2005; Torres et al., 2005; Torres et al., 2008; Helfer-Hungerbuehler et al., 2015b). Therefore, once FeLV infection is definitively established, proviral and viral RNA loads can be used to help distinguish progressive from regressive infection. In the field, given that it cannot usually be determined at what stage a FeLV-infected cat is at, the proviral and plasma viral RNA loads alone at a single time point are not sufficient to determine whether the cat has progressive or regressive infection. Therefore, repeated testing at 6-week intervals is recommended to clearly identify the course of infection.

### Focal Infection

Focal infection is sometimes also called “localised infection” or “atypical infection” because diagnostic tests in these cats can change over time and commonly do not follow a typical pattern. In these cats it can be frustrating to identify FeLV infection. In cats with focal infection, free FeLV p27 antigen can be present in the blood but not infectious virus; these cats have been described in earlier studies as ‘discordant cats’ (Jarrett et al., 1982b; Lutz et al., 1983b; Jarrett et al., 1991; Miyazawa and Jarrett 1997). In cats with focal infection, the cat’s immune system keeps FeLV replication sequestered to certain tissues, such as the spleen, lymph nodes, small intestine, urinary tract, or mammary glands (Pacitti et al., 1986; Hayes et al., 1989; Hoover and Mullins 1991; Jarrett et al., 1991; Miyazawa and Jarrett 1997). Production and release of FeLV antigen into the blood (but no or only minimal release of infected cells with provirus integration) in these cats can be intermittent or low-grade. Therefore, these cats can have weakly positive or discordant results in antigen tests, or positive and negative results can alternate. Cats with focal FeLV infection are rare and probably not a major epidemiological concern, but this infection outcome can lead to perplexing appearances of FeLV infections and confusing FeLV test results. One case of focal infection is well documented in a queen, where the virus had sequestered in the mammary glands; during a phase of negative FeLV antigen test results in the blood, the queen transmitted the virus to the kittens via the milk (Jarrett 1985; Pacitti et al., 1986).
Focal infections with discordant test results have been reported under experimental conditions (Jarrett et al., 1982a; Hayes et al., 1989; Hayes et al., 1992) and have also been observed in up to 10% of naturally FeLV-infected cats (Jarrett 1985; Jarrett et al., 1991; Miyazawa and Jarrett 1997; Westman et al., 2019a). In one study, about one-third of the cats with discordant FeLV test results were provirus PCR-positive and it was assumed that in these cats the bone marrow was infected (Miyazawa and Jarrett 1997). Finally, there are some cats with discordant or alternating test results in the early stage of FeLV infection, when the host-virus balance is not yet established.

## IMMUNITY

### Passive Immunity

Experimentally, susceptible kittens can be protected from FeLV infection following passive immunisation with high titre antisera against FeLV (Hoover et al., 1977). Even the transfer of serum with low titres of acquired neutralising antibodies from previously FeLV-infected aviraemic queens to their kittens provided protection from a large FeLV dose (Jarrett et al., 1977). It was therefore assumed that under natural conditions many sucking kittens of queens with FeLV neutralising antibodies are protected from infection during the period of greatest susceptibility (Jarrett et al., 1977). However, protection was not long-lasting; at the age of sixteen weeks, the antibodies were not detectable anymore and kittens were left unprotected (Jarrett et al., 1977). Moreover, once progressive infection has become established, passive immunisation with monoclonal antibodies was ineffective (Weijer et al., 1986b). Nonetheless, the observations with prophylactic passive immunisation suggest that antibodies have a role in providing protection, and it was reasonable to assume that production of an effective FeLV vaccine would be possible.

### Active Immune Response

Most cats that overcome FeLV viraemia exhibit high virus neutralising antibody titres (Russell and Jarrett 1978; Lutz et al., 1980a; Flynn et al., 2002). Cats with regressive infection have higher antibody titres than cats with progressive infection (Hofmann-Lehmann et al., 2001; Parr et al., 2021) and cats with high neutralising antibody titres are immune to new infection. Antibodies are directed against all components of the virus (Lutz et al., 1980a). Since not all immune cats develop high antibody titres, it was concluded that CTLs are also important in FeLV immunity (Lutz et al., 1980a). Indeed, CTLs specific for FeLV appear before virus neutralising antibodies, and following adoptive transfer of FeLV specific CTLs stimulated *in vitro*, the viral load in progressively FeLV-infected cats could be lowered (Flynn et al., 2000; Flynn et al., 2002). Protection in the absence of antibodies directed to FeLV can also be demonstrated in cats vaccinated with certain FeLV vaccines; use of a recombinant FeLV canarypox vaccine did not induce antibodies to FeLV and the cats were nonetheless effectively protected from progressive infection (Poulet et al., 2003; Hofmann-Lehmann et al., 2006). This observation also supported the idea that CTLs play an important role in protection against FeLV infection.
Antibody development was observed in some cats as the sole evidence of FeLV infection (Major et al., 2010). These cats had been exposed once intranasally to very low doses of FeLV (10,000 focus forming units). Since some of these cats developed antibodies, it was concluded that the virus had replicated somewhere to sufficient levels to trigger antibody synthesis. The observation that PCR analysis of several organs was negative indicates that further replication must have been controlled by the immune system.

## CLINICAL SIGNS

If FeLV infection is associated with clinical signs, it is usually through progressive FeLV infection as active virus replication generally is responsible for the development of clinical signs. However, certain diseases (such as lymphoma and bone marrow suppression) have also been described in regressively infected cats. Although even progressively infected cats can be clinically healthy for many years, they have a reduced life-expectancy (Addie et al., 2000; Levy et al., 2006b; Gleich and

Hartmann 2009; Gleich et al., 2009; Spada et al., 2018). The death rate of progressively infected cats in multicat households has been described in the older literature as approximately 50% in 2 years and 80% in 3 years (Cotter 1998; Levy 2000). However, survival rates for progressively infected cats kept indoors in single-cat households with good veterinary care today are significantly higher. Still, FeLV infection had the greatest impact on mortality in closed households with endemic feline coronavirus (FCoV), FeLV, FIV, or all of these infections (Addie et al., 2000; Gleich and Hartmann 2009). A large study in the United States compared the survival of more than 1000 progressively FeLV-infected cats to more than 8000 age- and sex-matched uninfected control cats and found that in progressively FeLV-infected cats, median survival was 2.4 years compared to 6.0 years for non-infected cats (Levy et al., 2006b).

FeLV can cause variable clinical signs (Hartmann 2011b; Hartmann 2012b). A variety of disease conditions can be associated with progressive FeLV infection, including bone marrow disorders (mainly anaemia), tumours (mainly lymphoma), immunosuppression leading to susceptibility to secondary infections, and some other clinical syndromes, such as immune-mediated diseases, FeLV-associated neuropathies, reproductive disorders, and fading kitten syndrome (Hoover and Mullins 1991; Crawford et al., 2001; Hartmann 2011b; Hartmann 2012b). Thus, progressive FeLV infection increases the risk for a wide variety of conditions, but it is not always possible to determine whether concurrent diseases are a consequence of FeLV infection or are independent events. In one study of 3712 FeLV-infected cats presented for veterinary care, 29% were free of clinical signs (O'Connor et al., 1991). Weight loss was the most common clinical sign reported in symptomatic cats (63%), followed by fever (42%), dehydration (35%), rhinitis (18%), diarrhoea (17%), conjunctivitis (17%), oral inflammation (15%), lymphadenopathy (13%), and abscesses (12%) (O'Connor et al., 1991). In a study of 8756 diseased FeLV-infected cats seen at veterinary medical teaching hospitals, anaemia (in 18% of infected cats) was the most common finding among sick cats; other findings included upper respiratory infections (11%), lymphoma (10%), myeloproliferative diseases (6%), stomatitis (5%), leukopenia (3%), haemoplasmosis (3%), lymphadenopathy (3%), and uveitis (2%) (Crawford et al., 2001).
In contrast to progressive infection, regressive FeLV infection does not have an impact on life-expectancy, unless infection is reactivated, and cats become viraemic and then are at risk for the same diseases as those with progressive infection. In addition, there are few clinical syndromes that can be caused by regressive infection even without reactivation. In these conditions, the provirus itself can disturb cellular mechanisms or act as an oncogenic substance leading to tumour development without necessity of replicating virus. One study found a high prevalence of regressive infection in older cats at necropsy and suggested an association with haematologic changes and inflammatory processes (Suntz et al., 2010). Therefore, regressive FeLV infections can rarely cause myelosuppression or lymphoma at least in some cats that have negative FeLV antigen test results.

Although the virus was named after the contagious tumours that first garnered its attention, most FeLV-infected cats are presented to the veterinarian not for neoplasia but for anaemia or immunosuppression. Of 8642 FeLV-infected cats examined at United States veterinary teaching hospitals, various co-infections (including feline infectious peritonitis (FIP), FIV infection, upper respiratory infection, haemotropic mycoplasmosis, and chronic gingivostomatitis) were the most frequent findings (15%), followed by anaemia (11%), lymphoma (6%), leukopenia or thrombocytopenia (5%), and leukaemia or myeloproliferative disease (4%) (Cotter 1991).
The exact mechanisms for the different clinical responses of progressively FeLV-infected cats are poorly understood. It is assumed that the clinical course is determined by a combination of viral and host factors (see risk factors above). Clinical signs that are associated with FeLV infection can be classified as tumours induced by FeLV, haematologic disorders, immunosuppression, immune-mediated diseases, and other syndromes (including reproductive disorders, fading kitten syndrome, and neuropathy).

**Tumours**

FeLV causes various tumours in cats (Fig. 10, Fig. 11, Fig. 12, Fig. 13, Fig. 14, Fig. 15), most commonly lymphoma and leukaemia and less commonly other haematopoietic tumours. In addition, unusual forms of lymphoma, such as feline neurolymphomatosis (Mandrioli et al., 2012) or other tumours, including osteochondromas, olfactory neuroblastoma, uterine adenocarcinoma, and cutaneous horns, have been described in FeLV-infected cats.
Feline oncornavirus cell membrane antigen (FOCMA), an antigen present on the surface of transformed cells, was detected in 1973 but remains a subject of discussion and confusion. Its value as clinical tool (either diagnostic or preventative) is without doubt limited. FOCMA was first detected on the surface of cultured lymphoma cells incubated with serum of cats that did not develop tumours, although they were infected with FeSV, a recombinant of FeLV with oncogenic potential (Essex et al., 1975; Snyder et al., 1978). FOCMA can be found on the surface of feline lymphoma and FeSV-induced fibrosarcoma cells but not on the surface of normal feline lymphocytes (Hardy et al., 1977; Vedbrat et al., 1983). FOCMA was first considered to be a cellular antigen that is expressed after FeLV infection or tumour transformation (Essex et al., 1976; Hardy et al., 1977; Snyder et al., 1978). It has also been proposed that FOCMA is a viral antigen of FeLV-(Vedbrat et al., 1983), but subsequently, it was shown that FOCMA and FeLV-C-gp70 are similar but not completely homologous (Snyder et al., 1983). Some authors believed that development of large amounts of antibodies against FOCMA could protect against the development of FeLV-induced lymphomas by complement-dependent lysis of tumour cells (Cotter et al., 1975; Essex et al., 1975; Grant et al., 1980). Evidence for this was provided when experimentally FeLV-infected kittens did not develop neoplasia if they produced or passively received sufficient amounts of antibodies against FOCMA (Essex et

al., 1971; Essex et al., 1975). Many cats with FeLV in cluster households have antibodies against FOCMA. Those with the highest titres are most likely to remain free of tumours. However, some cats that were initially viraemic with a high FOCMA antibody titre developed lymphoma or leukaemia months or years later after the titre had declined (Cotter 1998). Opinions about identity and importance of FOCMA are still diverse, but there is consensus that FOCMA can be considered a non-homogenous group of viral antigens that can, although not always, be present on the surface of FeLV-infected cells. At the least, presence of FOCMA antibodies indicate exposure to FeLV but does not mean more than this. Alternatively, FOCMA antibodies might provide some type of protective mechanism against tumour development.

***Lymphoma.*** In the 1960s, the most common primary feline malignancies were haematopoietic tumours, of which about 90% are lymphomas. Lymphoma and leukaemia account for about 30% of all feline tumours, which is the highest proportion recorded in any animal species (Dorn et al., 1967; Dorn et al., 1968; Crighton 1969; Hardy 1978; Hardy 1981a). Progressively FeLV-infected cats have an about 60-fold increased risk of developing lymphoma compared with non-infected cats, and lymphoma can affect up to 25% of cats with progressive FeLV infection. FeLV RNA can be detected in large amounts in these FeLV-associated lymphomas by in situ hybridisation (Szilasi et al., 2020).
Mediastinal (thymic) lymphoma Fig. 11, Fig. 13) is the most common form of FeLV-associated lymphoma, followed by multicentric lymphoma, although spinal (Fig. 10), renal (Fig. 12, Fig. 15), ocular (Bandinelli et al., 2020) and other forms of lymphoma are occasionally reported in progressively FeLV-infected cats, while intestinal (alimentary) lymphoma (Fig. 14) is only very rarely associated with FeLV (Hartmann 2015). As the prevalence of FeLV decreased since its discovery, so has the incidence of FeLV-associated lymphoma (Louwerens et al., 2005; Meichner et al., 2012). A study in Germany showed a decrease of progressive FeLV infection in cats with lymphomas from 59% in 1980 to 1995 to 20% in 1996 to 1999 (Meichner et al., 2012). Other studies worldwide confirmed a decrease of progressive FeLV infection in cats with lymphoma with current prevalences of 0 to 21% (Gabor et al., 2001; Teske et al., 2002; Louwerens et al., 2005; Brenn et al., 2008; Simon et al., 2008; Stützer et al., 2010). In a study in the Netherlands, only 6% of cats with lymphoma were progressively FeLV-infected, although 31% of these cats had mediastinal lymphoma, which have the strongest association with FeLV infection (Teske et al., 2002). However, in countries with high general FeLV prevalence, progressive FeLV infection is still common in cats with lymphoma, such as with prevalences of 52% (Leite-Filho et al., 2020), 57% (Cristo et al., 2019b), and even 91% (Horta et al., 2020) in cats with lymphoma, and 78% in cats with leukaemia as determined by immunostaining (Cristo et al., 2019a).

Fig. 10. Progressively FeLV-infected cat with limb paralysis due to spinal lymphoma ©Tadeusz Frymus, Warsaw University of Life Sciences

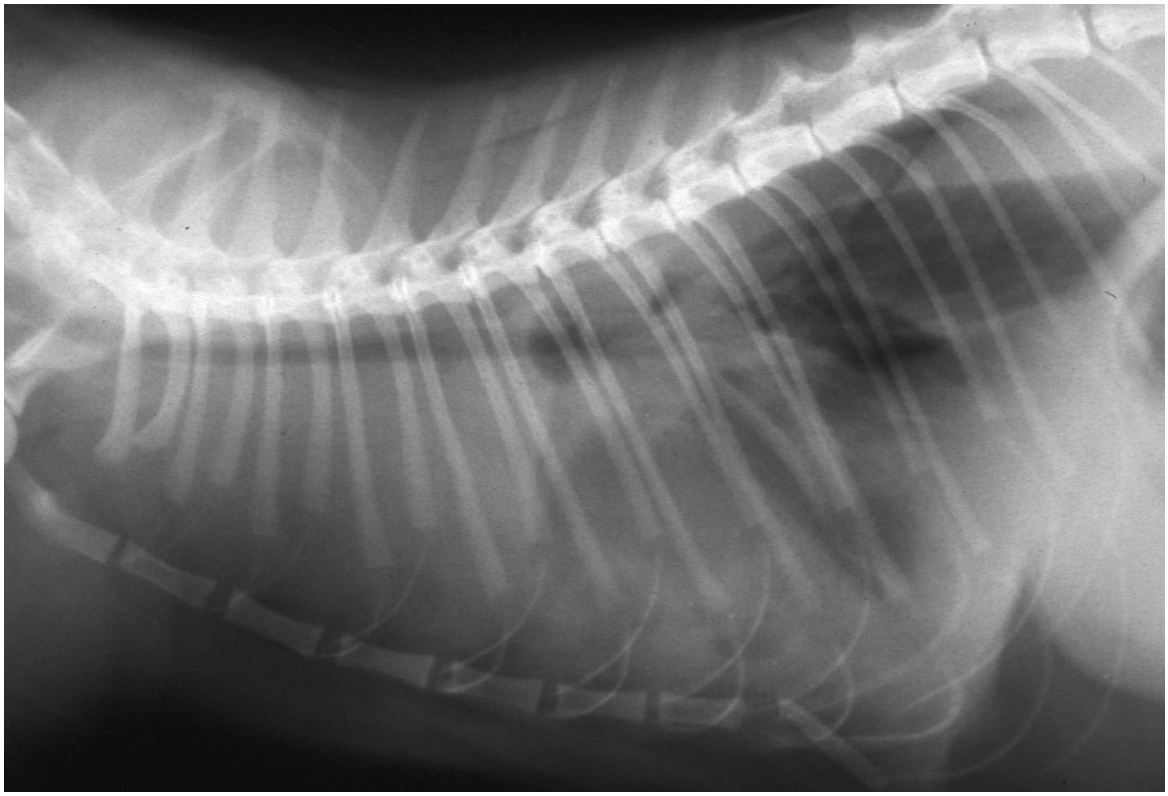

Fig. 11: Lateral thoracic radiograph of a progressively FeLV-infected cat with severe pleural effusion and mediastinal mass. The trachea is displaced dorsally, and the cardiac shadow is hardly visible ©Séverine Tasker, University of Bristol

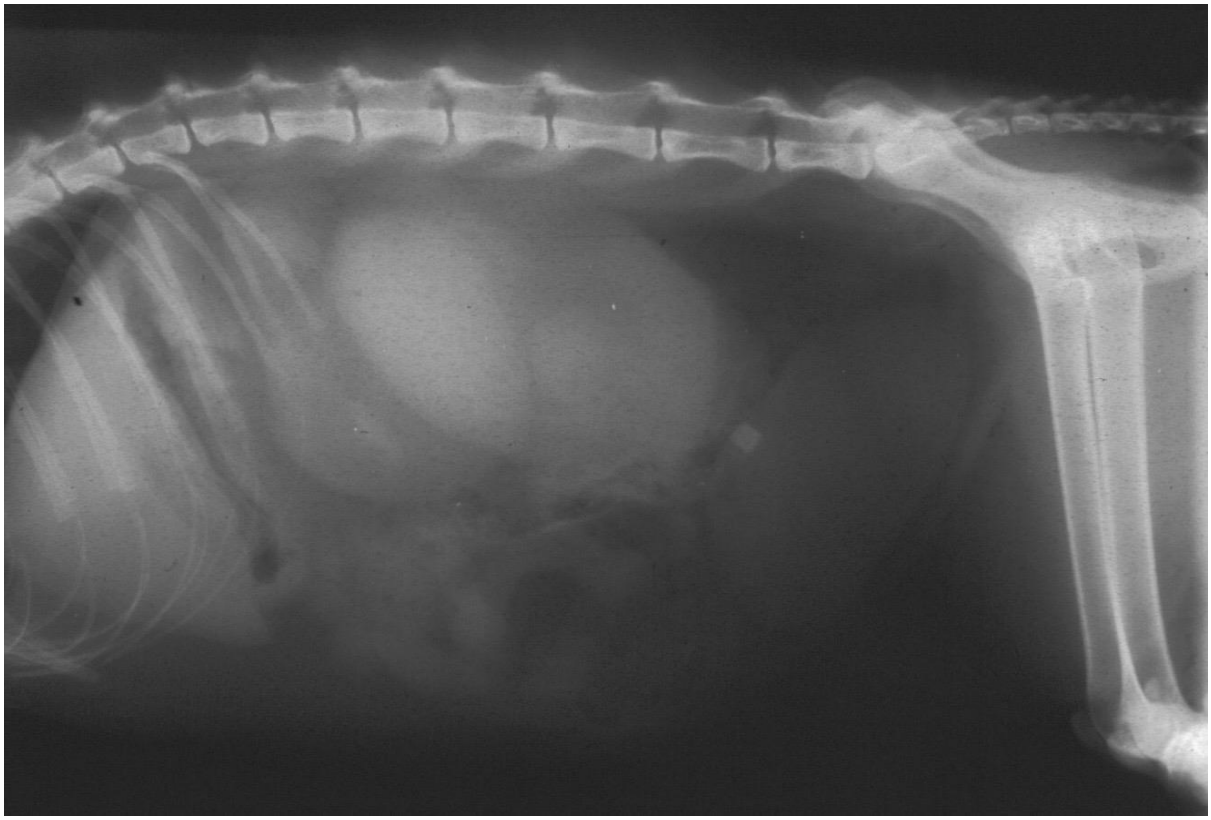

Fig. 12: Lateral abdominal radiograph of a cat with renal lymphoma ©Séverine Tasker, University of Bristol

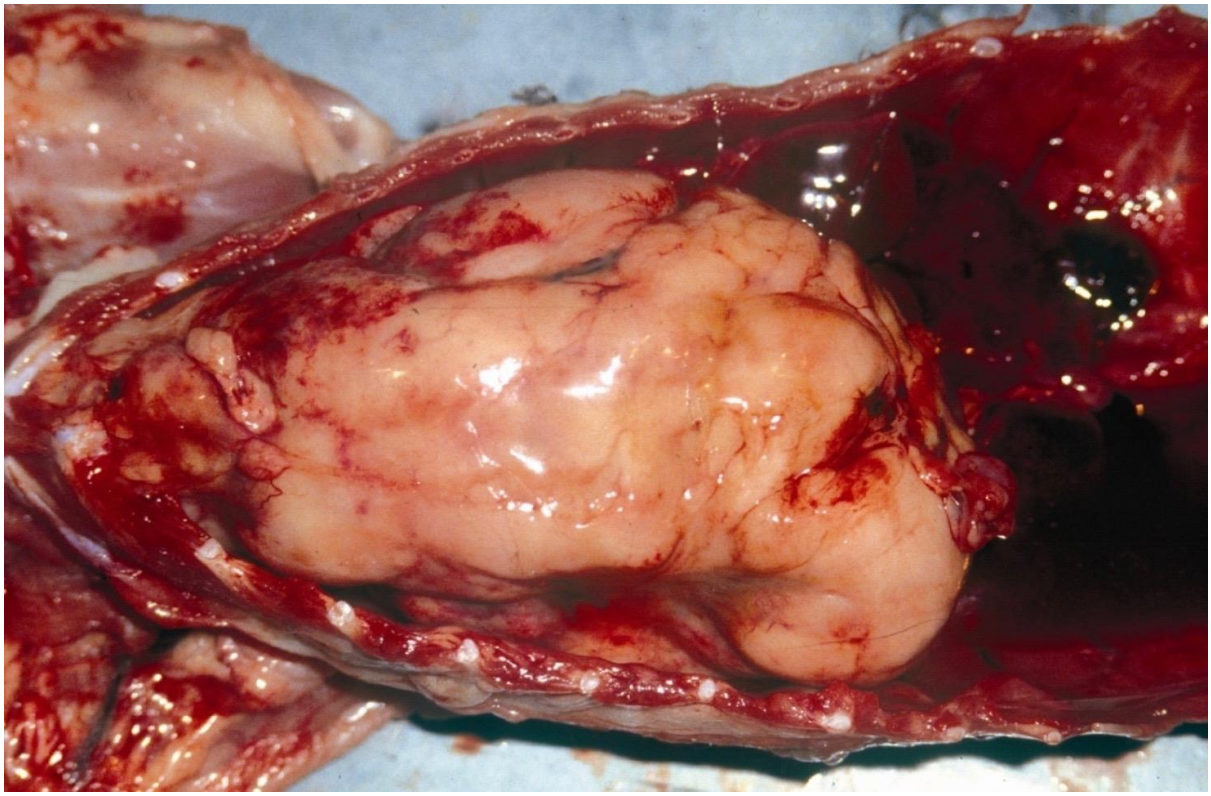

Fig. 13: *Post mortem situs* of a mediastinal (thymic) lymphoma in a progressively FeLV-infected cat ©Tadeusz Frymus, Warsaw University of Life Sciences

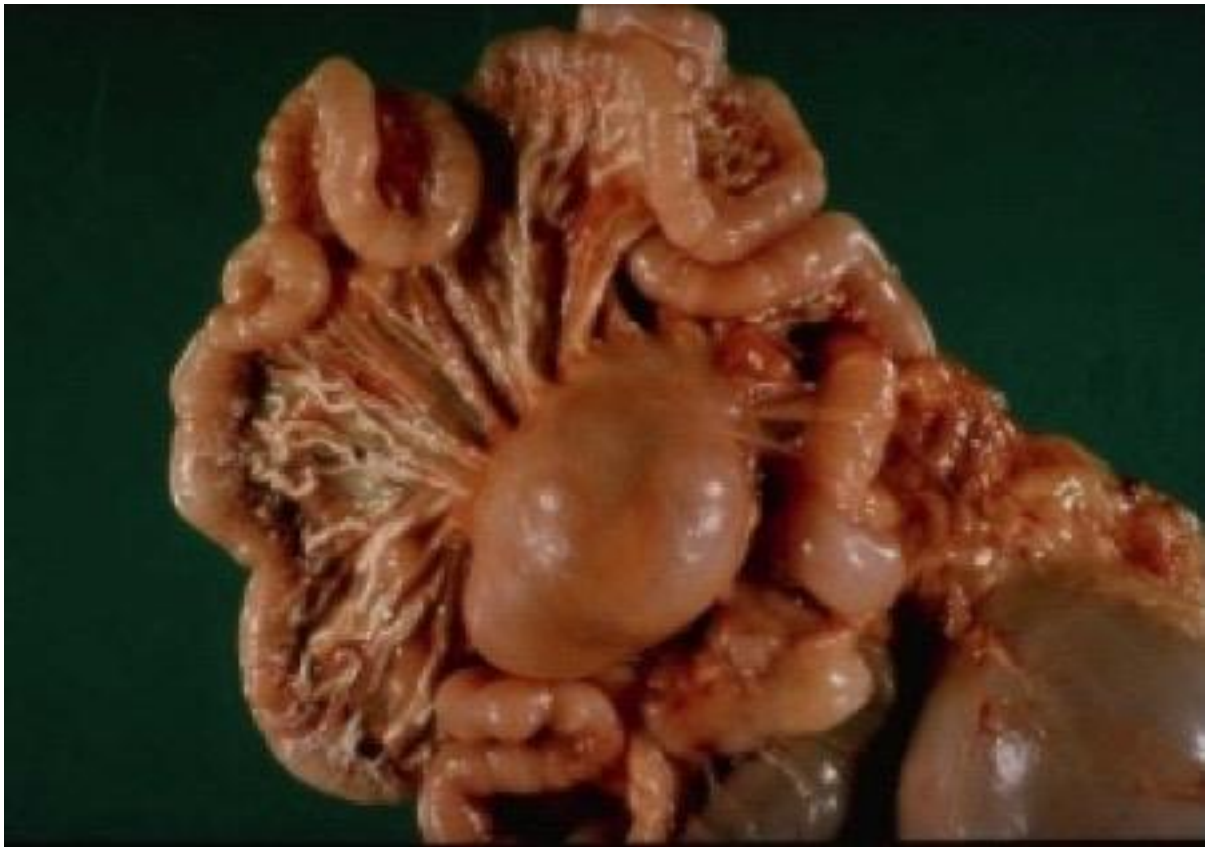

Fig. 14: Enlarged mesenteric lymph node in postmortem necropsy in a progressively FeLV-infected cat with intestinal lymphoma ©Hans Lutz, University of Zurich

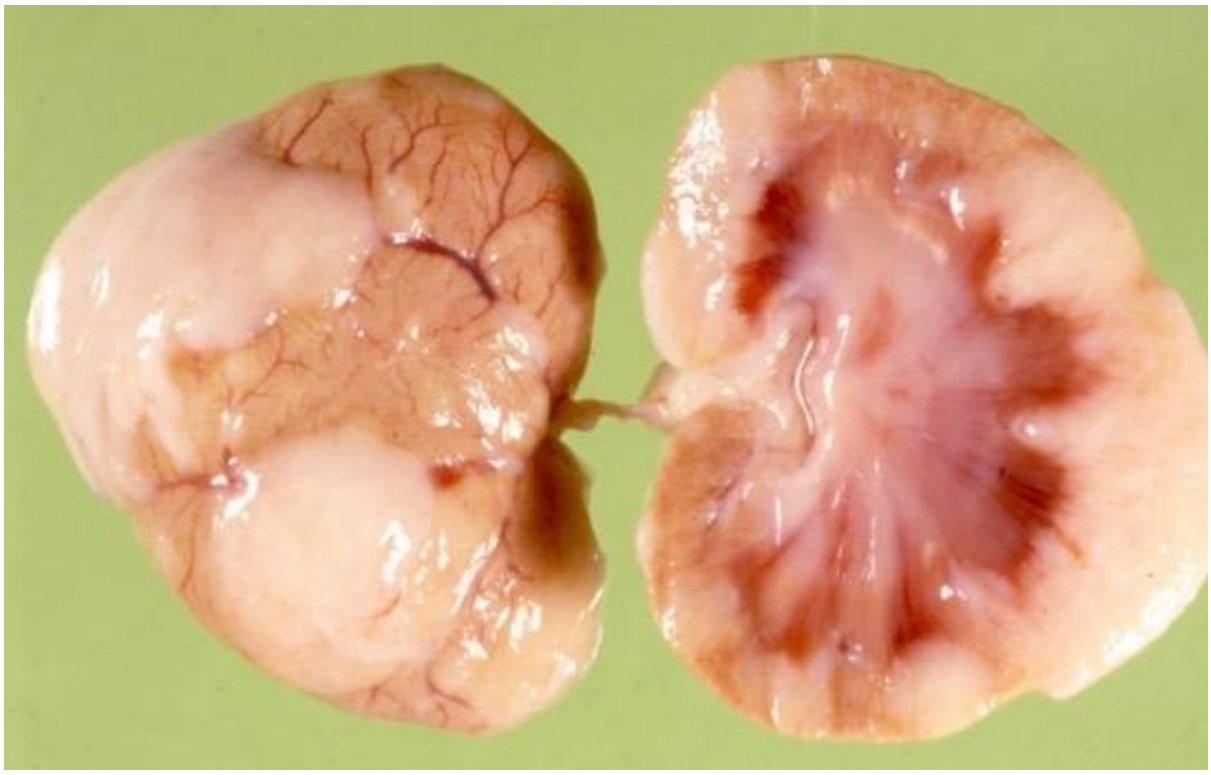

Fig. 15. Renal lymphoma in *post mortem* necropsy in a progressively FeLV-infected cat ©Regina Hofmann-Lehmann, University of Zurich

The association between FeLV and lymphoma has been clearly established (Beatty 2014; Hartmann 2015), because lymphoma have been induced in kittens by experimental FeLV infection (Rickard et al., 1969; Hardy et al., 1973; Jarrett et al., 1973); cats naturally infected with FeLV have a higher risk of developing lymphoma than uninfected cats (Hardy et al., 1973; Essex et al., 1975); and in the past, when the prevalence of FeLV was higher, most cats with lymphoma had progressive FeLV infection. Old studies from the 1970th to the early 1990th demonstrated that up to 80% of feline lymphoma and leukaemia cases were FeLV-related (Cotter et al., 1975; Francis et al., 1977; Francis et al., 1979a; Hardy et al., 1980; Francis et al., 1981; Reinacher 1987; Shelton et al., 1990). However, the prevalence of progressive FeLV infection in the cat population is decreasing (Cotter 1997; Dorny et al., 2002; Muirden 2002; Maruyama et al., 2003; Gleich et al., 2009) and thus, prevalence of progressive FeLV infection is also decreasing in cats with lymphoma worldwide (Teske et al., 2002; Louwerens et al., 2005; Brenn et al., 2008; Stützer et al., 2010; Meichner et al., 2012). The decrease in prevalence of FeLV infection in cats with lymphoma or leukaemia also indicates a shift in tumour causation (Beatty et al., 2011).
FeLV infection can cause oncogenesis indirectly by immunosuppression or more importantly by directly activating proto-oncogenes or disrupt tumour suppressor genes at or near the sites of FeLV proviral DNA integration (insertional mutagenesis) (Rezanka et al., 1992; Fujino et al., 2008; Levy et al., 2008a). This leads to disruption of the molecular regulatory circuits of cell physiology, the basic principle of tumour development. The most important mechanism for the development of malignancy is insertion of the FeLV genome into the cellular genome near a cellular oncogene (most commonly *myc*). This results in activation and over-expression of that gene and uncontrolled proliferation of these cells (clone). FeLV can also incorporate the oncogene to form a recombinant virus (e.g., FeLV-B, FeSV) containing cellular oncogene sequences that are then rearranged and activated. When they enter a new cell, these recombinant viruses are oncogenic. Thus, FeLV-induced neoplasms are caused, at least in part, by somatically acquired insertional mutagenesis in which the integrated provirus activates a proto-oncogene or disrupts a tumour suppressor gene. In a study of 119 cats with lymphoma, transduction of *myc* or proviral insertion at the *myc* locus had occurred in 38 cats (32%) (Tsatsanis et al., 1994). Notch2 transduction by FeLV was described in a naturally infected cat with multicentric lymphoma (Watanabe et al., 2014). Another study suggested that the U3-LTR region of FeLV transactivates cancer-related signalling pathways through production of a non-coding 104 base RNA transcript that activates NF kappaB (Forman et al., 2009). Twelve common integration sites for FeLV associated with lymphoma development have been identified in 6 loci: *c-myc*, *flvi-1*, *flvi-2* (contains *bmi-1*), *fit-1*, *pim-1* and *flit-1* (Fujino et al., 2008). Oncogenic association of the loci is based on the fact that c-myc is known as a proto-oncogene, *bmi-1* and *pim-1* have been recognized as *myc*-collaborators, fit-1 appears to be closely linked to *myb,* and *flit-1 insertion* was shown to be associated with over-expression of cellular genes, e.g., activin-A receptor type II-like 1 (ACVRL1). (Fujino et al., 2008). *Flit-1* seems to play an important role in the development of lymphoma and appears to represent a common novel FeLV proviral integration domain that can influence lymphomagenesis by insertional mutagenesis. Of 35 FeLV-related tumours, 5 of 25 thymic lymphomas demonstrated proviral insertion within *flit-1* locus, whereas 0 of 4 alimentary lymphomas, 0 of 5 multicentric lymphomas, and 0 of 1 T-lymphoid leukaemia had rearrangements in this region. Expression of ACVRL1 mRNA was detected in the 2 thymic lymphomas with *flit-1* rearrangement, whereas normal thymuses and 7 lymphoid tumours without *flit-1* rearrangement had no detectable ACVRL1 mRNA expression (Fujino et al., 2009). Some studies also showed that variations in the FeLV surface glycoprotein can determine the development of tumours (Bolin et al., 2011). In one study, oncogene-containing retroviruses were experimentally generated by recombination events between viral and cellular sequences, a phenomenon called "oncogene capture", and a novel FeLV, "FeLV-AKT" was designed that captured feline c-AKT1 in feline lymphoma. FeLV-AKT contains a *gag*-AKT fusion gene that encodes the myristoylated Gag matrix protein and the kinase domain of feline c-AKT1, but not its pleckstrin homology domain. It was therefore hypothesized that AKT might be involved in the mechanisms leading to tumours in cats (Kawamura et al., 2016).

Regressive FeLV infection can also be involved in tumour formation. Regressive FeLV infection has been identified as a risk factor for lymphoma (McLuckie et al., 2018). Cats from FeLV cluster households had a 40-fold higher rate of development of FeLV antigen-negative lymphoma than cats from the general population. FeLV proviral DNA was detected in lymphomas of FeLV antigen-negative cats (Jackson et al., 1993) and lymphomas have also occurred in FeLV antigen-negative laboratory cats that had been previously infected with FeLV (Rohn et al., 1994). Results of different studies concerning the prevalence of regressive FeLV infection in cats with lymphoma vary significantly (Gabor 1991; Jackson et al., 1993; Wang et al., 2001; Beatty et al., 2011; Stützer et al., 2011); however, the newer studies found evidence of provirus in only 10% (Beatty et al., 2011) or even in none of the tested cats (0/50) with FeLV antigen-negative lymphomas (Stützer et al., 2011), suggesting that regressive infection is only rarely involved in tumour development today.
The mechanism how regressive FeLV infection causes lymphomas relates to the fact that FeLV provirus can insert at many different sites in the host's genome, and can thereby interrupt or inactivate cellular genes in the infected cells, or regulatory features of viral DNA might alter expression of neighbouring genes. In addition, because bone marrow microenvironment cells (e.g., myelomonocytic progenitor cells and stromal fibroblasts) provide a reservoir for regressive FeLV infections in some cats, it is possible that the integrated provirus can alter cellular functions and contribute to the pathogenesis. Finally, FeLV can appropriate cellular genes, and several such transducted genes also present in cells of regressively infected cats have been implicated in viral oncogenesis (Rezanka et al., 1992; Sheets et al., 1993; Rohn et al., 1994). Overall, the most important oncogenetic mechanism seems to be by insertion of the FeLV genome into the cellular genome near a cellular oncogene (most commonly *myc*) (Tsatsanis et al., 1994; Fujino et al., 2008; Sumi et al., 2018) resulting in activation and over-expression of that gene. These effects lead to uncontrolled proliferation of these cells (clone).

Several studies have compared differences in presentation and outcome of lymphoma in FeLV antigen-negative (regressively infected) and FeLV antigen-positive (progressively infected) cats. In one study, no breed predisposition was found (Meichner et al., 2012), which is in contrast to the results of other studies in which there was a relatively high incidence of mediastinal lymphoma in young, FeLV antigen-negative Siamese-type cats (Day 1997; Gabor et al., 1998; Teske et al., 2002; Louwerens et al., 2005). These cats also have a predisposition for intestinal lymphoma (Rissetto et al., 2011), which suggests that breed predisposition or genetic factors also play a role in lymphoma pathogenesis.
The predominant anatomical locations of lymphoma in cats are alimentary (approximately 50%) and extranodal (approximately 25%) (Vail et al., 1998; Teske et al., 2002; Louwerens et al., 2005; Simon et al., 2008; Meichner et al., 2012). The majority of cats with alimentary and extranodal lymphoma (mainly kidney and nasopharyngeal) are FeLV antigen-negative (96% and 89%, respectively) (Meichner et al., 2012), which indicates a shift from progressive FeLV infection to other multifactorial aetiologies, such as chronic inflammation or environmental and genetic factors. The feline gastrointestinal tract, kidneys and nasopharyngeal region are sites where chronic lymphocytic or lymphoplasmacytic inflammation occurs frequently (e.g. inflammatory bowel disease (IBD), dietary allergy, tubulointerstitial nephritis, chronic rhinitis). In cats, chronic inflammation, such as IBD or dietary allergy, is thought to be a precursor of intestinal low-grade lymphoma (Willard et al., 2002; Richter 2003) and progression of IBD to lymphoma is reported (Davenport et al., 1987). This suggests that cats might be predisposed to the development of tumours at or near sites of chronic inflammation. In the past, renal and multicentric lymphomas were frequently associated with progressive FeLV infection; several decades ago, 25% to 31% of renal lymphoma cases were associated with FeLV infection (Francis et al., 1979a; Mooney et al., 1987; Vail et al., 1998) compared with 0% to 6% in more recent studies (Gabor et al., 2001; Teske et al., 2002; Taylor et al., 2009; Meichner et al., 2012). A similar situation is seen in cats with multicentric lymphoma; in the 1980th and 1990th, up to 69% of all lymphomas were multicentric, and 31% to 65% of these cats had progressive FeLV infection (Francis et al., 1979a; Jackson et al., 1993; Vail et al., 1998) compared with 0% to 13% of cats in more recent studies (Gabor et al., 2001; Teske et al., 2002; Simon et al., 2008; Meichner et al., 2012). Mediastinal lymphoma is uncommon today (10%) (Meichner et al., 2012), but many affected cats have progressive FeLV infection with a prevalence ranging from 19% to 73% (Hardy 1981a; Jackson et al., 1993; Day 1997; Vail et al., 1998; Meichner et al., 2012). In a study from the Netherlands, only 19% of cats with mediastinal lymphoma were progressively FeLV-infected, but of the cats with lymphoma, all progressively infected cats had mediastinal lymphoma. This most likely reflects the very low prevalence of FeLV (0.3%) among cats in this country (Teske et al., 2002). In a study in UK, 9% of cats with mediastinal lymphoma were progressively FeLV-infected, which is higher than the general prevalence (Fabrizio et al., 2014).

The results of studies on FeLV as a negative prognostic factor with regard to remission and survival times in lymphoma patients are contradictory (Mooney et al., 1987; Vail et al., 1998; Teske et al., 2002; Brenn et al., 2008). In one study, FeLV antigen-negative cats with lymphoma had significantly longer remission times (472 days) than FeLV antigen-positive cats (25 days) following treatment (Meichner et al., 2012). In another study, the median remission and survival times for FeLV antigen-positive cats were 27 and 37 days and for FeLV antigen-negative cats, 146 and 170 days (Vail et al., 1998). The prognosis for cats with lymphoma and progressive FeLV infection is poor because of bone marrow suppression, which is usually exacerbated by chemotherapy and can frequently delay treatment. Immunosuppression caused by FeLV infection also is aggravated by chemotherapy, leading to secondary infection that can cause overt clinical signs and impair quality of life. Furthermore, FeLV-associated lymphomas are associated with a higher rate of mitoses (Valli et al., 2000), possibly indicating a more aggressive biological behaviour that negatively affects outcome. The prognosis is also guarded because of the theoretical risk of development of additional lymphoid malignancies in cats with FeLV-associated lymphoma. During virus replication, FeLV is integrated into the host genome and recombination with endogenous FeLV-related sequences could form new and more pathogenic variants, such as FeLV subgroup B, with the potential of new lymphoma formation at any time. Finally, owners of cats with progressive FeLV infection and lymphoma often themselves elect euthanasia and do not comply with treatment.

***Leukaemia.*** Leukaemia can involve lymphoid cells (most common) but also all other haematopoietic cell lines. Thus, lymphoid and myeloid (including granulocytic, erythroid, and megakaryocytic (Molossi et al., 2021)) leukaemia types occur. More than half of the cats with non-lymphoid leukaemia have progressive FeLV infection. All haematopoietic cell lines are susceptible to transformation by FeLV, resulting in myeloproliferative disease or myelodysplastic syndrome (MDS). The prognosis for cats with myeloproliferative diseases in general is poor.
In acute leukaemia or MDS of any type, the bone marrow is filled with blast cells, and normal haematopoiesis is suppressed (Hisasue et al., 2001). Diagnosis of acute leukaemia is established by complete blood count (CBC) and bone marrow examination. Cytologic abnormalities of bone marrow include increased cellularity, megaloblastic maturation, increased myelofibrosis, and immature blast cells (Shelton and Linenberger 1995). In cats with acute leukaemia that have large numbers of circulating blast cells, the CBC in itself might be diagnostic. Although classifications have been proposed for the acute leukaemias, and flow cytometry or histochemical stains analysis can be used for further characterisation, it can be difficult to identify the predominant cell type due to lack of marker expression or limited availability of species-specific antibodies. Transformation, especially for the non-lymphoid leukaemias, usually occurs at or very close to the stem-cell level, so more than one cell line can be affected. In some cats with acute leukaemia, FeLV infection is found; a cat with a rare form of acute myelomonocytic leukaemia and FeLV infection (Mylonakis et al., 2008) and a cat with acute monoblastic leukaemia and FeLV infection have been described (Prihirunkit et al., 2008). A study focusing on acute myelocytic

leukaemia (AML) found that certain changes of the LTR of FeLV in these cats differ from the LTRs of other known FeLV strains in that it has three tandem direct 47-bp repeats in URE, and that FeLV variants that bear URE repeats in their LTR are strongly associated with the induction of both MDS and AML in cats. When cats were injected with FeLV clone33 (originating from a cat with AML), 41% of them developed MDS characterized by peripheral blood cytopenias and dysplastic changes in the bone marrow, and some of the cats with MDS eventually developed AML. The bone marrow of most cats with FeLV clone33-induced MDS produced fewer erythroid and myeloid colonies in culture with erythropoietin or granulocyte-macrophage colony-stimulating factor than bone marrow from normal control cats. Furthermore, the bone marrow of some of the cats expressed high levels of the apoptosis-related genes tumour necrosis factor-alpha and survivin. Analysis of the proviral sequences obtained from 13 cats with naturally occurring MDS also found the characteristic URE repeats (Hisasue et al., 2009).
Chronic leukaemias are rare in cats and sometimes associated with FeLV (Kyle and Wright 2010; Weiss 2010). They include well differentiated chronic lymphocytic leukaemia, chronic myelogenous leukaemia, polycythemia vera, and essential thrombocythemia. In erythraemic myelosis, proliferation of erythrocyte precursors is usually associated with FeLV-C, and most are FeLV antigen-positive. Cats with this disorder have a very low haematocrit (HCT) (12% to 15%) with normal neutrophil counts and variable thrombocytopenia. The anaemia is usually non-regenerative or poorly regenerative, and the HCT does not increase with time. Despite the lack of regeneration, the mean corpuscular volume (MCV) and numbers of nucleated erythrocytes are usually high. Abnormal erythrocyte stages are found in bone marrow and often in peripheral blood. MDS can result as a clonal proliferation of haematopoietic cells that is a preleukemic state of acute myeloid leukaemia (Hisasue et al., 2000; Shimoda et al., 2000a).
Eosinophilic leukaemia can be a subgroup of chronic myelogenous leukaemia and has been seen in association with FeLV. A case report of a cat with chronic eosinophilic leukaemia associated with FeLV infection has been published (Gelain et al., 2006). The differentiation between hypereosinophilic syndrome (severe reactive eosinophilia) and malignancy is difficult because both have been associated with large numbers of morphologically normal eosinophils in the marrow, peripheral blood, and other organs (Cotter 1998; Hartmann 2002).
Polycytemia vera characterized by uncontrolled production of red blood cells can also be caused by FeLV. A case of essential thrombocythemia in a cat associated with FeLV was described in the Netherlands (De Bosschere and Vander Stichele 2014).

***Fibrosarcoma.*** Fibrosarcomas that are associated with FeLV are caused by FeSV, a recombinant virus that develops *de novo* in FeLV-A-infected cats by recombination of the FeLV-A genome with cellular oncogenes. Through a process of genetic recombination, FeSV acquires one of several oncogenes, such as *fes, fms*, or *fgr.* As a result, FeSV is an acutely transforming (tumour-causing) virus, causing a polyclonal malignancy with multifocal tumours arising simultaneously after a short incubation period. With the decrease in FeLV prevalence, FeSV has become very uncommon. FeSV-induced fibrosarcomas are multicentric and usually occur in young cats. Several strains of FeSV that have been identified from naturally occurring tumours are defective. They are unable to replicate without the presence of FeLV-A as a helper virus that supplies proteins (such as those coded by the *env* gene) to FeSV. The host range for FeSV depends on the helper FeLV-A. By manipulation of the helper virus in the laboratory, FeSV can enter cells of species not naturally susceptible to infection. Experimental inoculation of FeSV has produced tumours in cats, rabbits, dogs, sheep, rats, and non-human primates (Theilen et al., 1970). Many of the FeSV-induced fibrosarcomas regress spontaneously, even after reaching a large size (Cotter 1998). Fibrosarcoma cells express FOCMA similarly to lymphomas. Experimental infection with FeSV causes tumours that progress in some cats and regress in others. Those in which the tumours regress, have high FOCMA antibody titres.

Fibrosarcomas caused by various strains of FeSV tend to grow rapidly, often with multiple cutaneous or subcutaneous nodules that are locally invasive and metastasize to the lung and other sites. FeSV-induced fibrosarcomas have to be differentiated from solitary fibrosarcomas in old cats not caused by FeSV; these feline injection site–associated sarcomas (FISS) are slower growing, locally invasive, slower metastasizing, and occasionally curable by excision combined with radiation and/or gene therapy. They are caused subsequent to granulomatous inflammatory reaction at the injection site after inoculation of adjuvant-containing vaccines or other injections or irritations (Hartmann et al., 2015; Hartmann et al., 2019). It has been demonstrated that neither FeSV nor FeLV play any role in the development of FISS (Ellis et al., 1996b).
In addition to fibrosarcomas, FeSV has experimentally caused melanomas, showing that FeSV can transform cells of ectodermal and of mesodermal origin (Cotter 1990). Intradermal or intraocular inoculation of FeSV into kittens produced melanomas in the skin or anterior chamber of the eye (Cotter 1998). However, FeSV has not been associated with naturally occurring melanomas of cats.

***Other Tumours.*** A few other tumours have been found in progressively FeLV-infected cats; some of them might have an association with FeLV, and some have been observed by chance simultaneously in a FeLV-infected cat.

*Iris melanomas*, for example, are probably not associated with FeLV infections, as once was believed as a result of one study, in which three of 18 eyes had positive test results for FeLV-FeSV proviral DNA (Stiles et al., 1999). In a later study, however, immunohistochemical staining and PCR did not reveal FeLV or FeSV in the ocular tissues of any cats with this disorder (Cullen et al., 2002).

*Uterine adenocarcinoma* are uncommon malignant tumours that have been poorly characterized to date. A case report described an uterine adenocarcinoma in a two-year old female Persian cat with progressive FeLV infection. A cross-section of the uterine wall revealed a thickened uterine horn, and histopathology diagnosed uterine adenocarcinoma that had metastasized to the omentum (Cho et al., 2011). It is unclear, however, whether FeLV played a role in the tumourgenesis.

*Osteochondromas* (cartilaginous exostoses on flat bones) have been described in FeLV-infected cats. Although histologically benign, they can cause significant morbidity if they occur in an area, such as a vertebra, and put pressure on the spinal cord or nerve roots (Pool 1972; Lott-Stolz 1988). A case report of a six-year-old neutered male Burmese cat with a progressive FeLV infection and solitary extraskeletal osteochondroma was published. The cat presented with a rapidly growing, solid, non-painful mass on the craniolateral aspect of the left elbow. Radiographs revealed an oval, well circumscribed 2.0 cm × 1.5 cm × 1.5 cm mineralised mass separated from the underlying bone. Surgical excisional biopsy confirmed the diagnosis (Rosa and Kirberger 2012). The pathogenesis of these osteochondromas is unknown.

*Spontaneous feline olfactory neuroblastomas* are aggressive, histologically inhomogeneous tumours of the tasting and smelling epithelium of nose and pharynx and have a high metastasis rate. Budding FeLV particles were found in the tumours and lymph node metastases, and FeLV DNA was found in tumour tissue (Schrenzel et al., 1990). Two of three cats described had positive FeLV antigen tests. The exact role of FeLV in the genesis of these tumours is uncertain.

Several rare skin disorders also have been described in FeLV-infected cats. *Cutaneous horns* are a benign hyperplasia of keratinocytes that have been described in FeLV-infected cats (Pedersen 1991b). The exact role of FeLV in the pathogenesis is unclear. *FeLV-induced giant-cell dermatosis* was diagnosed in a FeLV antigen-positive cat with nonregenerative anaemia (Favrot et al., 2005). Histological examination revealed ulcerative dermatitis with folliculitis, dyskeratotic keratinocytes and syncytia formation. A second cat that was FeLV-antigen negative was diagnosed with a *cutaneous non-epitheliotropic T-cell lymphoma* (Favrot et al., 2005). In both cases, FeLV DNA was demonstrated by PCR and viral proteins by immunohistochemistry in the pathologically changed skin samples. Plasma cell pododermatitis was associated with progressive FeLV and concomitant FIV infection in 2-year-old, intact male, mixed-breed cat that was presented with alopecia, skin peeling, and erythematous swelling in the left metacarpal paw pad (Biezus et al., 2020).

Haematologic Disorders

Haematologic disorders described in association with FeLV include anaemia (non-regenerative or regenerative) (Mackey et al., 1975); persistent, transient, or cyclic neutropenia; panleukopenia-like syndrome; platelet abnormalities (thrombocytopenia and platelet function abnormalities); and aplastic anaemia (pancytopenia). In a recent study from Iran including 31 cats that had been preselected for having clinical signs of an FeLV infection and at least a single lineage hematologic cytopenia (anaemia, thrombocytopenia, neutropenia), FeLV RNA was detected in all cats by RT-qPCR in the whole blood, bone marrow and spleen with highest copy numbers in the spleen; 24 out of 31 cats were also positive for the FeLV antigen (Abdollahi-Pirbazari et al., 2019). Of the 31 FeLV RNA-positive cats, 25 had anaemia, 17 had thrombocytopenia and nine had neutropenia; of the 24 antigen-positive cats, 18 had anaemia, 14 had thrombocytopenia and eight had neutropenia (Abdollahi-Pirbazari et al., 2019). For most mechanisms in which FeLV causes bone marrow suppression, active virus replication is required. However, it has been demonstrated that in some antigen-negative cats, regressive FeLV infection can be responsible for bone marrow suppression. In a study including 37 cats with myelosuppression that had negative FeLV antigen test results, two cats (5%) were found regressively infected with FeLV (both had non-regenerative anaemia) (Stützer et al., 2010). In another study, 1/65 (2 %) cats with cytopenias (regenerative or non-regenerative) were transiently positive for FeLV DNA in blood (Beatty et al., 2011). In these cats, FeLV provirus might have interrupted or inactivated cellular genes in the infected cells, or regulatory features of viral DNA might have altered expression of neighbouring genes. Additionally, cell function of provirus-containing myelomonocytic progenitor and stromal fibroblasts that provide bone marrow microenvironment might have been altered. Alternatively, FeLV provirus might have caused bone marrow disorders by inducing the expression of antigens on the cell surface, resulting in an immune-mediated destruction of the cell.

***Anaemia.*** Anaemia (Fig. 16) a is a major non-neoplastic complication that occurs in a majority of symptomatic FeLV-infected cats (Gleich and Hartmann 2009). In the older literature, it has been stated that more than two thirds of all non-regenerative anaemias in cats are the result of FeLV infection. While this might still be true in countries with a high FeLV prevalence (Abdollahi-Pirbazari et al., 2019), in most European countries this would now be overestimated because of the decrease in overall FeLV prevalence. In a study investigating 79 anaemic cats, FeLV was found in only two of 79 cats (both of them had haemolytic anaemia) (Laberke 2009). Anaemia in FeLV-infected cats can have various causes. Approximately 10% of FeLV-associated anaemias are regenerative, characterized by a high reticulocyte count, high MCV (macrocytosis), and presence of anisocytosis, nucleated erythrocytes, and polychromasia (Mackey et al., 1975; Shelton and Linenberger 1995). Regardless of the cause, regenerative FeLV-associated anaemias usually have a favourable response to treatment. Most FeLV-associated anaemias, however, are non-regenerative and caused by the bone marrow suppressive effect of the virus resulting from primary infection of haematopoietic stem cells and infection of stroma cells that constitute the supporting environment for haematopoietic cells. *In vitro* exposure of normal feline bone marrow to some strains of FeLV causes suppression of erythrogenesis (Cotter 1998). In addition to the direct effect of the virus on erythropoiesis, secondary disorders can cause non-regenerative anaemia in FeLV-infected cats.

Fig. 16. Pale conjunctivae due to anaemia in a cat with progressive FeLV infection ©Tadeusz Frymus, Warsaw University of Life Sciences

*Haemolytic anaemia caused by secondary infections* (regenerative anaemia) can occur in FeLV-infected cats with immunosuppression. Clinical signs associated with haemolytic anaemia are lethargy, anorexia, depression, pale mucous membranes or icterus, dehydration, and splenomegaly. Fever can be present in association with the secondary infection. The most common secondary infections responsible for haemolytic anaemia in FeLV-infected cats are haemotropic *Mycoplasma* spp. infections (George et al., 2002; Harrus et al., 2002).

*FeLV-induced immune-mediated haemolytic anaemia* (IMHA)(regenerative anaemia) also has been described in progressively FeLV-infected cats. It is suspected that FeLV can induce an immune-mediated response leading to secondary IMHA with positive Coombs' test result, autoagglutination, and spherocytosis. IMHA occurs less frequently in cats than in dogs, but FeLV infection is a potential trigger. In a study on IMHA in cats, two of 19 cats were FeLV antigen-positive (Kohn et al., 2006). However, in a more extensive study, Coombs' positive results in cats with anaemia were not significantly associated with retrovirus or haemotropic *Mycoplasma* spp. infection (Tasker et al., 2010). In cats with feline babesiosis, concurrent FeLV infection has a negative impact on the response to treatment and outcome (Schoeman et al., 2001).

*Anaemia of blood loss* (regenerative anaemia) can be present in a few cats with FeLV infection. It is seen in cats that have haemorrhage due to FeLV-associated thrombocytopenia or platelet functional abnormalities.

*Pure red cell aplasia (*PRCA) (non-regenerative anaemia) is a severe anaemia (HCT below 15%) without regeneration. It can be caused by infection with FeLV-C through interactions of FeLV-C with cell surface receptors in the bone marrow (Tailor et al., 1999; Quigley et al., 2000). The subgroup FeLV-C receptor FLVCR is a 12-transmembrane domain surface protein that exports haem from cells, and it was shown to be required for erythroid development (Philip et al., 2015) .The cell surface receptor interactions block the differentiation of erythroid progenitors between burst-forming units and colony-forming units by interfering with signal transduction pathways essential for erythropoiesis (Quackenbush et al., 1990; Shelton and Linenberger 1995; Young et al., 2000). Bone marrow examination shows an almost complete lack of erythroid precursors (at least of the late forms) with normal myeloid and megakaryocytic precursors and an increased myeloid-erythroid ratio (Cotter 1998; Levy 2000). These cats typically have macrocytosis (or rarely normocytosis) without reticulocytes. Whenever macrocytic anaemia (MCV greater than 55 fl) occurs in a cat in absence of reticulocytosis, FeLV infection should be suspected. Macrocytosis is caused through the FeLV-induced defect by skipped mitoses in cell division during erythropoiesis. These cats do not have folate or vitamin $B_{12}$ deficiencies. Iron is present in macrophages but not erythrocyte precursors; however, iron kinetics are normal. Serum erythropoietin levels are markedly increased, indicating

that anaemia is not caused by an erythropoietin deficiency (Levy 2000). Treatment with immunosuppressive drugs (glucocorticoids and ciclosporin or cyclophosphamide) can result in resolution of anaemia within three to five weeks; however, relapse usually occurs when treatment is discontinued (Stokol and Blue 1999). An atypical presentation of pure red cell aplasia was reported in a nine-month-old, female, domestic longhair kitten with progressive FeLV infection. Histological examination of the bone marrow revealed an almost complete absence of erythroid precursor cells, consistent with pure red cell aplasia, and mild to moderate myelofibrosis. The cat also had very unusual central nervous system defects (Southard et al., 2016).

*Anaemia of chronic disease* or *anaemia of chronic inflammation* (non-regenerative anaemia) is caused by excessive inflammatory cytokine production in progressively FeLV-infected cats especially those with secondary diseases, such as chronic infections or tumours. It is characterized by a mild anaemia (HCT 20% to 30%) (Laberke 2009). The HCT often increases spontaneously if the secondary problem is treated successfully, even if the cat continues to have positive FeLV antigen test results.

*Anaemia caused by crowding out* (non-regenerative anaemia) can be present if infectious agents or neoplastic cells infiltrate the bone marrow and replace erythrocyte precursor cells. Haematopoietic tumours ("myeloproliferative disorders"), including lymphoma or leukaemia, as well as secondary infectious diseases, such as systemic mycosis or mycobacteriosis, can cause severe anaemia by "crowding out" bone marrow cells (Molossi et al., 2021). MDS, characterized by peripheral blood cytopenias and dysplastic changes in the bone marrow, is a pre-stage of AML. It was found that changes in the LTR of FeLV (presence of three tandem direct 47-bp repeats in the URE) are strongly associated with the induction of MDS (Hisasue et al., 2009). Myelofibrosis, another cause of bone marrow suppression, is a condition characterized by abnormal proliferation of fibroblasts resulting from chronic stimulation of the bone marrow, such as chronic bone marrow activity from hyperplastic or neoplastic regeneration caused by FeLV. In severe cases, the entire endosteum within the medullary cavity can be obliterated. To diagnose this condition, a fine-needle aspiration of the bone marrow is often non-diagnostic and a bone marrow core biopsy is necessary.

*Aplastic anaemia* or severe *pancytopenia* (non-regenerative anaemia) can be present in FeLV-infected cats and involves all cell lines. Bone marrow cytology is usually hypocellular or can show necrosis (Shimoda et al., 2000b). Cats with pancytopenia often had positive test results for FeLV antigen in earlier times, but in a study of 13 cats with aplastic anaemia from 1996 to 2004, only two of 13 were found to be FeLV antigen-positive (Weiss 2006). In this condition, the virus probably affects precursors near the stem cell level. In some cats, cyclic haematopoiesis with periodic fluctuation in reticulocytes, granulocytes, and platelets can be seen. Alteration of accessory cells within the bone marrow microenvironment providing the structural framework, cytoadhesive molecules, and growth-regulatory cytokines necessary for normal haematopoiesis might be the cause. FeLV can affect bone marrow accessory cell viability, growth, production, or all of these of haematopoietic progenitor growth-regulating substances by altering cytokine mRNA levels in general and strain-specific patterns (Linenberger and Abkowitz 1992; Linenberger et al., 1995; Linenberger and Deng 1999). In bone marrow cytology, few if any precursors can be found, and core biopsy specimens are usually needed to obtain a diagnosis. The aplastic marrow can represent a more advanced stage of myelosuppression than PRCA. Bone marrow transplantation has not been successful in these cats. A few cats might respond at least temporarily to immunosuppression with glucocorticoids.

***Platelet Abnormalities.*** FeLV infection can cause decreased platelet counts. It also can be responsible for platelet function deficits.

*Thrombocytopenia* can occur secondary to decreased platelet production from FeLV-induced bone marrow suppression or haematopoietic tumours. Platelets harbour FeLV proteins as a result of infection. In addition, megakaryocytes, the bone marrow precursors of platelets, are frequent targets of progressive FeLV infection. Immune-mediated thrombocytopenia, which rarely occurs as a single disease entity in cats, often accompanies IMHA in cats with underlying FeLV infection. Thrombocytopenia can result in bleeding tendencies. Prevalence of thrombocytopenia and association with FeLV was evaluated retrospectively in a referral population of cats in the UK. Prevalence of thrombocytopenia was 6% of cats, and of those, 11% were progressively FeLV-infected, which is lower than reported previously (Ellis et al., 2018).

*Thrombocytopathy* in FeLV-infected cats involves platelet changes in size, shape, and function. The life span of platelets is shortened in some FeLV-infected cats. Giant platelets and thrombocytosis have been observed in some progressively FeLV-infected cats (Shelton and Linenberger 1995).

***White Blood Cell Abnormalities.*** FeLV can cause a reduction in numbers or impaired function of white blood cells (Lafrado and Olsen 1986; Hoffmann-Jagielska et al., 2005; Wardini et al., 2010).

*Lymphopenia* is seen in many cats with progressive FeLV infection. Affected cats can develop thymic atrophy and depletion of lymph node paracortical zones after infection. In some cats, lymphopenia is characterized by preferential loss of $CD4^+$ helper T cells, resulting in an inverted CD4/CD8 ratio (Quackenbush et al., 1990). More commonly, substantial losses of helper cells and cytotoxic suppressor cells (CD8+ cells) can occur (Hoffmann-Fezer et al., 1996), and in long-term FeLV

infection, a significant decrease in both CD4+ and, to a lesser degree, CD8+ lymphocytes was demonstrated (Hofmann-Lehmann et al., 1997).

*Neutropenia* is common in FeLV-infected cats (Brown and Rogers 2001), and generally occurs alone or in conjunction with other cytopenias. In some cases, myeloid hypoplasia of all granulocytic stages is observed, suggesting direct cytopathic infection of neutrophil precursors by FeLV. In some neutropenic FeLV-infected cats, an arrest in bone marrow maturation can occur at the myelocyte and metamyelocyte stages. It has been hypothesized that an immune-mediated mechanism might be responsible in cases in which neutrophil counts responds to glucocorticoid treatment ("glucocorticoid-responsive neutropenia") (Stavroulaki et al., 2020). Cyclic neutropenia also has been reported in cats with progressive FeLV infection and usually is effectively treated with glucocorticoids, suggesting that immune-mediated mechanisms are also likely in this syndrome. The cycles are usually regular, ranging from eight to 14 days. Bone marrow cytology during the neutropenic phase can indicate either granulocytic hyperplasia or hypoplasia, with a disproportionate number of cells in the promyelocytic stage. Cats with neutropenia commonly have recurrent fever or persistent bacterial infections. Some cats show persistent gingivitis, occasionally without the usual signs of inflammation, such as hyperaemia and purulent exudate, because granulocytes are necessary for the inflammatory response (Cotter 1998). In addition to problems associated with low neutrophil counts, neutrophils of progressively FeLV-infected cats can have decreased chemotactic and phagocytic function (Hoffmann-Jagielska et al., 2005).

*Myeloblastopenia* (a similar syndrome has been described earlier as *feline panleukopenia-like syndrome (FPLS)* or *FeLV-associated enteritis (FAE)*), consists of severe leukopenia (< 3 x $10^9$/l) with enteritis and destruction of intestinal crypt epithelium that mimics feline panleukopenia caused by feline panleukopenia virus (FPV) infection. FPV antigen has been demonstrated by immunofluorescence in intestinal sections of cats that died from this syndrome after being experimentally infected with FeLV (Lutz et al., 1995). FPV was also demonstrated by electron microscopy despite negative FPV antigen blood tests. It appears that this syndrome might not be caused by FeLV itself, as previously thought, but by co-infection with FPV. The syndrome also has been referred to as FAE in cats with progressive FeLV infection because the clinical signs observed are usually caused be gastrointestinal disorders, including haemorrhagic diarrhoea, vomiting, oral ulceration or gingivitis, anorexia, and weight loss (Kipar et al., 2000; Kipar et al., 2001). It is still unclear whether all these syndromes are simply caused by co-infection with FPV (and even modified live FPV vaccines have been discussed) or if they are caused by FeLV itself (Lutz et al., 1995). In experimental studies, a similar syndrome could be induced, leading to enteritis with proliferation of FeLV antigen within the enterocytes, when cats had been experimentally infected with FeLV-FAIDS variants of FeLV. FeLV-FAIDS infection begins with a prodromal period of lymphoid hyperplasia associated with viral replication in lymphoid follicles, followed by lymphoid depletion associated with extinction of viral replication. Cats developed enterocolitis with crypt necrosis and villous atrophy (Hoover et al., 1987). Intractable diarrhoea and weight loss as well as immunodeficiency characterized by lymphopenia, suppressed lymphocyte stimulation, impaired cutaneous allograft rejection, hypogammaglobulinemia, and opportunistic infections, such as respiratory disease or chronic gingivostomatitis have been described. These observations suggest that the development of FPLS and/or FAE might be FeLV strain-dependent, but it is unclear if this syndrome occurs in nature.

**Immunosuppression**

Diseases secondary to immunosuppression account for a large portion of the morbidity and mortality in FeLV-infected cats (Ogilvie et al., 1988; Pardi et al., 1991; Diehl and Hoover 1992; Sykes 2010). Although FeLV certainly can suppress immune function, it should not be assumed that all concurrent infections are a direct consequence of FeLV infection. Progressively FeLV-infected cats are predisposed to secondary infections primarily because they can develop immunosuppression similar to that in human patients infected with HIV, and immunosuppression is more severe than the one caused by FIV infection. Evaluation of the true immune status of FeLV-infected cats is hampered by the lack of well-characterized tests. Thus, clinicians primarily depend on CBC and clinical presentation for diagnosing immune dysfunction. Some laboratories offer selective counts of CD4+ and CD8+ cells, but results of these measurement will not change treatment or management decisions and thus the value of these measurements in naturally infected cat is questionable (Hoffmann-Fezer et al., 1996). The exact mechanisms by which FeLV damages the immune system are poorly understood, as is why different animals have such varying degrees of immunosuppression in response to the same virus. Immunosuppression is occasionally associated with unintegrated viral DNA from replication-defective viral variants (Overbaugh et al., 1988a). Some of these pathogenic immunosuppressive variants, such as FeLV-T, have been studied experimentally. FeLV-T requires a membrane-spanning receptor molecule (Pit1) and a second co-receptor protein (FeLIX) to infect T lymphocytes (Lauring et al., 2002). The latter protein is an endogenously expressed protein encoded by an endogenous provirus arising from FeLV-A, which is similar to the FeLV receptor-binding protein of FeLV-B (Barnett et al., 2003). Affected cats can develop thymic atrophy and depletion of lymph node paracortical zones after infection.
Lymphopenia and neutropenia are common in progressively FeLV-infected cats; this contributes to the immunosuppression. In addition, neutrophils of viraemic cats have decreased chemotactic and phagocytic function compared with those of normal cats (Hoffmann-Jagielska et al., 2005). This abnormality persists for an unknown period, even if FeLV viraemia is transient in regressively infected cats. After experimental infection, both CD4+ and, to a lesser degree, CD8+ lymphocytes are decreased in long-term progressively FeLV infected cats (Hofmann-Lehmann et al., 1997). This preferential loss of CD4+ helper T cells results in an inverted CD4/CD8 ratio; however, this is more typical of FIV

infection (Quackenbush et al., 1990; Hoffmann-Fezer et al., 1996; Hofmann-Lehmann et al., 1997). More commonly, substantial losses of CD4+ helper T cells and CD8+ cytotoxic suppressor cells occur (Hoffmann-Fezer et al., 1996). Many immune function tests of naturally FeLV-infected cats have been reported to be abnormal, including poor response to T-cell mitogens, prolonged allograft reaction, reduced immunoglobulin production, depressed neutrophil function, and complement depletion. Interleukin (IL)-2 and IL-4 are decreased in some cats (Linenberger and Deng 1999; Levy 2000), but FeLV does not appear to suppress IL-1 production from infected macrophages. Increased TNF-alpha levels have been observed in serum of FeLV-infected cats (Lehmann et al., 1992) and as well as in FeLV-infected cells in culture. Each cytokine plays a vital role in the generation of a normal immune response, and the excess production of certain cytokines, such as TNF-alpha, can also cause illness. T-cells of FeLV-infected cats produce significantly lower levels of B-cell stimulatory factors than do those of normal cats (this defect becomes progressively more severe over time) (Diehl and Hoover 1992), but when B-cells of FeLV-infected cats are stimulated *in vitro* by uninfected T-cells, their function remains normal.
Primary and secondary humoral antibody responses to specific antigens are delayed and decreased in FeLV-infected cats, and this can also alter the response to vaccination. In vaccination studies, FeLV-infected cats have not been consistently able to mount an adequate immune response to vaccines, such as rabies (Franchini 1990). Therefore, protection in a FeLV-infected cat after vaccination is not comparable to that in a healthy cat, and more frequent application of core components (e.g., yearly) must be considered (ABCD Guidelines Vaccination in Immunosuppressed cats (Hartmann et al., 2017)).

Immunosuppression can lead to an increased susceptibility to develop tumours and secondary infections. From a clinical standpoint, it is important to realize that many of these secondary diseases are treatable. Many reports exist of progressively FeLV-infected cats suffering from concurrent viral, bacterial, parasitic, and fungal infections, but few studies prove that these cats indeed have a higher rate of infection than those without FeLV infection or that they have a less favourable response to therapy. Thus, although FeLV is well known to suppress immune function, it should not be assumed that all concurrent infections are the result of the immunosuppressive effect of FeLV infection. In a study evaluating FeLV-infected shelter cats, upper respiratory infection was the most common comorbidity in FeLV-infected cats (16%) at the time of admission in the shelter; this prevalence was, however, not significantly different from the prevalence of upper respiratory infection in the cats that were not infected with FeLV (20%) (Lockhart et al., 2020). Besides feline upper respiratory tract disease, examples of secondary infectious diseases that can be associated with progressive FeLV infection include FIP and coccidiosis (Reinacher 1989; Reinacher et al., 1995). One study in 81 cats in Brazil investigated if FeLV infection influences pathogenicity of feline foamy virus (FFV) infection and found that the amount of FFV shedding was higher in cats coinfected with FeLV, but there was no influence of FeLV infection on FFV pathogenicity (Cavalcante et al., 2018). However interestingly, in a domestic cat breeding colony, FFV viral load was correlated with FeLV progression (Powers et al., 2018). An association of the presence of feline gamma-herpesvirus 1 (FcaGHV1) and FeLV antigenemia was demonstrated in studies from Singapore and Switzerland (Beatty 2014; Novacco et al., 2019), while regressively FeLV-infected cats (provirus-positive) were not more frequently FcaGHV1-infected (Novacco et al., 2019). High FcaGHV1 loads in blood were found more frequently in progressively FeLV-infected cats than in non-FeLV-infected cats (Novacco et al., 2019). However, these results (association with progressive infection and higher FcaGHV1 loads) could not be confirmed in another study (McLuckie et al., 2017). The differences in observed results could be due to different study populations although geographic influences, e.g. due to differences in the prevailing virus isolates cannot be fully excluded. A few studies have focused on the role and the influence of FeLV infection on haemotropic *Mycoplasma* spp. infection with controversial results, because in some studies, *Mycoplasma* spp. infection was associated with FeLV infection (Bauer et al., 2008; Sykes et al., 2008; Laberke et al., 2010; Stojanovic and Foley 2011; de Bortoli et al., 2012; Bergmann et al., 2017a), whereas in others it was not (Willi et al., 2006; Macieira et al., 2008; Jenkins et al., 2013). FeLV did not influence clinical and laboratory parameters in cats with *Mycoplasma* spp. infection under field conditions (Munhoz et al., 2018). An experimental study investigated FeLV and haemoplasma coinfection and it was found that a pre-existing FeLV infection can have an aggravating effect on the clinical signs of haemoplasma infection (George et al., 2002). An association between *Bartonella* spp. infection and FeLV infection has been described (Sato et al., 2017). Cats with progressive FeLV infection were more likely to develop and not eliminate *Bartonella henselae* infection; however, the course and clinical outcome of *Bartonella henselae* infection was not different in cats to those that were not co-infected with FeLV (Buchmann et al., 2010; Laberke et al., 2010; de Bortoli et al., 2012; Bergmann et al., 2017b). No association was found between in FeLV and rickettsial infections (Bergmann et al., 2015). Presence of antibodies against *Toxoplasma gondii* was also not associated with the presence of FeLV infection (Abdou et al., 2013), but fatal cases of toxoplasmosis have been described in progressively FeLV-infected cats (Pena et al., 2017; Zandona et al., 2018). No association was found with feline leishmaniasis in several studies (Martin-Sanchez et al., 2007; Sobrinho et al., 2012; Spada et al., 2016; Bezerra et al., 2019) although in one study, presence of *Leishmania* spp. antibodies and DNA was significantly associated with FeLV infection (Sherry et al., 2011). In one large study in USA and Canada including 34,975 cats, infection with FeLV or FIV increased the risk of heartworm infection, but this could also be explained by the common risk factor of outdoor lifestyle (Levy et al., 2017a). There was no association with sporotrichosis in a study in Brazil (Kitada et al., 2014), and no significant association was observed in cats with sporotrichosis between those with retrovirus coinfections and those without concerning treatment outcome or healing time of the skin lesions (de Souza et al., 2018). However, progressive FeLV infection can change the severity of the clinical presentation in cats with sporotrichosis, and immunological differences have been observed in coinfected cats. Of 30 cats with sporotrichosis (including three cats with FIV and five cats with progressive FeLV infection), progressively FeLV-infected cats had higher IL-10 levels and lower IL-4 levels, and all cats with poor general

condition had progressive FeLV or FIV infection, but the retrovirus status was not associated with outcome of sporotrichosis (de Miranda et al., 2018).
Skin infections have been described in some FeLV-infected cats. Progressively FeLV-infected cats have a greater diversity of cutaneous and mucosal microflora compared with uninfected cats (Sierra et al., 2000), and infections associated with dermatologic conditions are usually caused by the immunosuppression (Piscopo 2000). Traumatic injuries can be complicated by secondary bacterial infections or abscesses. Otitis externa and miliary dermatitis can develop from ectoparasites or allergies but persist because of secondary bacterial infections.
The association of chronic gingivostomatitis and FeLV was subject of several studies. Most of the studies did not find an association between chronic gingivostomatitis and progressive FeLV infection (Quimby et al., 2008; Belgard 2010), although one study showed that cats with any oral inflammatory disease were more likely to have FeLV infection than orally healthy cats (Kornya et al., 2014). One study investigated biopsy sample of oral mucosal biopsies of 27 cats with chronic gingivostomatitis and detected presence of FeLV antigen by immunohistochemistry in the epithelium and inflammatory infiltrate of 30% of the cats, while FIV antigen was identified only in one cat, and feline calicivirus (FCV) antigen in none of the cats, indicating a potential role of FeLV in this diseases complex (Rolim et al., 2017).

**Immune-Mediated Diseases**

FeLV-infected cats can rarely have immune-mediated diseases caused by an overactive or dysregulated immune response to the virus, although this is much less common than in FIV-infected cats. Generally humoral immunity to specific stimulation decreases in FeLV-infected cats, but nonspecific increases of IgG and IgM sometimes also have been noted. The loss of T-cell activity and the formation of antigen antibody complexes contribute to the immune dysregulation (Pedersen 1988). Antigens that can lead to antigen-antibody complex formation include not only whole virus particles but also free gp70, p27, or p15E proteins (Day et al., 1980 ; Tuomari et al., 1984). Immune-mediated diseases described in FeLV-infected cats include IMHA (Kohn et al., 2006), glomerulonephritis (Anderson and Jarrett 1971), uveitis with immune complex deposition in iris and ciliary body (Brightman et al., 1991; Jinks et al., 2016), and polyarthritis (Pedersen 1991a). Chronic progressive polyarthritis can be triggered by FeLV. According to older studies, in about 20% of cats with polyarthritis, FeLV seems to be an associated agent (Pedersen 1991a), but a similar syndrome can also be caused by FFV infection, and concurrent FIV infection can also occur (Oohashi et al., 2010).
One important immune-mediated disease in cats is glomerulonephritis although it is much less common than in dogs, and in cats is much less commonly seen than chronic kidney disease (CKD) caused by tubulointerstitial nephritis particularly in cats of older age (Hartmann et al., 2020). Generally, glomerulopathies can be categorized in immune-complex glomerulonephritis (ICGN) and non-immune-complex glomerulopathies, such as glomerulosclerosis, glomerular atrophy, amyloidosis, or glomerular disease as a consequence of severe chronic interstitial nephritis or renal dysplasia (Cianciolo et al., 2013; Rossi et al., 2019). For ICGN in general, chronic inflammatory conditions, often associated with chronic persistent infections, primary immune-mediated diseases, and tumours are relevant causes. In a study on glomerulopathies in cats that included 37 cats with ICGN and 31 cats with glomerulopathies not associated with immune complex deposition, all cats that had retrovirus infection and proteinuria had ICGN (Rossi et al., 2019), and the predominance of glomerular disease in retrovirus-infected cats versus tubulointerstitial injury was in accordance with previous histopathologic studies (Poli et al., 1993; Poli et al., 2012). However although cats with ICGN in this study were significantly more frequently FIV- or FeLV-infected than cats with non-immune-complex glomerulonephritis, FIV was three times more frequent among the retrovirus-infected cats and the significance of FeLV by itself was not investigated (Rossi et al., 2019). In an experimental study, in cats with long-term FeLV infection urea was increased compared to uninfected control cats (Hofmann-Lehmann et al., 1997). The latter could potentially indicate mild kidney dysfunction since FeLV infection has been associated with glomerulonephritis partly due to deposition of circulating immune complexes. Glomerular disease in FIV-infected cats is likely the result of an immune-mediated response caused by hypergammaglobulinemia (Ackley et al., 1990; Flynn et al., 1994; Gleich et al., 2009) through an excessive antibody response against the chronic persistent infection. Hypergammaglobulinemia reflects polyclonal B-cell stimulation (Flynn et al., 1994), and the produced antibodies are not neutralising and thus, can lead to antigen antibody complex formation. Hypergammaglobulinemia also can be caused by excessive production of autoantibodies (Pennisi et al., 1994). When comparing plasma electrophoretograms, hypergammaglobulinemia and hyperproteinaemia are significantly more common in FIV-infected versus non-infected cats (Miro et al., 2007; Gleich et al., 2009). In progressively FeLV-infected cats with glomerulonephritis, an antigen antibody complex aetiology would also be expected. However, in one study, progressively FeLV-infected cats did not have significantly more commonly hypergammaglobulinemia in plasma electrophoretogram, which would likely be present in immune complex-associated diseases, in contrast to FIV-infected cats (Miro et al., 2007). In addition, hyperproteinaemia was not a laboratory change significantly more commonly present in progressively FeLV-infected cats than in uninfected cats, in contrast to findings in cats with FIV infection (Day et al., 1980; Tuomari et al., 1984; Miro et al., 2007; Gleich and Hartmann 2009). In conclusion, cats with FeLV infection can also succumb to glomerulopathies, but this is generally through a different mechanism than in FIV-infected cats. In FeLV-infected cats, kidney disease usually develops because the virus can induce formation of renal lymphoma, and this renal function impairment is rather caused by the tumour than by the infection. In earlier studies, ICGN was described in FeLV-infected cats (Glick et al., 1978; Slauson and Lewis 1979; Jeraj et al., 1985) but in most of these cases, cats had FeLV-associated lymphoma and thus, likely lymphoma-induced immune-complex disease. Therefore, FeLV itself does not seem to play a major role in the development of glomerulopathies other than being an important neoplasia-promoting factor in cats (Hartmann et al., 2020).

**Other Syndromes**

Other syndromes directly caused by FeLV infection include FeLV-associated neuropathy, reproductive disorders, and fading kitten syndrome.

***Neuropathies.*** Neurologic dysfunction has been described in progressively FeLV-infected cats. Most neurologic signs seen in FeLV-infected cats are caused by lymphoma and lymphocytic infiltrations in brain or spinal cord (Fig. 10) leading to compression (Szilasi et al., 2020). Two cases of cerebellar lymphoma in FeLV-infected cats have been described (Yoshino et al., 2017; Pardo et al., 2019). One four-year-old cat exhibited neurologic signs, such as wobbling, right head tilt, and intention tremor, caused by a cerebellar Hodgkin lymphoma-like tumour whose cells were positive for FeLV antigen (Yoshino et al., 2017). The other one was an eight-month-old cat with progressive FeLV infection that was presented with altered consciousness, symmetric ataxia, hypermetric gait, vertical positional nystagmus, mydriasis, strabismus, intention tremor of the head, and increased patellar reflexes caused by a lymphoma in the right cerebellar hemisphere (Pardo et al., 2019). Neurologic signs can also be induced by secondary infectious diseases, such as cryprococcosis, toxoplasmosis, FIP, or even unusual other infectious diseases, such as encephalitozoonosis or listeriosis (Raith et al., 2010). However, in some cases no other infections or tumours are detected with diagnostic imaging methods or at necropsy. Direct neurotoxic effects of FeLV have been discussed as pathogenetic mechanisms in these cases. Anisocoria, mydriasis, central blindness, Horner's syndrome, or urinary incontinency caused by myelopathy (Carmichael et al., 2002) have been described in progressively FeLV-infected cats without morphologic changes. Envelope glycoproteins of retroviruses can produce increased intracellular free calcium leading to neuronal death, as observed in HIV-infected humans. A polypeptide of the FeLV envelope was found to cause dose-dependent neurotoxicity associated with alterations in intracellular calcium ion concentration, neuronal survival, and neurite outgrowth. The polypeptide of a FeLV-C strain was significantly more neurotoxic than the same peptide derived from a FeLV-A strain (Fails et al., 1997; Mitchell et al., 1997). It also has been suggested that FeLV can lead to disruption of intracellular thiamine uptake by blocking a putative thiamine receptor (THTR1), the receptor for FeLV-A, which might contribute to the neuropathogenesis (Mendoza et al., 2013).
Clinical signs in 16 cats with progressive FeLV infection and neurologic signs consisted of abnormal vocalization, hyperesthesia, and paresis progressing to paralysis. Some cats developed anisocoria or urinary incontinence during the course of their illness. Others had concurrent FeLV-related problems, such as myelodysplastic disease. The clinical course of affected cats involved gradually progressive neurologic dysfunction. Microscopically, white-matter degeneration with dilation of myelin sheaths and swollen axons was identified in the spinal cords and brainstems of affected animals (Carmichael et al., 2002). Immunohistochemical staining of affected tissues revealed consistent expression of FeLV p27 antigens in neurons, endothelial cells, and glial cells, and proviral DNA was amplified from multiple sections of spinal cord (Carmichael et al., 2002).

***Reproductive Disorders*.** Progressively FeLV-infected queens can transmit FeLV transplacentally (Essex 1977). Reproductive failure in form of foetal resorption, abortion, and neonatal death is common if *in utero* FeLV infection occurs. The apparent infertility is likely caused by early resorption of foetuses. Abortions usually occur late in gestation, with expulsion of normal-appearing foetuses. Bacterial endometritis can accompany these abortions, particularly in cats with neutropenia (Cotter 1998). One study investigated queens with reproductive failures such as abortion, stillbirth and neonatal mortality in the absence of genetic, traumatic, hormonal or nutritional problems, or recurrence of the problem. The queens were tested for viral infections, including FeLV; none of the tested 26 cats was FeLV PCR-positive (Oliveira et al., 2018).

***Fading Kitten Syndrome.*** Kittens from infected queens can be infected transplacentally and also can experience heavy exposure also during birth and throughout the nursing period. Some kittens become immune, but most become progressively infected and die at an early age of the so-called fading kitten syndrome, characterized by failure to nurse, dehydration, hypothermia, thymic atrophy, and death usually within the first two weeks of life (Levy 2000). Fading kitten syndrome generally includes non-infectious and infectious causes for neonatal death (birth to weaning age) (Bucheler 1999). A possible association of thymus dysfunction with fading syndromes has been suggested (Roth 1987). Non-infectious causes are mostly responsible for mortality in the first week of life and include congenital disorders, low birth weights, trauma, malnutrition, environmental causes, and neonatal isoerythroylsis, while in infectious causes, including FeLV, death occurs more commonly at about three to four weeks of age (Bucheler 1999).

## LABORATORY AND DIAGNOSTIC IMAGING FINDINGS

### Laboratory Changes

#### *Haematology*

Most cats with FeLV infection, even those with the progressive form, do not show CBC abnormalities. Thus, the CBC in progressively FeLV-infected cats can be normal or show regenerative or non-regenerative anaemia, neutropenia, lymphopenia, monocytopenia, and/or thrombocytopenia. Evidence of agglutination can be present in cats with IMHA. Moderate to marked leukocytosis due to neutophilia and increased band neutrophils can also be present. Large numbers of circulating blasts, megakaryocytes or dysplastic cells (such as erythrocytes with giant Howell-Jolly bodies) can be found in cats with leukaemia or MDS (Fig. 17). When compared to uninfected cats, progressively FeLV-infected cats were nearly four-fold more likely to be anaemic, five-fold more likely to be thrombocytopenic, four-fold more likely to be neutropenic, and three-fold more likely to have lymphocytosis (Gleich and Hartmann 2009). These findings were also confirmed in a Brazilian study including 153 FeLV antigen-positive cats (31% of 493 cats): antigen-positive cats had higher odds to anaemia, leukopenia and lymphopenia compared to FeLV antigen-negative cats (da Costa et al., 2017). In an experimental study, where cats were under observation for more than six years after FeLV infection, no changes in total white blood cell count and absolute differential counts were found compared to uninfected cats (Hofmann-Lehmann et al., 1997). However, FeLV infection led to a decrease in CD4+ and to a lesser degree in CD8+ T lymphocytes even in cats with regressive infection, which could be associated with changes in the immune function of these animals. Moreover, mean corpuscular volume and mean corpuscular haemoglobin of red blood cells were increased in these long-term FeLV-infected cats (Hofmann-Lehmann et al., 1997). Similar observations have been described in an old study (Weiser and Kociba 1983) and were recently confirmed in naturally infected cats in Brazil, where symptomatic progressively infected cats that were presented with macrocytic anaemia and haematological disorders were associated with higher viral blood and proviral bone marrow loads (Duda et al., 2020).

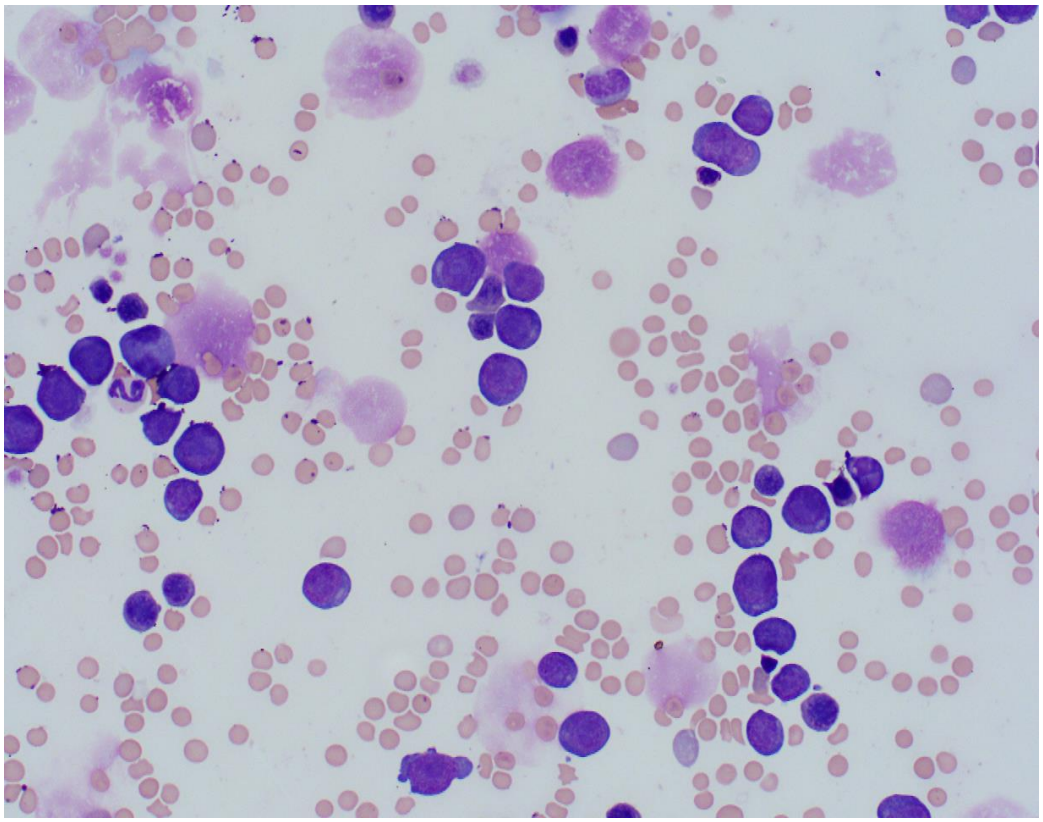

Fig. 17: Blood smear of a progressively FeLV-infected cat with acute erythroid leukaemia: The density of the red blood cells is decreased. Beside moderate amounts of nuclear protein of smashed cells and some nucleated red blood cells (metarubricytes and polychromatophilic rubricytes), some large round cells are present. They have small to moderate amounts of dark basophilic cytoplasm, occasionally with a small perinuclear clearance zone and a centrically to slightly eccentrically placed nucleus with fine chromatin pattern and one or several indistinct nucleoli. Based on morphology, an

erythroid origin is the primary differential diagnosis; further diagnostic testing by flow cytometry is however required to confirm this suspicion and rule out lymphoid or myeloid origin.

***Serum Biochemistry***

Findings on serum biochemistry analysis are non-specific and reflect underlying secondary disease processes. Hyperbilirubinemia and bilirubinuria can be present in cats with immune-mediated haemolytic anaemia or secondary haemoplasma infection (Gleich and Hartmann 2009).

***Urinalysis***

Findings on urinalysis are often non-specific. Cats with FeLV-associated glomerulonephritis can be proteinuric. Some cats have evidence of bacterial urinary tract infections secondary to their immunosuppression. Urine culture and susceptibility testing of a urine specimen obtained via cystocentesis are indicated in cats with suspected urinary tract infection.

***Bone Marrow Cytology and Histology***

Both bone marrow aspirate and core biopsy specimens should be obtained in cats with pancytopenia or non-regenerative anaemia. If aspirate results are not diagnostic, the core biopsy should be submitted for interpretation, because bone marrow aspirates from cats with aplastic anaemia or myelofibrosis are typically of low cellularity. Bone marrow findings in cats with progressive FeLV infection can include evidence of neoplastic lymphoid, erythroid, or myeloid cells (which circulate in the peripheral blood of cats with leukaemia (Fig. 17)); myelodysplasia; hypoplasia or aplasia of erythroid, myeloid, or megakaryocyte cell lines; erythroid, myeloid, and megakaryocyte hyperplasia despite peripheral cytopenias; and megakaryocyte hypoplasia. Cytochemical stains that identify cells of the myeloid lineage (such as alkaline phosphatase, peroxidase, Sudan black B, and nonspecific esterase), immunocytochemistry, or flow cytometry using antibodies that target cell surface cluster of differentiation (CD) molecules might be needed to definitively identify the cell type involved in some acute undifferentiated leukaemias (Sykes 2013).

**Diagnostic Imaging**

***Radiographs***

Imaging findings in cats with FeLV infection can reflect a secondary disease process and are extremely variable. Cats with FeLV-associated thymic lymphoma have a mediastinal mass on thoracic radiography that can be accompanied by mild to severe pleural effusion (Fig. 11). Renal lymphoma can appear as enlargement of one or both kidneys (Fig. 12).

***Sonography***

Abdominal sonography in cats with multicentric lymphoma can reveal hypoechoic and enlarged abdominal lymph nodes and enlargement, hypoechogenicity, or mottling of the spleen, liver, or kidneys. Increased hepatic echogenicity can also occur with liver lymphoma. Increase in kidney size can be detected in cats with renal lymphoma. Intestinal masses with loss of normal bowel wall layering can be detected in cats with intestinal lymphoma. Splenomegaly can sometimes be detected in cats with immune-mediated cytopenias.

**Gross necropsy and histopathologic findings**

Gross and histopathologic findings in cats with progressive FeLV infection usually reflect secondary disease processes. Lymphoma (Fig. 13, g. 14, Fig. 15), and evidence of myelodysplasia or leukaemias are the most common findings but secondary infections also can be detected. Reactive lymphoid hyperplasia is a common finding in cats with progressive FeLV infection. Histology of the bone marrow can reveal lymphoma, leukaemias, or myelodysplasia. The intestinal tracts of cats with FeLV-associated enteritis can show crypt cell necrosis and regeneration, lymphoplasmacytic infiltrates, and blunting and fusion of villi (Reinacher 1987). CNS lesions can include loss of axons and dilated myelin sheaths within the spinal cord (Carmichael et al., 2002). Immunohistochemical stains can be applied to tissues, e.g., bone marrow or tumour tissue, to confirm the presence of FeLV antigen (Stützer et al., 2010; Suntz et al., 2010; Stützer et al., 2011; Santagostino et al., 2015).

## DIAGNOSIS

**Approach to the Diagnosis**

Testing for FeLV and consequently preventing exposure of healthy cats to FeLV-infected cats is the most effective way to prevent the spread of infection (Levy et al., 2008a; Little et al., 2011). The American Association of Feline Practitioners (AAFP) has established guidelines for testing cats for FeLV (Levy et al., 2008a; Little et al., 2020). According to these

guidelines, the FeLV status of all cats should be known, because the infection has serious health consequences that influence patient management, both in illness and for wellness care. Accurate diagnosis of infection is important for both uninfected and infected cats. Identification and segregation of progressively infected cats is the most effective method for preventing new infections in other cats. Failure to identify infected cats can lead to inadvertent exposure and transmission to uninfected cats. Misdiagnosis of infection in uninfected cats can lead to unnecessary changes in lifestyle or even euthanasia (Little et al., 2020). To completely eliminate any risk when bringing in a new cat into an established household, a follow-up test should be performed six weeks after the initial test or after a possible exposure to FeLV because cats could be in the early stage of infection at the time of the first test (Hofmann-Lehmann and Hartmann 2020). Moreover, the test should be performed before bringing the cat into the home (Levy et al., 2001; Little et al., 2020).
Cats can be tested at any age, because the screening tests detect antigen and not antibodies, and thus, neither maternal antibodies nor antibodies from vaccination interfere with testing. It has to be realized, however, that kittens infected by some form of maternal transmission might not test positive for weeks to months after birth (Levy and Crawford 2005). Vaccination against FeLV usually does not compromise testing, because FeLV tests detect antigen and not antibodies. However, blood collected immediately after vaccination could contain detectable FeLV antigens from the vaccine itself; thus, diagnostic samples should be collected before FeLV vaccine administration (Levy et al., 2008a). It is not known how long this test interference persists.

**Detection of FeLV Infection**

Diagnosing FeLV infection is difficult, due to different stages of infection and courses of disease, that can vary over time (Lutz et al., 1983b; Hofmann-Lehmann et al., 2001; Torres et al., 2005), the performance of various tests, that determine different viral and immunological parameter (Levy et al., 2008a; Lutz et al., 2009) and different interpretation of these tests (Hartmann and Hofmann-Lehmann 2020a; Hofmann-Lehmann and Hartmann 2020). Moreover, the interaction between the virus and the cat's immune system and the balance of who gains and keeps the upper hand , the host or the virus (Fig. 6), can vary over time (Hofmann-Lehmann et al., 2001; Flynn et al., 2002; Hofmann-Lehmann et al., 2008). Nevertheless, veterinary practitioners must make important decisions based on test results at a certain point of time. For these reasons, ABCD has developed a tree for the diagnosis of FeLV infection aimed to be a useful tool for veterinarians in practice dealing with potentially FeLV-infected cats (Fig. 18). This diagnostic tree is based on FeLV risk assessment as well as the cat's clinical presentation, it takes into account the different test characteristics, the timespan over which a test will produce positive results, and the positive and negative predictive value of tests. It also highlights the steps for confirmation of results as well as for repeated testing to determine the different courses of FeLV infection. Thus, it integrates the knowledge one should have about FeLV for a reliable and accurate diagnosis of FeLV.

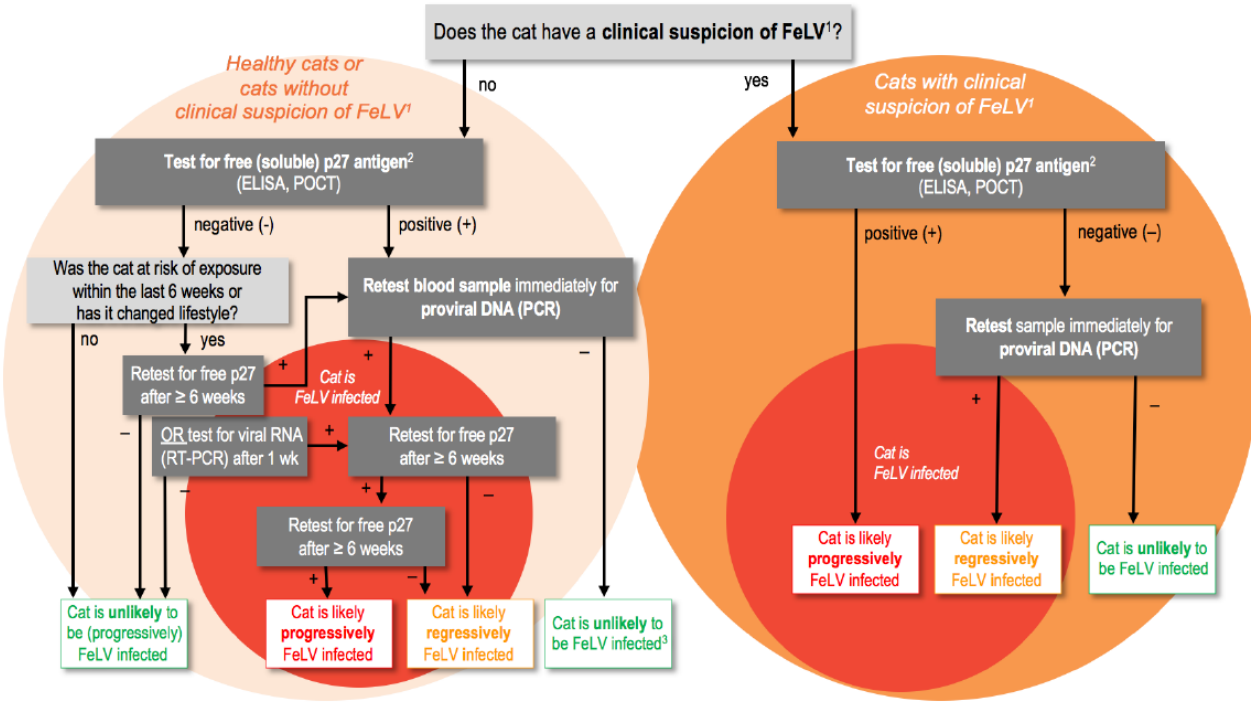


Fig. 18: FeLV diagnostic tree. [1]For risk factors or clinical disorders associated with FeLV, see Table 7. [2]Whenever testing for free FeLV p27 antigen of blood samples is suggested (ELISA, POCT) in any of the boxes in the figure, alternatively testing for

viral RNA of saliva samples (RT-PCR) can be used. [3]In very rare cases, a focal FeLV infection can be the reason for such a result (positive in free FeLV p27 antigen and negative in provirus-PCR, both from blood samples). POCT = point-of-care test

Table 7: Risk factors and clinical problems that can be associated with FeLV infection

1. Mixed breed, free-ranging or feral cat, cat from household with FeLV-positive cat(s)
2. Cats from an area with high FeLV prevalence
3. Presence of neoplasia (lymphoma, leukaemia other neoplasia)
4. Bone marrow suppression (n on-regenerative anaemia, thrombocytopenia, neutropenia, pancytopenia)
5. Chronic or recurrent infections suggesting immunosuppression
6. Chronic gingivostomatitis
7. Immune-mediated haemolytic anaemia
8. Neurological signs (peripheral >> CNS)
9. Reproductive disorders
10. Fading kitten syndrome
11. Immune-mediated uveitis
12. Immune-mediated polyarthritis

For diagnosis of FeLV infection, usually direct methods of virus detection are preferred. Direct FeLV detection methods include detection of free FeLV p27 antigen by ELISA or immunomigration methods or cell-bound FeLV antigens by IFA, detection of proviral DNA (PCR) or plasma viral RNA (RT-PCR) or virus isolation. Routine tests (POC tests) are available that detect free FeLV p27 antigen produced and shed abundantly by virus infected cells in blood; these tests are based on the principals of ELISA or a similar immunochromatography technique. Detection of antibodies (indirect detection methods) is not widely available, although a new POC test has recently become available in Europe. Measurement of antibodies is necessary to detect abortive infection. Presence of neutralising antibodies (only tested in a few laboratories worldwide) can indicate protection against new infection.
The first tests that become positive after experimental FeLV infection are usually virus isolation and RT-PCR for the detection of viral RNA, followed within a few days by DNA (provirus) PCR, then ELISA, and later by IFA. Cats with progressive infection are usually positive in all those tests.

Table 8: Specifics of FeLV detection methods modified from (Hofmann-Lehmann and Hartmann 2020)

| **Detection of** | **Material** | **Method** | **Detects viremia/ antigenemia*** | **Detects** latency of the virus during regressive infection (presence of provirus) | **Earliest positive result after infection** | **Availability** |
|---|---|---|---|---|---|---|
| Replicating virus | Blood (whole blood) | Virus isolation | Yes | Only if bone marrow is treated in vitro with high dose glucocorticoids | Weeks 1-2 | Specialized laboratories; usually not for routine diagnostics |
| Free FeLV p27 antigen | Blood (preferentially plasma or serum) | POC test, plate-based ELISA | Yes | No | Week 3-6 | POC test available worldwide; plate-based ELISA in specialized laboratories |
| Cell-associated FeLV p27 antigen in neutrophils and platelets | Blood (blood smear) | IFA | Yes | No | Usually 3 weeks after free FeLV p27 antigen test | Specialized laboratories; usually not for routine diagnostics or screening purposes |

| | | | | | | |
|---|---|---|---|---|---|---|
| Proviral DNA ("provirus") | Blood (whole blood) | PCR | Not directly (but high proviral loads in viraemic/ antigenemic cats**) | Yes | Weeks 1-2 | Specialized laboratories |
| Plasma viral RNA | Blood (plasma or serum) | RT-PCR | Not directly (but high viral RNA loads in viraemic/ antigenemic cats**) | No | Week 1 | Specialized laboratories |
| Viral RNA in saliva | Saliva (samples can be pooled in the lab**) | RT-PCR | Yes (viral RNA in saliva correlates well with antigenemia) | No | Weeks 1-2 | Specialized laboratories |
| Neutralising antibodies to FeLV | Blood (plasma or serum) | *In vitro* neutralisation | No | Yes (regressively infected cats have neutralising antibodies) | Week 2 at the earliest | Specialized laboratories |
| Antibodies to FeLV | Blood (plasma or serum) | ELISA (whole virus, SU), Western blot, neutralisation, p15E POC test | No | Yes (regressively infected cats have antibodies to p15E) | Week 2 at the earliest | Specialized laboratory***; POC test available, but not yet validated in the field |

*Antigenaemia is a measure for viraemia in most cats. **Quantitative (real-time) PCR or RT-PCR in specialized laboratories is necessary to determine quantitative results and to have a sufficiently high sensitivity. *** Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland; University of Glasgow Centre for Virus Research, Glasgow, Scotland (Parr et al., 2021). IFA = immunofluorescence assay; ELISA = enzyme linked immunosorbent assay; POC = point-of-care.

In practice, diagnosis of FeLV infection is usually based only on the detection of free FeLV p27 antigen mostly using POC tests. However, when measuring FeLV antigen, only progressively infected cats, and some cats with regressive infection during very early infection (transient viraemia) or after reactivation of the infection can be detected. Most cats with regressive infection and cats with abortive infections are not identified using FeLV p27 antigen tests. PCR that detects genome-integrated FeLV provirus reveals a higher number of FeLV-infected cats than antigen tests routinely used for detection of FeLV infection, since also regressively infected cats are FeLV provirus-positive (Hofmann-Lehmann et al., 2001; Gomes-Keller et al., 2006a; Englert et al., 2012).

Table 9: FeLV infection courses and test results, modified from (Hartmann and Hofmann-Lehmann 2020a)

| **Parameter** | **Progressive Infection** | **Regressive Infection** | **Focal Infection (rare)** | **Abortive Infection** | **No Infection** |
|---|---|---|---|---|---|
| FeLV infection status & immune response | Persistent viremia (ineffective immune response) | Undetectable or transient viremia (effective immune response) | Discordant FeLV results (effective immune response) | Virus undetectable (highly effective immune response) | No FeLV infection (no immune response) |
| Free FeLV p27 antigen in blood (ELISA or immunomigration on blood samples) | Positive (≈3-6 weeks after infection) | Always negative or only short-term positive during transient viremia (or positive after reactivation)* | Alternating or low positive | Negative | Negative |
| Intracellular FeLV p27 antigen (IFA on blood smear) | Positive (≈3 weeks after free FeLV p27 antigen testing) | Always negative or only short-term positive during transient viremia (or positive after reactivation)* | Negative or alternating | Negative | Negative |

| | | | | | |
|---|---|---|---|---|---|
| Proviral FeLV DNA (PCR of whole blood) | Positive (≈2 weeks after infection) | Positive (≈2 weeks after infection) | Negative or low positive | Negative | Negative |
| Anti-FeLV antibodies (different tests on serum & plasma) | Negative (or low titres) | Positive (high titres) | Positive (high titres) | Positive (variable titres) | Negative (possibly positive if vaccinated) |
| Replicating virus (virus isolation from blood samples) | Positive | Always negative or only short-term (i.e., first few weeks) positive during transient viremia (or positive after reactivation)* | Negative | Negative | Negative |
| Viral RNA (RT-PCR of blood samples) | Positive (≈1 week after infection) | Usually negative | Usually negative | Negative | Negative |
| Viral shedding | Yes | No (only during transient viraemia or after reactivation) | Unlikely | No | No |
| Consequences | FeLV-associated disease is common, poor long-term prognosis | Usually no clinical signs, rarely FeLV-associated lymphoma or bone marrow suppression can be caused by regressive infection | Unlikely | None | None |
| Usefulness of vaccination | No | No | Unlikely | Unlikely | Yes |

*Some regressively infected cats never develop detectable antigenemia or viremia.

***Detection of Intracellular gag Proteins using the Immunofluorescence Assay***

Historically, the first method that allowed FeLV detection in progressively infected cats under field conditions was the indirect immunofluorescence assay (IFA), introduced in 1973 (Hardy et al., 1973). It was based on the observation that granulocytes, lymphocytes, and platelets in progressively infected cats contain gag components including FeLV p27 antigen (Fig. 2), which can be detected by IFA in blood smears after bone marrow infection (Fig. 19). Thus, IFA becomes positive later (about three weeks) than the ELISA (Table 8) and only in the phase of the second viraemia, when precursor cells in the bone marrow are infected (Fig. 3 ). This also means that positive IFA results are more likely associated with progressive infection (Hardy 1981b; Hardy 1991; Levy 2000). The diagnostic sensitivity of IFA is significantly less than that of the ELISA (Hawks et al., 1991). If a progressively infected cat has leukopenia or if only a small percentage of peripheral leukocytes are infected, the presence of FeLV infection can be overlooked using IFA tests. Furthermore, eosinophils can bind the FITC conjugates used for IFA resulting in false positive tests if slides are not read carefully and by an experienced person (Floyd et al., 1983). False-positive results can also occur when smears are too thick, background fluorescence is high, or the test is prepared and interpreted by inexperienced personnel. Using anti-coagulated blood rather than fresh blood for preparing smears can also cause errors (Jarrett 1995; Weijer and van Herwijnen 1995). IFA requires special processing and fluorescent microscopy and must be performed by a qualified reference laboratory. Variations in quality control among facilities have been reported, and careful attention should be paid to the selection of the reference laboratory (Levy 2000).

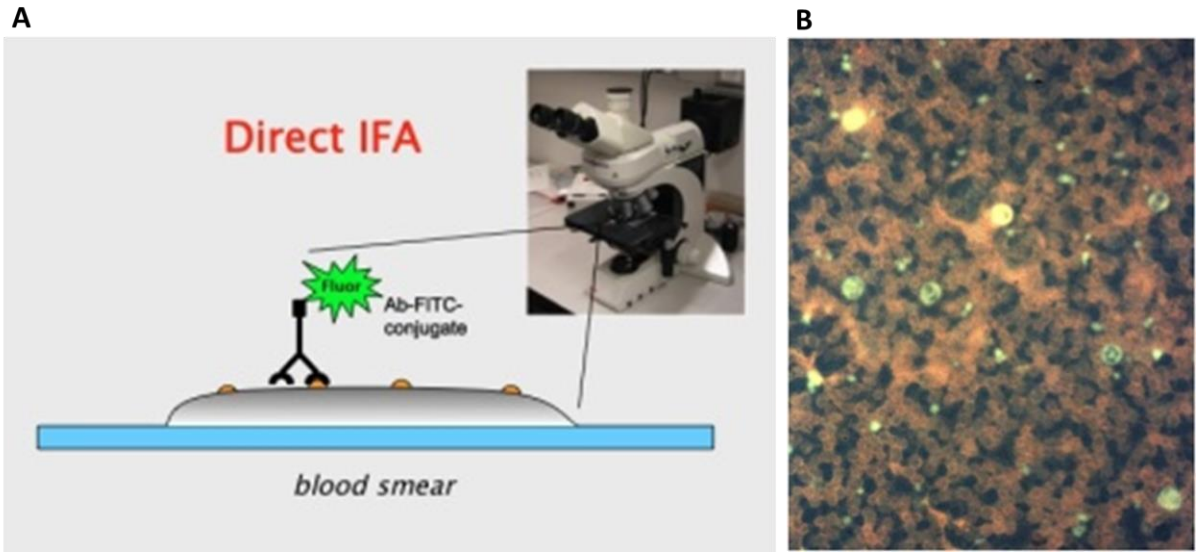


Fig. 19. A) The principle of an immunofluorescence assay for antigen detection in cells ©Hans Lutz; B) Immunofluorescence test on a blood smear ©Diane Addie

For IFA, two or more blood smears of good quality should be air-dried and mailed, unfixed, to the laboratory. However, IFA is not recommended as a screening test because cats that are in the first weeks of viraemia, but already infectious to others, are not detected. IFA is still in use in some countries, e.g., USA. It can be used for prognostic reasons or to confirm positive and suspicious ELISA results (Hardy and Zuckerman 1991).

***Detection of Free FeLV p27 Antigen using ELISA or Similar Immunochromatography***

FeLV p27 antigen can be detected using POC tests based on an ELISA or similar immunochromatography principle (so-called immunomigration test) or using plate-based ELISA in specialized laboratories. FeLV p27 antigen detection is recommended as screening FeLV tests for progressive infection; these tests also detect regressively infected cats in the phase of transient viraemia (Barr 1996). A positive whole blood, serum, or plasma FeLV p27 antigen result usually indicates that the cat is viraemic, with few exceptions (Jarrett et al., 1982b; Lutz et al., 1983b; Jarrett et al., 1991) (see also discordant cats and focal infection). FeLV antigen tests turn positive early in infection, during the primary viraemia and within approximately three weeks after infection, already before the bone marrow is affected. In experimental settings, most cats have positive ELISA results within four weeks after exposure (Jarrett et al., 1982a).

It is recommended that the FeLV status of every cat should be known (Levy et al., 2008a; Little et al., 2020). Usually FeLV p27 antigen tests, sometimes in combination with PCR tests, depending on the situation, are used to achieve this (Table 9). Now POC tests that detect FeLV p27 antigen in combination with p15E antibodies might give more information than conventional POC tests, but these new tests still have to be evaluated.

Table 10: Testing for FeLV p27 antigen should be conducted in these cats or scenarios

| |
|---|
| 1. All cats with unknown FeLV status<br>2. Cats suspected of having an FeLV infection for any reason<br>3. Sick cats presented for veterinary examination<br>4. Healthy cats prior to FeLV vaccination<br>5. Cats with an unknown FeLV history<br>6. For detection of FeLV shedders, such as in a multi-cat environment<br>7. Prior to introducing a new cat into an environment |

*Detection of free FeLV p27 antigen using ELISA*

In 1979, the first commercial ELISA was licensed. These assays detect free (soluble) FeLV p27 antigen. The initial ELISA proved very sensitive in detecting already low concentrations of antigen in serum of infected cats (Lopez et al., 1989). However, the assay was based on polyclonal antibodies and not very specific. The tests had the advantage of allowing quantitation of p27 but had a tendency to produce false-positive results as the antibodies did not only detect viral proteins but occasionally also non-viral components (Lutz et al., 1980b; Lutz et al., 1980c). Improved ELISA based on monoclonal antibodies to p27 were introduced later to detect p27 capsid protein of FeLV present in blood or serum (Lutz et al., 1983a;

Lutz et al., 1983b). The sandwich ELISA was based on three different epitopes of FeLV p27 antigen that did not cross-react with proteins of other retroviruses (Fig. 20); thus, the resulting test was more specific (Lutz et al., 1983a). ELISA are available as plate-based assays usually run in specialised laboratories or as POC tests for in-house diagnostic.

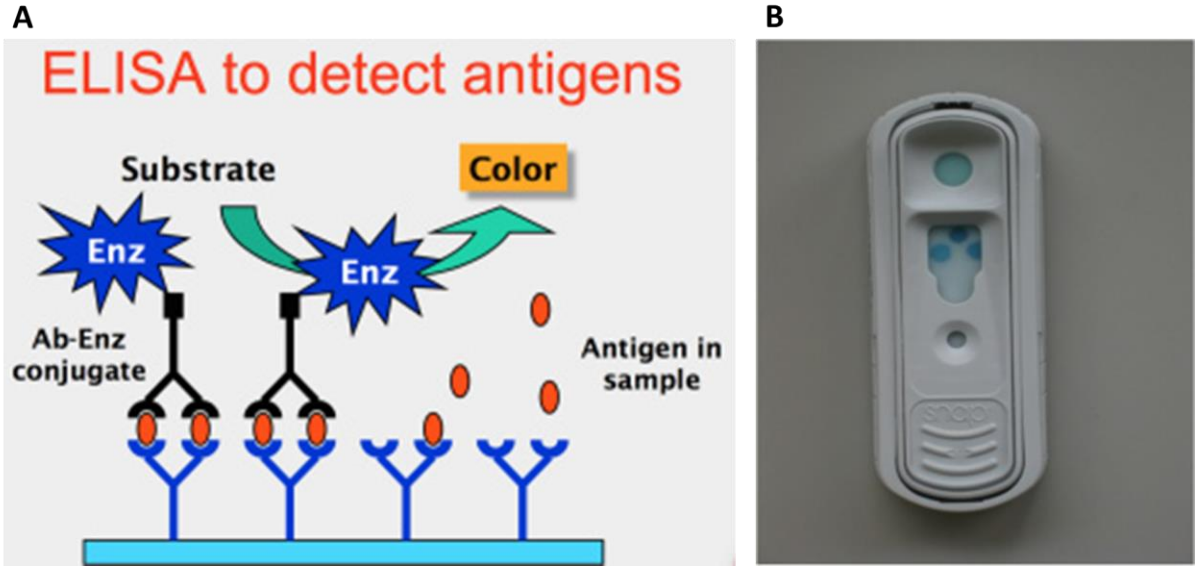


Fig. 20. A) The principle of the sandwich ELISA for antigen detection ©Hans Lutz. This FeLV assay (Lutz et al., 1983a) utilizes a single monoclonal antibody specific for an epitope (A, blue antibodies) of p27 fixed to a solid phase (light blue, ELISA plate well bottom). The serum sample to be tested is mixed with one or two additional monoclonal antibodies specific for epitopes B and C of p27 (black antibodies), and the mixture is then added to the solid phase (ELISA plate well). Hence the presence of p27 in the serum (red dots) leads to insolubilisation of the enzyme-conjugated (Enz) antibodies (black antibodies) and the resulting colour change, when a substrate for the enzyme is added, is indicative for the presence of p27, a marker of progressive infection or of transient viremia in some cats with the regressive form of infection during the early phase. B) An ELISA-based POC test for FeLV p27 antigen detection ©Hans Lutz.

ELISA procedures have the advantage of high diagnostic sensitivity and specificity. However, even improved ELISA and immunomigration tests can have false-positive results. Although ELISA can be performed on serum, plasma, or whole blood, in some studies, higher rates of false-positive results were recorded when whole blood samples were used, particularly when the samples were haemolysed (Barr 1996). Thus, plate-based ELISA as well as ELISA-based POC tests should preferentially be performed with plasma or serum and not whole blood. False-positive results were also a problem in some test systems that used murine-derived reagents in cats that had naturally occurring anti-mouse antibodies (Lopez et al., 1989), which are present in about 1% to 2% of all cats. Improved tests have solved that problem by including additional control steps. Technical and user errors contribute to false-positive results as well (Hardy and Zuckerman 1991; Macy 1991). These errors are most likely to occur during the washing steps of kits using micro-well or plate formats. Immunomigration tests eliminate separate washing steps and include positive and negative controls for each test sample.

Some ELISA have been developed for tear and saliva samples. Testing of tears or saliva for FeLV p27 antigen, however, is not recommended because antigen shedding is intermittent resulting in false-negative results (Benveniste et al., 1975; Hawkins 1986; Lutz and Jarrett 1987; Hawkins 1991; Babyak et al., 1996).

One advantage of plate-based ELISA is that they can be used to quantify FeLV p27 antigen loads (Lutz et al., 1980c; Lutz et al., 1983a; Beall et al., 2019). A positive correlation was demonstrated between FeLV p27 antigen loads and proviral DNA loads as determined by quantitative PCR (Tandon et al., 2005; Beall et al., 2019). In a recent study it was found that samples with high proviral DNA loads (at least $1\times10^6$ copies/mL of whole blood), typically had FeLV p27 antigen concentrations greater than 30 ng/mL in plasma, and samples with proviral DNA loads below this level had concentrations of FeLV p27 antigen in plasma that were less than 10 ng/mL (Beall et al., 2019). The authors suggested that the concentration of FeLV p27 antigen at a given point in time can help to indicate the likelihood of progressive or regressive infection. This had indeed been demonstrated in an experimental study, where cats with regressive FeLV infection had lower FeLV p27 antigen loads compared to progressively infected cats starting four weeks after infection (Hofmann-Lehmann et al., 2001; Helfer-Hungerbuehler et al., 2015b). However, FeLV p27 antigen loads correlate more strongly with plasma and saliva viral RNA loads than with proviral loads (Tandon et al., 2005; Gomes-Keller et al., 2006a). Nonetheless, FeLV p27 antigen loads could be used to help indicate the current stage of infection and be a prognostic marker to monitor the outcome of infection over time (Beall et al., 2019). Asymptomatic cats with progressive FeLV infection had lower FeLV p27 antigen loads than diseased progressively FeLV-infected cats (Helfer-Hungerbuehler et al., 2015b). In a longitudinal

study in cats that tested positive at intake in a cat shelter offering an adoption program, a correlation of FeLV p27 antigen and provirus blood levels and the survival of the cats during a four year observation period was found (Beall et al., 2021). Cats were tested monthly for six months and then followed after adoption for four years. At that time, the median survival time of cats with high p27 antigen loads (37.5 ng/ml) and/or proviral loads (> 4 x $10^5$/copies/ml) was 1.37 years (95% CI 0.83-2.02), while most of the cats with low initial loads were still alive (93.1% survival). Further prospective studies are needed to better understand how quantitation of FeLV p27 antigen could help to indicate the current stage of infection and the prognosis of the cat.

*Detection of free FeLV p27 antigen using immunomigration tests*

Immunomigration tests are another form of POC tests; they are also immunological assays like the ELISA but they use small beads of less than one micron in size that are coated to the revealing antibodies rather than enzymes like (Fig. 21). The diagnostic sensitivity and specificity of immunomigration tests was shown to be comparable to those of the ELISA (Robinson et al., 1998; Hartmann et al., 2001; Hartmann et al., 2007; Pinches et al., 2007a; Sand et al., 2010; Hartmann 2017). Immunomigration tests contain a filtering membrane, so whole blood and serum/plasma do not produce different results (Hartmann et al., 2001).

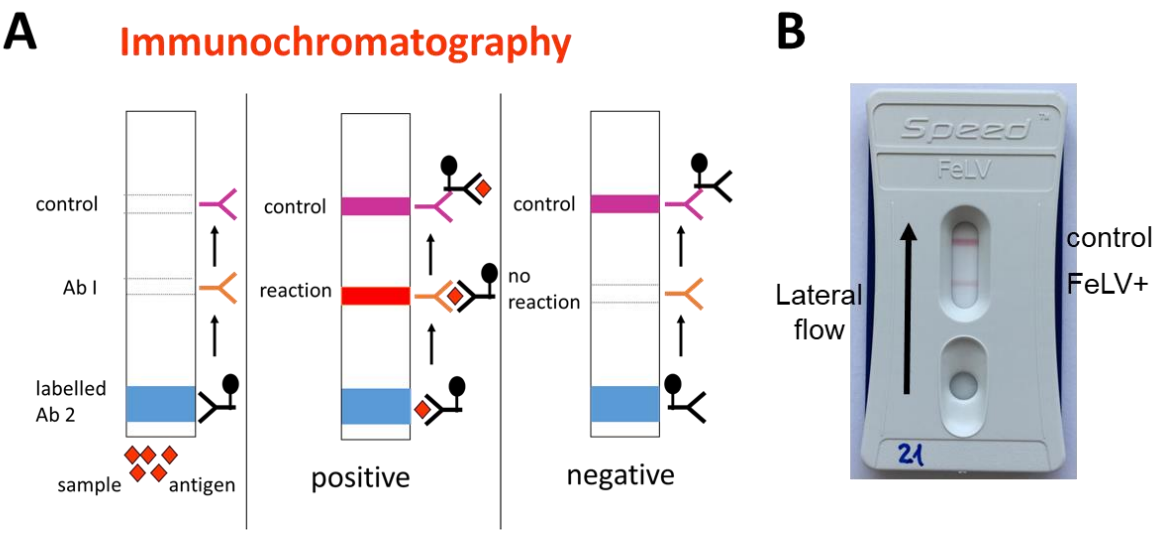


Fig. 21. A) The principle of an immunomigration test (lateral flow) for antigen detection ©Hans Lutz; B) An immunomigration POC test for FeLV antigen detection.

Several comparison studies between different POC tests for the detection of FeLV p27 antigen have been performed (Hartmann et al., 2001; Hartmann et al., 2007; Pinches et al., 2007a; Sand et al., 2010; Kim et al., 2014; Levy et al., 2017b; Westman et al., 2019b). These comparison studies are, however, difficult to compare, due to differences in study design, especially concerning the reference standards used. In addition, tests with similar names can differ between countries or might have undergone design changes over time. It is difficult to select the gold standard for FeLV diagnostic comparison studies, since references tests, such as PCR, detect different virus components and thus different outcome of FeLV infection, and therefore results are not directly comparable. However, in most of these studies, sensitivities and specificities of the different tests were similar. In a study in the USA, four different FeLV POC tests were compared using 146 FeLV antigen-positive and 154 FeLV antigen-negative serum or plasma samples (Levy et al., 2017b). The results of two plate-based ELISA tests were used as the gold standard for the determination of the true FeLV infection status. Diagnostic sensitivity and specificity for the included cat population were between 85.6% and 100% and between 85.7% and 100%, respectively (Levy et al., 2017b). A large study from Australia using EDTA whole blood and comparing with proviral PCR results found, of course (since antigen-negative regressively infected cats were also included), much lower sensitivities for the evaluated three POC tests of 57% to 63%; at the same time they found a high specificity (94% and 98%) (Westman et al., 2017). Thus, the sensitivity and specificity largely depended on the tested cat population. A comparison of different FeLV POC tests using saliva revealed a lower diagnostic sensitivity and confirmed that saliva is not an appropriate matrix for FeLV antigen testing (Westman et al., 2017; Westman et al., 2019b).

The reliability of a test (its predictive values) strongly depends on the prevalence of infection within a cat population (Levy et al., 2017b). Positive predictive values of most tests in the comparison studies were between 50% and 100%, whereas negative predictive values were 94% to close to 100% (Hartmann et al., 2001; Hartmann et al., 2007; Sand et al., 2010; Liu et al., 2016; Westman et al., 2017). False-positive results, therefore, are more important nowadays because the decrease in FeLV prevalence led to lower positive predictive values in most geographic areas. In an Australian study, 21-38% of false positive FeLV p27 antigen results were reported in a cat population with a FeLV sample prevalence of 8% (Westman et al., 2019b). In preselected cat populations with an expected high prevalence, e.g., in cats with thymic lymphoma, a positive

test result is likely to be more accurate, whereas in a lower-risk population, such as a closed breeding colony known to be free of FeLV, a positive test should be viewed with more suspicion, and confirmatory tests should be performed (see also Fig. 18). Thus, positive FeLV p27 antigen test results have to be interpreted carefully, and confirmatory tests have to be considered after a positive result (see Table 12 and Table 12). In general, negative test results are highly reliable because of the low FeLV prevalence in most populations (Burling et al., 2017; Studer et al., 2019), unless the cat has undergone a potential infection risk very recently. It usually takes at least 3 weeks (sometimes even longer) after FeLV exposure before FeLV antigen can be detected in the peripheral blood of an infected cat. In cats with a potential recent FeLV exposure risk and a negative FeLV p27 antigen result, RT-PCR for the detection of viral RNA in blood or saliva can be used; RT-PCR tests positive earlier after infection (Table 8).

Table 11: Calculated positive and negative predictive values for three POC tests in hypothetical populations of cats with low risk (1% prevalence) to high risk (50% prevalence) of FeLV infection (modified from (Levy et al., 2017b)).

| Prevalence in hypothetical populations | Sensitivity/ specificity of FeLV POC tests | 1% (FeLV prevalence observed in most European countries) | | 5% (FeLV prevalence observed in some European countries) | | 10% (FeLV prevalence in some Asian countries) | | 25% (FeLV prevalence in high-risk situations, e.g., stray cat populations) | | 50% (FeLV prevalence in very high-risk situations, e.g., FeLV-endemic households) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PPV | NPV | PPV | NPV | PPV | NPV | PPV | NPV | PPV | NPV |
| Test A | 89%/96% | 17 % | 100 % | 51 % | 99 % | 69 % | 99 % | 87 % | 96 % | 95 % | 90 % |
| Test B | 92%/96% | 17 % | 100 % | 52 % | 100 % | 69 % | 99 % | 87 % | 97 % | 95 % | 92 % |
| Test C | 86%/86% | 6 % | 100 % | 24 % | 99 % | 40 % | 98 % | 67 % | 95 % | 86 % | 86 % |

PPV = positive predictive value; NPV = negative predictive value

For confirmation of positive FeLV p27 antigen results, provirus PCR is recommended. If PCR is not readily available or too expensive to perform, a second POC can be performed to decrease the likelihood of a false-positive result (preferentially a test from of a different brand). If the second test is positive, this increases the predictive value of the positive result (Hartmann et al., 2001; Westman et al., 2019b). Retesting should be performed immediately and has nothing to do with the different outcomes of FeLV infection; it is used to compensate for the low predictive value of positive results in an environment with a low FeLV prevalence. The diagnostic tree for the diagnosis of FeLV infection (Fig. 18) takes these factors, such as low prevalence, already into account. It indicates at which steps confirmation of results is necessary to reach a reliable interpretation of the test results (Fig. 18).

Table 12: When to immediately repeat an FeLV POC test

If a positive result:
- Is found in cats from areas with low prevalence of FeLV infection
- Is found in low-risk cats
- Would lead to euthanasia (e.g., shelter situation)

If a negative result:
- Is found in high-risk cats
- Is found in cats traveling from a high-risk area or country

It is important to correctly interpret FeLV p27 antigen test results (Table 13 and Table 14).
If the cat is negative but recent FeLV exposure cannot be excluded, the cat should be tested immediately using RT-PCR or it should be retested for FeLV p27 antigen in approximately 6 weeks (Fig. 18). During this time span, the cat should be kept separated from further potential infection risks, and not to pose a risk for other cats, should it indeed be FeLV infected.
A confirmed positive FeLV antigen test result (whether a single result or repeatedly positive result over time) should never be a death sentence for a cat. However, the cat should be kept separated from other cats since it poses an infection risk to naïve cats.
FeLV p27 antigen positive cats present an infection risk and should always be kept separated from FeLV-negative companions, regardless of their health status and until retesting negative at a later time point.

Table 13: Interpretation of a negative FeLV p27 antigen test result

| A negative FeLV p27 antigen test result indicates that the cat |
|---|
| • was not exposed to FeLV (uninfected)<br>• is immune to FeLV (e.g., has been vaccinated), has overcome antigenaemia (is regressively infected)<br>• has abortive FeLV infection or<br>• is not yet positive because it is still in a very early stage of FeLV infection. |

Table 14: Interpretation of a positive FeLV p27 antigen test result

| A confirmed positive FeLV p27 antigen test result indicates that the cat |
|---|
| • is antigenaemic and shedding FeLV at the time of testing<br>• can undergo either regressive or progressive infection (for differentiation see below) |

To distinguish between regressive and progressive infection, repeated testing of FeLV p27 positive cats is necessary. Cats in which an antigen-positive test result has been confirmed can overcome viraemia (regressive infection) after weeks, usually by twelve weeks – in rare cases even later; in one documented case more than one year after infection (Hofmann-Lehmann et al., 1995). Thus, positively tested cats should be retested for FeLV p27 antigen six weeks after the first positive test result (Fig. 18). If a cat still then has a positive result, it should be retested after another six weeks. If at this time the cat still has a positive result, it is most likely progressively infected and will have positive results for the rest of its life.

Another method without the retesting delay is to immediately test a cat with a positive ELISA results with IFA. If the IFA is positive, the likelihood of a regressive infection is small, because less than 10% of the positive IFA results are caused by a transient viraemia (Hardy 1981a; Jarrett et al., 1982a; Lutz et al., 1983a; Hardy and Zuckerman 1991; Jarrett et al., 1991). A small number of cats (2-3%) with discordant test results that develop persistently positive results in ELISA and negative results in IFA and virus isolation, can have focal or atypical infections that are kept localized by the cats' immune systems; such cats are potential sources of infection (Lutz et al., 1980c; Jarrett et al., 1991). A negative ELISA but positive IFA is always a false result, either a false-negative ELISA result (which is very unlikely) or a false-positive IFA result.

***Detection of Replicating Virus by Virus Isolation using Cell Culture***

Virus isolation using cell culture was originally developed to identify FeLV-infected cats and was considered the ultimate criterion for progressive FeLV infection (Jarrett et al., 1968; Jarrett 1980; Jarrett et al., 1982b; Devauchelle 2001). Virus isolation tests positive at least one week earlier than FeLV antigen detection (Table 8). However, it might not be practicable for routine diagnosis because it is time-consuming to perform and requires specialised laboratories. Moreover, only few laboratories still offer virus isolation today. It can still be used for the confirmation of positive antigen test results and to investigate suspicious samples.

***Detection of FeLV Genome using Molecular Assays (PCR, RT-PCR)***

PCR and RT-PCR detect viral nucleic acid sequences; PCR detects proviral DNA (provirus integrated into the cellular genome of the cat), while RT-PCR detects viral RNA (cell-associated or free replicating virus). Both molecular methods can be performed on blood, saliva, bone marrow, and other tissues (Gomes-Keller et al., 2006b; Hofmann-Lehmann et al., 2008; Cattori et al., 2009; Helfer-Hungerbuehler et al., 2015b). Molecular methods are very sensitive because the process involves amplification of FeLV gene sequences to enhance detection. When performed under optimal conditions, molecular methods are the most sensitive tests for FeLV diagnosis and can help resolve cases with discordant antigen test results. However, they must be performed by well-equipped and well-trained laboratories because minor alterations in sample handling can destroy the nucleic acid material or introduce minute amounts of cross-contamination, leading to either false-negative or false-positive results, respectively. Technical errors can reduce sensitivity and specificity of results from molecular methods. There are no comparative studies of the diagnostic accuracy of various commercial laboratories offering FeLV PCR. In addition, molecular methods are highly target specific. As a retrovirus, FeLV mutates naturally, and minor strain variations can prevent binding of the primers, a step necessary to amplify the viral genome. To reduce the risk that cats infected with mutated FeLV might have negative test reactions with a specific PCR or RT-PCR, the assays usually target conserved regions of the FeLV genome; however, different assays might vary in their analytical sensitivity and specificity. Moreover, primers and probe must be designed to distinguish endogenous FeLV-like sequences from exogenous FeLV infection. With all these issues in mind, PCR has greatly enhanced the possibilities of detecting FeLV infection in blood, cultures, solid tissue, and fixed specimens.
In addition, quantitative real-time PCR and RT-PCR is used to quantify provirus and viral RNA loads, respectively (Hofmann-Lehmann et al., 2001; Tandon et al., 2005; Cattori et al., 2006; Arjona et al., 2007; Pinches et al., 2007b; Cattori et al., 2008; Torres et al., 2008). Using quantitative PCR, it has been shown that proviral loads in regressively infected cats that mount an effective immune response were much lower (300-fold less) than proviral loads in progressively infected cats (Hofmann-Lehmann et al., 2001).

*Detection of proviral DNA using PCR*

PCR detects the provirus integrated into the cellular genome of the cat and can be performed on blood, bone marrow, and other tissues (Table 15). Whole blood is mostly used for the detection of proviral DNA. To omit venipuncture, PCR was suggested from oral, conjunctival and rectal swabs; however, the diagnostic sensitivity was lower compared to whole blood (86%, 90% and 74%, respectively) (Victor et al., 2020).

Table 15: Testing for proviral DNA using PCR is helpful in these cats or scenarios

1. Confirmation of FeLV p27 antigen results: One important indication for PCR detecting FeLV DNA (provirus) in whole blood is to confirm a positive or inconclusive FeLV p27 antigen test result. This becomes increasingly important with a decreasing prevalence of FeLV and thus, a decreasing positive predictive value of positive antigen test results. True positive ELISA results are accompanied by high proviral loads in the blood (Hofmann-Lehmann et al., 2001; Hofmann-Lehmann et al., 2006; Beall et al., 2019).
2. Application during early infection: PCR from whole blood can be helpful in the very early phase of FeLV infection, as provirus PCR is positive earlier than antigen detection (Hofmann-Lehmann et al., 2006) (Table 8).
3. Detection of regressive infection: Studies using PCR found that 1.2% to 10% of cats with negative antigen tests were positive for FeLV provirus by PCR (regressive infection) (Hofmann-Lehmann et al., 2001; Gomes-Keller et al., 2006a; Englert et al., 2012). Most of these cats remain antigen-negative, do not shed virus, and are unlikely to ever develop FeLV-associated diseases, but some can reactivate and then, shed FeLV and suffer from FeLV-associated problems (Helfer-Hungerbuehler et al., 2015b).
4. Detection of FeLV infection in multi-cat households: Confirm absence of FeLV or to identify regressively infected cats (provirus carriers) in a multi-cat environment, such as a breeding colonies and catteries.
5. Clarification of obscure clinical cases with suspected FeLV infection but absence of FeLV antigenaemia: Provirus carriers have been detected among cats with lymphoma, bone marrow-suppressive syndrome, or stomatitis (Hayes et al., 1992; Jackson et al., 1993; Jackson et al., 1996; Uthman 1996; Stützer et al., 2010; Stützer et al., 2011; Meichner et al., 2012).
6. Testing of blood donors: Blood donors (and blood products) as well as tissue donors should be free of progressive and regressive FeLV infection prior to transfusion.

Correct interpretation of positive and negative PCR results is important. A positive provirus PCR result indicates that the cat has been exposed to FeLV and has developed either progressive or regressive infection (Table 9). It confirms a positive or questionable FeLV p27 antigen test results. Some laboratories also provide the provirus load (quantitative value). Progressively infected cats have higher provirus loads than regressively infected cats several weeks into infection (Fig. 9); however, not during early infection. Since in naturally infected cats the time point of infection is usually unknown, the provirus load usually cannot be used to differentiate these two infection courses, and therefore, FeLV p27 antigen testing is necessary to recognize antigenaemic cats (if not done already) and repeated testing is necessary to differentiate regressively and progressively infected cats.

A negative provirus PCR result indicates that the cat does not have provirus integrated into its genome. This is in accordance with no exposure to FeLV, presence of an abortive infection, or a very early stage of FeLV infection (first one to two weeks after FeLV exposure; Table 8).

False-positive PCR results can be caused by laboratory contamination. False negative results can be result from inappropriate assay design or test material or due to laboratory errors. It is important only to use well-experienced reference laboratories where the sensitivity and specificity of the PCR assay is known, and appropriate controls are performed.

*Detection of viral RNA using RT-PCR*

The detection of viral RNA recently has been proposed as a diagnostic tool and added a new aspect to the diagnosis of FeLV infection (Tandon et al., 2005; Torres et al., 2008). Viral RNA present in whole blood, serum, plasma or saliva is detected by RT-PCR. RNA RT-PCR does not provide the same information as DNA (provirus) PCR. If performed on blood (or serum or plasma), RT-PCR always reacts positive, when FeLV p27 antigen tests are positive. Detection of viral RNA in saliva is a reliable parameter of viraemia (Gomes-Keller et al., 2006a; Gomes-Keller et al., 2006b; Cattori et al., 2009). RT-PCR can thus be used as confirmatory test of positive FeLV p27 antigen test results. However, in some cats with regressive infection, plasma viral RNA can be found also in the absence of FeLV antigen (Gomes-Keller et al., 2006a; Hofmann-Lehmann et al., 2007; Hofmann-Lehmann et al., 2008).

RT-PCR from plasma and saliva can be helpful in the very early phase of infection. Detection of viral RNA by RT-PCR from plasma or saliva is positive as early as one week after FeLV exposure and with that earlier than FeLV p27 antigen testing and also earlier than provirus PCR from blood (Table 8) (Hofmann-Lehmann et al., 2006; Cattori et al., 2009).

The use of saliva as the substrate for RT-PCR represents an alternative for assessing the infectious state of a cat without leading to unnecessary stress to the patient commonly associated with blood sampling. Moreover, the sample collection

does not require trained personnel. FeLV RNA was stable for more than 64 days in saliva samples stored at room temperature (Gomes-Keller et al., 2006a; Gomes-Keller et al., 2006b; Englert et al., 2012). When in a multi-cat environment the cost of testing is a limitation, pooled saliva samples can be used for RT-PCR, as the assay is sufficiently sensitive to detect a single infected cat in a pool of up to 30 samples (Gomes-Keller et al., 2006b).

Interpretation of RT-PCR (viral RNA) results differs from those of PCR (proviral DNA) results. If the FeLV RT-PCR result from saliva in a single cat is positive, the cat is antigenaemic and an FeLV shedder at the time it was tested. Thus, interpretation is the same as for a cat that tests positive for free FeLV p27 antigen. If a pooled saliva sample from a multi-cat environment is positive, subsequent testing of individual cats is necessary to detect the FeLV shedder(s) within the group of tested cats, either by using RT-PCR on single saliva swabs or FeLV p27 antigen testing of blood from individual cats. As for proviral PCR, only experienced laboratories should be used for these analyses.
If a positive RT-PCR result from blood occurs in an aviraemic (antigen-negative) cat, this can occur during the very early phase of the infection (Table 8), in cats with regressive infection (with potentially a higher risk of reactivation), or possibly in a cat with focal infection.

***Indirect Detection of FeLV Infection using Antibody Testing***

**Biological active virus-neutralising antibodies** can be measured using cell culture assays as a predictor of protection. However, virus neutralisation tests are performed only in specialised laboratories and only used infrequently for routine diagnostic purposes. Testing for FeLV neutralising antibodies can be used to differentiate FeLV disease outcomes (Lutz et al., 1983b; Hofmann-Lehmann et al., 2001; Hofmann-Lehmann et al., 2007). Most cats with regressive infection exhibit a strong neutralising immune response, while progressively infected cats have low levels of or no neutralising antibodies against FeLV (Fig. 6). The connection between presence of antibodies and immunity is not absolute, because some vaccinated cats will not develop antibodies (Englert et al., 2012) and there will be cats that are protected against FeLV despite the absence of detectable antibodies (Poulet et al., 2003; Sparkes 2003; Hofmann-Lehmann et al., 2006; Langhammer et al., 2006; Boenzli et al., 2014).

The observation that antibodies can develop as the sole parameter of exposure to FeLV (Gomes-Keller et al., 2009; Major et al., 2010) led to the investigation of various antibody tests against different antigens to assess their diagnostic usefulness. In contrast to published results (Fontenot et al., 1992), a recombinant preparation of a FeLV transmembrane protein **p15E** (Fig. 2) proved highly effective for the detection of antibodies induced by FeLV infection and most promising for the diagnosis of previous FeLV exposure (Boenzli et al., 2014). In naturally infected cats, the p15E ELISA showed a diagnostic sensitivity of 77.1% and a specificity of 85.6% when compared with provirus PCR results (Boenzli et al., 2014). Use of the p15E antibody test in combination with FeLV p27 antigen testing might offer most promise for recognising all FeLV exposed cats. The antibody test is expected to be positive in cats with regressive or abortive infection and the antigen test will recognise all cats with progressive FeLV infection. Antibody testing can help to reveal the FeLV status of a population. Recently, the first routine diagnostic p15E POC test became available commercially; however, there is not yet sufficient data on whether this test reliably detects cats with abortive FeLV infection under field conditions.

Another antigen that has been used in an antibody test to detect the humoral immune response to FeLV is the FeLV surface antigen, **FeLV-SU** (Lehmann et al., 1991; Parr et al., 2021). An increase in anti-SU antibodies has been demonstrated after experimental FeLV infections (Hofmann-Lehmann et al., 1995; Hofmann-Lehmann et al., 2007). Measuring antibodies to FeLV-A SU and FeLV-B SU in 123 naturally infected cats demonstrated a correlation between the strength of the humoral immune response to the SU protein and the outcome of exposure (Parr et al., 2021). Cats with regressive infection had higher anti-SU antibody responses than cats with other infection outcomes. Moreover, in Australian cats correlation was demonstrated between neutralising antibodies and anti FeLV-SU antibodies determined by ELISA (Westman et al., 2021): cats with neutralising antibodies displayed significantly higher anti-SU antibody responses compared to neutralising antibody-negative cats. Thus, the easier to determine anti-SU antibody ELISA responses complements the use of viral diagnostic tests to define the FeLV infection outcome or could even be used as a surrogate for neutralising antibodies, thereby allowing the rapid identification of regressively infected cats that are unlikely to develop FeLV-related disease (Parr et al., 2021). In a study assessing the status of FeLV infection in the pet cat population of southern Germany, 78/476 cats (16%) were found to have anti FeLV-SU antibodies despite having negative FeLV p27 antigen and provirus PCR test results and 23 of these 78 cats were known to never have been vaccinated against FeLV (4.8%) (Englert et al., 2012); therefore, it was assumed that these cats had undergone abortive FeLV infection. Similarly, but using a neutralisation assay, 47 of 440 client-owned Australian cats with outdoor access (11%) were assumed to have abortive infection (Westman et al., 2019a).

In some research laboratories, the so-called FOCMA (feline oncornavirus-associated cell membrane antigen) antibody 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 (Vedbrat et al., 1983). Today, this test is not considered to be of clinical value.

## TREATMENT

### Antimicrobial Treatment

#### *Antiviral Chemotherapy*

Antiviral drugs have been commonly used in FeLV-infected cats. They can be divided into different classes based on their interference with the viral replication cycle (Table 16). Unfortunately, few large controlled studies have been conducted in naturally infected cats and most of them did not shown any efficacy of the used antiviral drugs (Hartmann et al., 1992a; Hartmann et al., 1992b; Hartmann et al., 1998; Hartmann et al., 1999; McCaw et al., 2001; Stuetzer et al., 2013).

Table 16: Classes of currently available antiviral compounds

- Entry inhibitors
  - Receptor blockers
  - Fusion inhibitors
- Ion channel blockers
- Reverse transcriptase inhibitors
  - Nucleoside reverse transcriptase inhibitors
  - Nucleotide reverse transcriptase inhibitors
  - Non-nucleoside reverse transcriptase inhibitors
- Integrase inhibitors
- DNA/RNA synthesis inhibitors
  - Nucleoside DNA/RNA synthesis inhibitors
  - Nucleotide DNA/RNA synthesis inhibitors
- Protease inhibitors
- Neuraminidase inhibitors

Most antivirals used in cats are licensed for humans and are specifically intended for treatment of HIV infection. Some of these, such as nucleoside analogues, can also be used to treat FeLV infection but are usually less effective against FeLV when compared to FIV (Hartmann 2012a), as FeLV is not as closely related to HIV. All antiviral compounds interfere with one or more steps of the virus replication process (Table 17). Based upon this, the drugs can be assigned to different drug classes (De Clercq 1995; De Clercq 2009; Palmisano and Vella 2011). Potential targets in the retroviral replication process for antiviral drugs include binding of virus to specific cell surface receptors, entry into the cell and uncoating of the virus, reverse transcription of viral genome, integration of proviral DNA into host genome, viral protein processing, virion assembly and maturation, and virion release (De Clercq 1995; Hartmann 2012a; Mohammadi and Bienzle 2012).

Table 17: Classes, mechanism of action, subcategories, potential to have anti-retrovirus activity, examples of compounds and efficacy for treatment of FeLV infection

| **Class** | **Stage of replication inhibited by the drug** | **Subcategory** | **Compounds** | **Comment** |
|---|---|---|---|---|
| Entry inhibitors | virus attachment | receptor blockers | plerixafor | likely ineffective against FeLV due to the different cellular receptor |
| Ion channel blockers | uncoating | | amantadine | efficacy against FeLV unknown |
| Reverse transcriptase inhibitors | reverse transcription | nucleoside reverse transcriptase inhibitors | zidovudine<br>didanosine<br>zalcitabine<br>decitabine<br>gemcitabine | various FeLV efficacy studies – see Table 18 |
| | | nucleotide reverse transcriptase inhibitors | adefovir<br>tenofovir | |
| | | non-nucleoside reverse transcriptase inhibitors | suramin | |

| | | | | |
|---|---|---|---|---|
| Integrase inhibitors | integration of proviral DNA | | raltegravir | limited FeLV efficacy studies – see Table 18 |
| RNA (DNA) synthesis inhibitors | RNA (DNA) synthesis | nucleoside RNA (DNA) synthesis inhibitors<br><br>nucleotide RNA (DNA) synthesis inhibitors | vidarabine<br><br>foscarnet<br>ribavirin | efficacy against FeLV unknown or only *in vitro* and too toxic |
| Protease inhibitors | maturation | | nelfinavir | efficacy against FeLV unknown, but likely ineffective |
| Neuraminidase inhibitors | extrusion | | oseltamivir | not effective because FeLV does not possess a neuraminidase enzyme |

Although many new, no yet marketed drugs and other therapeutic approaches, such as the small interfering RNA molecules (Lehmann et al., 2015) or synthetic peptides derived from the FeLV transmembrane domain (Boenzli et al., 2011) are under investigation and might be interesting approaches for the future, and although there is a need for new antiretroviral treatment options for FeLV infection (Greggs et al., 2011), this section will focus on drugs that are currently commercially available. For some compounds evidence of efficacy is weak and thus, sometimes only expert opinion, case reports, studies in other species, or pathophysiological justification are available (Table 18).

Table 18: Summary on studies on efficacy of antiviral drugs for treatment of FeLV infection (including EBM grades for judgment of the available efficacy data including expert opinion of the authors)

| DRUG CLASS | DRUG | EFFICACY *IN VITRO* | CONTROLLED STUDY *IN VIVO* | EFFICACY *IN VIVO* | Comments | EBM LEVEL (I - IV) |
|---|---|---|---|---|---|---|
| **Entry inhibitors** | | | | | | |
| | plerixafor | no | no | n. d. | likely ineffective due to the different cellular receptor | IV |
| **Ion channel blockers** | | | | | | |
| | amantadin | no | no | n. d. | efficacy unknown | IV |
| **Nucleoside Reverse Transcriptase Inhibitors** | | | | | | |
| | Zidovudine | yes (Tavares et al., 1987; Tavares et al., 1989; Hoover et al., 1990; Zeidner et al., 1990c; Mathes et al., 1992; Mukherji et al., 1994) | yes | unclear (Zeidner et al., 1990a; Zeidner et al., 1990c; Stuetzer et al., 2013) | only weakly effective | I |
| | Didanosine | yes (Tavares et al., 1989; Zeidner et al., 1990c) | no | n. d. | possibly effective, but no *in vivo* data available | IV |
| | Zalcitabine | yes (Zeidner et al., 1990c) | yes | no (Hoover et al., 1989; Zeidner et al., 1989; Polas et al., 1990) | not very effective, but relatively toxic | II |
| | Decitabine | yes (Greggs et al., 2012) | no | n. d. | possibly effective, but no *in vivo* data available | IV |
| | Gemcitabine | yes (Greggs et al., 2012) | no | n. d. | possibly effective, but no *in vivo* data available | IV |
| **Nucleotide Reverse Transcriptase Inhibitors** | | | | | | |
| | Adefovir | yes (Hoover et al., 1991) | yes | unclear (Hoover et al., 1991; Hartmann | poorly effective, but relatively toxic | I |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | et al., 1992b) | | |
| | Tenofovir | yes (Greggs et al., 2012) | no | n. d. | possibly effective, but likely also relatively toxic | IV |
| **Non-Nucleoside Reverse Transcriptase Inhibitors** | | | | | | |
| | Suramin | no | no | n. d. | weak efficacy in uncontrolled experimental studies and case reports | III |
| **Nucleoside RNA (DNA) Synthesis Inhibitors** | | | | | | |
| | Vidarabine | no | no | n. d. | efficacy unknown, but too toxic | IV |
| **Nucleotide RNA (DNA) Synthesis Inhibitors** | | | | | | |
| | Foscarnet | yes (Swenson et al., 1991) | no | n. d. | effective *in vitro*, but too toxic | IV |
| | Ribavirin | Yes (Greene and Watson 1998b) | no | n. d. | effective *in vitro*, but too toxic | IV |
| **Integrase Inhibitor** | | | | | | |
| | Raltegravir | yes (Zhu et al., 2007; Cattori et al., 2011; Greggs et al., 2012) | yes | yes (Boesch et al., 2015) | mildly effective with reduction of viraemia in one experimental study, but viraemia relapsed after drug removal | II |
| **Protease inhibitors** | | | | | | |
| | nelfinavir | no | no | n. d. | efficacy unknown, but likely ineffective | IV |
| | remdesivir | no | no | n. d. | efficacy unknown, but likely ineffective | IV |
| **Neuraminidase inhibitors** | | | | | | |
| | oseltamivir | no | no | n. d. | not effective because FeLV does not possess a neuraminidase enzyme | IV |

*n. d.*, not determined.
EBM, evidence based medicine (Lloret 2009)
EBM grade I = This is the best evidence, comprising data obtained from properly designed, randomised controlled clinical trials in the target species (in this context cats).
EBM grade II = Data obtained from properly designed, randomised controlled studies in the target species with spontaneous disease in an experimental setting.
EBM grade III = Data based on non-randomised clinical trials, multiple case series, other experimental studies, and dramatic results from uncontrolled studies.
EBM grade IV = Expert opinion, case reports, studies in other species, pathophysiological justification.

***Entry Inhibitors***

Entry inhibitors either work as receptor blockers or fusion inhibitors. Fusion inhibitors have so far not been used in veterinary medicine. Receptor blockers are either homologues or antagonists of either the main receptors or of co-receptors. If these receptor blockers bind to the virus or to the cellular receptor, they lead to inhibition of attachment of the virus to the cell surface. Most of these receptor homologues/antagonists are highly selective for HIV and not useful for veterinary medicine. One exception that can be used in cats with FIV infection are bicyclams (e.g., plerixafor), because of the similarity between HIV and FIV infection concerning chemokine receptor usage for infection (Rucker et al., 1997; Willett and Hosie 1999; Hartmann et al., 2012). Efficacy of receptor homologues/antagonists against FeLV has not been determined, but they are likely ineffective due to the difference in receptors that FeLV uses for cell entry (Helfer-Hungerbuehler et al., 2011).

***Ion Channel Blockers***

Uncoating of viruses can be blocked by ion channel blockers, such as amantadine, that bind to M2 proteins that function as ion channels for proton influx into the virus. This leads to a pH change that causes inhibition of fusion of virus and endosomal membrane and thus, inhibits the internalization of the virus by endocytosis. Those compounds are mainly used against influenza in humans. Their usefulness against FeLV is unknown.

***Nucleoside Reverse Transcriptase Inhibitors***

The retroviral enzyme reverse transcriptase transcribes the viral RNA into proviral DNA, which is subsequently integrated into the host cell's genome (Mitsuya and Broder 1987). This is an important step in the retroviral replication cycle, and the compounds that inhibit this step have become the cornerstone of successful anti-HIV therapy (Cihlar and Ray 2010). Reverse transcriptase inhibitors can be divided into three categories, nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (De Clercq 2009). Of these, nucleoside reverse transcriptase inhibitors are the most used ones. Nucleoside reverse transcriptase inhibitors are analogues of endogenous 2´-deoxynucleosides (De Clercq 2009; Cihlar and Ray 2010). Nucleosides are the building blocks of nucleic acids and are composed of a nitrogenous base and a 5-carbon sugar (ribose or deoxyribose). Like natural nucleosides, nucleoside reverse transcriptase inhibitors require intracellular enzymatic activation through three phosphorylation steps to their 5´-triphosphate form (nucleotide) (De Clercq 2009). In their active form, they compete with the endogenous nucleotides at the catalytic, i.e., substrate-binding, site of reverse transcriptase and are incorporated into the elongating proviral deoxyribonucleic acid (DNA) strand (De Clercq 2009; Cihlar and Ray 2010), thus functioning as competitive substrate inhibitors (De Clercq 2009). However, in comparison to the natural nucleotides, nucleoside reverse transcriptase inhibitors lack the 3´-hydroxyl group on the deoxyribose moiety, and this leads to strand termination as the subsequent nucleotide cannot form the next 5´-3´ phosphodiester bond necessary to extend the DNA strand (Mohammadi and Bienzle 2012; Tressler and Godfrey 2012).

***Zidovudine*** (3´-azido-3´-deoxythymidine, AZT) is a thymidine analogue and was the first drug to be approved by the FDA for the treatment of HIV infection and still remains an important component for HIV treatment (Ezzell 1987; Tressler and Godfrey 2012). Zidovudine is available as injection, capsules, tablets, or syrup, with syrup being the most practical form for cats due to the possibility of exact dosing. It has good oral bioavailability in cats and is well distributed to tissues, including the central nervous system. It is metabolized to an inactive form by the liver and excreted by the kidneys. Dosage reduction has been recommended for cats with renal failure. Adverse effects can mimic FeLV-associated cytopenias as some cats treated with zidovudin can develop dose-related haematologic adverse effects, most commonly non-regenerative anaemia and neutropenia. Therefore, only lower dosages of zidovudin (5 mg/kg q12h) should be used in FeLV-infected cats, and complete blood counts should be monitored during treatment. Zidovudine is effective against FeLV *in vitro* (Tavares et al., 1987; Tavares et al., 1989; Hoover et al., 1990; Zeidner et al., 1990c; Mathes et al., 1992; Mukherji et al., 1994). Zidovudin was also shown to be effective in treating cats experimentally infected with FeLV when treatment was initiated less than three weeks after infection. When treated less than one week after challenge, cats were protected from bone marrow infection and persistent viraemia (Zeidner et al., 1990a; Zeidner et al., 1990c). Improvement in stomatitis, reduced antigenemia, and decrease of incidence of lymphoma have been reported in naturally FeLV-infected cats (Hartmann et al., 1992b; Nelson et al., 1995). However, in a placebo-controlled blinded study in which naturally FeLV-infected cats were treated with zidovudine for six weeks, however, treatment did not lead to a significant improvement of clinical, laboratory, immunologic, or virologic parameters (Stuetzer et al., 2013).Thus, overall therapeutic efficacy of zidovudine in FeLV-infected cats is limited.

***Didanosine*** (2',3'-dideoxyinosine, ddI) is also on the market to treat HIV infection in humans. Didanosine is active against FeLV *in vitro* (Tavares et al., 1989; Mukherji et al., 1994), but its *in vivo* efficacy is unknown.

***Zalcitabine*** (2',3'-dideoxycytidine, ddC) was also approved to treat HIV infection in humans (Broder 1990). Zalcitabine is effective against FeLV *in vitro* and has been used in experimental studies to treat FeLV-infected cats (Hoover et al., 1989; Tavares et al., 1989; Zeidner et al., 1989; Polas et al., 1990). It has a very short half-life and therefore, was administered either via IV bolus or controlled-release subcutaneous implants (Polas et al., 1990). Due to its toxicity, zalcitabine should not be used in concentrations over 5 mg/kg/h continuous infusion in feline patients (Polas et al., 1990). Controlled-release delivery of zalcitabine inhibited *de novo* FeLV replication and delayed onset of viraemia after experimental infection; however, when treatment was discontinued, an equivalent incidence and level of viraemia were established rapidly (Hoover et al., 1989). In a study evaluating the prophylactic antiviral activity against FeLV, zalcitabine was administered by continuous IV infusion for 28 days. Higher doses were extremely toxic, causing death in eight of ten cats. Even lower doses had side effects in form of thrombocytopenia, and only one of ten cats remained FeLV antigen-negative, although onset of viraemia was delayed for several weeks (Polas et al., 1990).

***Decitabine*** (5-aza-2'-deoxycytidine) was approved to treat myelodysplastic syndromes in humans, but also has anti-HIV activity in cell culture. Decitabine is a nucleoside reverse transcriptase inhibitor but also acts as a demethylation agent (and

therefore has antiproliferative effects). Decitabine is active against FeLV *in vitro* (Greggs et al., 2012), but its efficacy *in vivo* is so far unknown.

***Gemcitabine*** (2',2'-difluordesoxycytidin) was approved to treat tumours, such as pancreatic cancer, but also has anti-HIV activity in cell culture. Cytopenias and gastrointestinal toxicity occurred in some of the cats, when gemcitabine was used to treat carcinomas (Martinez-Ruzafa et al., 2012). Gemcitabine is active against FeLV *in vitro* (Greggs et al., 2012), but its efficacy *in vivo* is unknown so far.

***Nucleotide Reverse Transcriptase Inhibitors***

Nucleotide RT inhibitors also interact with the catalytic site of the reverse transcriptase and are incorporated into the elongating proviral DNA strand, subsequently causing strand termination (Ravichandran et al., 2008; De Clercq 2009) .They compete with the natural nucleotides and thus, also function as competitive substrate inhibitors. However, in contrast to nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors contain a phosphate group and therefore need only two intracellular phosphorylation steps (and not three) to be converted into their active forms (De Clercq 2009). This circumvents the first and often rate-limiting phosphorylation step (Ravichandran et al., 2008; Cihlar and Ray 2010).

***Adefovir*** (2-(6-amino-9H-purin-9-yl)-ethoxy-methyl-phosphonic acid, PMEA) is active against herpesviruses, hepadnaviruses (hepatitis B), and retroviruses (De Clercq 2007). Adfovir is not licensed as HIV drug but was approved to treat chronic hepatitis B in an orally available form (bis-POM PMEA). Adefovir is active against FeLV *in vitro* (Hoover et al., 1991). In one *in vivo* study in experimentally infected cats, adefovir prevented the development of persistent FeLV viraemia if used early in the infection (Hoover et al., 1991), but it was not effective in a placebo-controlled double-blinded study in naturally infected cats, when used for three weeks (Hartmann et al., 1992b).

***Tenofovir*** (2R-1-(6-amino-9H-purin-9-yl)-propan-2-yl-oxy-methyl-phosphonic acid, PMPA) has a narrower spectrum than adefovir, in that it no longer extends to herpes viruses, but is confined to hepadna and retroviruses (De Clercq 2007). Tenofovir is licensed for treatment of HIV infection and the only approved nucleotide reverse transcriptase inhibitor for the treatment of HIV infection. It is marketed as the prodrug tenofovir disoproxil fumarate (TDF) (Cihlar and Ray 2010). Tenofovir has also been shown to be effective against FeLV *in vitro* (Greggs et al., 2012). However, *in vivo* data in FeLV-infected cats are not available.

***Non-Nucleoside Reverse Transcriptase Inhibitors***

Most of the non-nucleoside reverse transcriptase inhibitors are highly specific for HIV. Unlike nucleoside and nucleotide reverse transcriptase inhibitors, which bind to the catalytic site of reverse transcriptase, non-nucleoside reverse transcriptase inhibitors interact with an allosteric site of the enzyme (De Clercq 2009) and are not incorporated into the proviral DNA strand (Ravichandran et al., 2008). They are classified as non-competitive inhibitors of reverse transcriptase and do not require intracellular activation to inhibit the enzyme (Ravichandran et al., 2008; Mohammadi and Bienzle 2012). Non-nucleoside reverse transcriptase inhibitors are a group of structurally diverse compounds that all bind a single site in the HIV RT enzyme (Xia et al., 2007). The interaction with the allosteric site which is located in close proximity to the catalytic site leads to a number of conformational changes within the reverse transcriptase (Xia et al., 2007; Das et al., 2012). Amongst other effects, these changes cause a decrease in the interaction between the DNA primer and the polymerase domain of the enzyme and thus, result in inhibition of virus replication (Xia et al., 2007; Das et al., 2012).

***Suramin*** (1-(3-benzamido-4-methylbenzamido)-naphthalene4,6,8-trisulfonic acid sym-3'-urea sodium salt), a sulphated naphthylamine and trypan red derivative, is one of the oldest known antimicrobial agents. Suramin inhibits reverse transcriptase by interacting with the template-primer binding site of the enzyme (De Clercq 1979), and therefore, has also been used early on in humans with HIV infection (Broder et al., 1985). Suramin has been given as antitrypanosomal agent or for treatment of some tumours, such as prostate tumours in humans (Garcia-Schurmann et al., 1999) and osteosarcoma in dogs (Alvarez et al., 2014). Suramin is associated with a significant number of severe adverse effects, such as nausea and anaphylactic shock as immediate reactions during administration in humans and peripheral neuritis leading to palmar-plantar hyperaesthesia, photophobia, skin reactions, agranulocytosis, haemolytic anaemia, and destruction of the adrenal cortex as later adverse effects (Broder et al., 1985; Dorfinger et al., 1991; O'Donnell et al., 1992; Garcia-Schurmann et al., 1999; Kaur et al., 2002). Suramin was not investigated against FeLV *in vitro* but has been used to treat progressively FeLV-infected cats, although only a limited number of cats were evaluated. In a field study, serum viral infectivity ceased transiently in two cats with naturally acquired FeLV infection but returned to high levels approximately 14 days after treatment was stopped (Cogan et al., 1986). In another uncontrolled study, six anaemic FeLV-infected cats received suramin, and within four to 14 days, erythropoiesis improved. However, progenitor cells remained infected (Abkowitz 1991).

***Nucleoside RNA (DNA) Synthesis Inhibitors***

The nucleoside analogue RNA (or DNA) synthesis inhibitors act similarly to the nucleotide analogues that inhibit reverse transcriptase, but are not restricted to retroviruses, instead have a broader spectrum of activity, and are mainly used to treat herpesvirus infections. Several of them (e.g., acyclovir, penciclovir) are activated intracellularly by the herpesviral enzyme thymidine kinase, which phosphorylates them to a monophosphate form, and thus, these drugs are only effective against herpesvirus infections (De Clercq 1982; Maggs and Clarke 2004; Williams et al., 2004; van der Meulen et al., 2006). Some nucleoside analogue RNA (or DNA) synthesis inhibitors, such as vidarabine however, have also some activity against retroviruses, despite being mainly used against herpesvirus infection (Hartmann 2011a).

***Vidarabine*** (9-β-D-arabinofuranosyladenine monohydrate, adenine arabinoside) is effective against herpesviruses, poxviruses, and retroviruses. Vidarabine is nonselective in its effect and associated with notable host toxicity, especially if administered systemically (Lauter et al., 1976). Another major disadvantage of vidarabine is its poor solubility; therefore, if it is given systemically, vidarabine must be administered intravenously and in large volumes of fluid over extended periods. It is rapidly deaminated by adenosine deaminase to hypoxanthine arabinoside. Adverse effects include local irritation at infusion sites, nausea, vomiting, and diarrhea. The drug also causes bone marrow suppression, resulting in anaemia, neutropenia, and thrombocytopenia. Systemic toxicity restricts its use in veterinary practice mainly to topical ophthalmic treatment, such as for treatment of feline herpesvirus-associated ocular changes (Maggs 2010). Therefore, it is not useful for treatment of FeLV infection, and studies on its efficacy against FeLV have not been performed.

***Nucleotide RNA (DNA) Synthesis Inhibitors***

Nucleotide synthesis inhibitors interfere with DNA and RNA synthesis, but not by mimicking nucleosides. They usually have a broad antiviral spectrum but also marked toxicity. They can be divided into two subclasses, inhibitors of nucleic acid synthesis, so-called pyrophosphate analogues that directly inhibit DNA and RNA polymerase (e.g., foscarnet) and inhibitors of triphosphate synthesis that inhibit the enzyme inosine monophosphate dehydrogenase (essential for synthesis of nucleotides) and thus prevent nucleotide production (e.g., ribavirin).

***Foscarnet*** (phosphonoformic acid, PFA) has a wide spectrum against DNA and RNA viruses, including retroviruses. Foscarnet is mainly administered IV by continuous infusion because of its short half-life, that also has been demonstrated in cats (Straw et al., 1992). It only inhibits virus replication during its application; thus, after treatment is stopped, virus replication is re-activated. Oral application is possible but can cause irritation of mucous membranes and oral bleeding. Foscarnet has many adverse effects in humans as well as in cats, such as nephrotoxicity and myelosuppression. It is also toxic to epithelial cells and mucous membranes, and gastrointestinal adverse effects and ulcerations of genital epithelium can occur. In addition, it chelates divalent cations, such as calcium, so that hypocalcemia, hypomagnesemia, and hypokalemia can develop (Ryrfeldt et al., 1992; Gerard and Salmon-Ceron 1995). In cats, foscarnet has very low oral bioavailability (8%) (De Clercq 2013). Foscarnet is active against FeLV *in vitro* (Swenson et al., 1991), but *in vivo* data are not available.

***Ribavirin*** (1-β-D-ribofuranosyl-1 H-1,2,4-triazole-3-carboxamide, RTCA) has strong *in vitro* antiviral activity against a variety of DNA and RNA viruses (Beaucourt and Vignuzzi 2014); however, therapeutic concentrations are difficult to achieve *in vivo* because of its toxicity, and cats are extremely sensitive to the adverse effects. Thus, systemic application of ribavirin is not indicated (Lafeuillade et al., 2001). Adverse effects in cats in several studies (even using low doses) mainly included haemolysis that developed as a result of sequestration of the drug in erythrocytes (Povey 1978; Weiss et al., 1993a). In addition, there was a dose-related toxic effect on bone marrow, primarily on megakaryocytes (resulting in thrombocytopenia and haemorrhage) and erythroid precursors. With prolonged treatment or higher doses, the drug also suppressed production of neutrophilic granulocytes. Liver toxicity also occurred. An approach to decrease the toxicity of ribavirin by incorporating ribavirin into lecithin-containing liposomes and giving it at lower doses to cats was not successful (Weiss et al., 1993b). Ribavirin is active against FeLV *in vitro* (Greene and Watson 1998b). No studies, however, are published on its effect in FeLV-infected cats, and due to its toxicity, its use is not recommended.

***Integrase Inhibitors***

The enzyme integrase catalyses strand transfer (3'-end joining), which inserts both viral DNA ends into a host cell chromosome. The high degree of conservation of integrase active sites across many retroviruses suggests that FeLV can be sensitive to integrase inhibitors (Cattori et al., 2011) .The mechanism of their action is inhibition of integration of the proviral DNA that is produced by reverse transcription of the viral RNA (Greggs et al., 2012).

***Raltegravir*** is approved for treatment of HIV infections and is used in combination with other anti-retrovirus drugs. Raltegravir has been shown to be highly effective *in vitro* against FeLV in several feline cell lines (Cattori et al., 2011; Greggs et al., 2012). The effective concentration of raltegravir had no effect on cell viability and did not induce apoptosis, suggesting that this could be an effective and safe drug also *in vivo* (Zhu et al., 2007). However, raltegravir is partly

eliminated as glucuronide, a metabolic pathway that is not very efficient in cats, and this potentially increases the risk of toxicity due to drug accumulation (Liles et al., 2003). One *in vivo* study evaluated efficacy and safety of raltegravir in seven cats with experimental progressive FeLV infection (Boesch et al., 2015). Raltegravir was administered at 10-15 mg/kg q12h PO for six and a half weeks, and then at 20-25 mg/kg q12h PO for two and a half weeks. The drug reached sufficient plasma concentrations with both doses. Raltegravir was well tolerated and no adverse effects were noted. Since a sufficient number of progressively infected cats was not available, a historic control group was used (cats infected with the same virus during a previous study (Gomes-Keller 2011)). The treatment was successful in reducing viraemia in each cat, with up to 1 log10 reduction in viral RNA in four of seven cats; however, viraemia rebounded in all but one cat after drug removal. In one cat, the FeLV RNA load reduction was still approximately fivefold at the end of the study eight weeks after the end of the treatment and this cat had developed antibodies to FeLV, but only after the treatment had been stopped (Boesch et al., 2015).

***Proteinase Inhibitors***

Protease inhibitors, such as nelfinavir or remdesivir, prevent viral replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors that are necessary for the production of infectious viral particles (Hartmann 2011a). Most proteinase inhibitors currently on the market are highly selective for HIV or coronaviruses and have not been evaluated for their efficacy against FeLV but are most likely ineffective.

***Neuraminidase Inhibitors***

Neuraminidase inhibitors, such as oseltamivir, are blockers of viral extrusion. They inhibit virus replication through binding in a gap of the viral enzyme neuraminidase, a glycoprotein on the viral surface (Hartmann 2011a). Retroviruses do not possess a neuraminidase, and thus, these compounds are not useful to treat FeLV infection.

**Immunomodulatory Therapy**

Immunomodulators are also referred to as biologic response modifiers. Their use for treatment of infectious diseases might be beneficial when compromise of the immune system impairs effective antimicrobial drug treatment (Table 19). These substances modify the responses of immunocompetent cells through cytokines or other mechanisms. Some of them not only have an effect on the immune system but also a true antiviral activity (e.g., interferons).

Table 19: Classes of currently available immunomodulatory compounds

- Naturally occurring cytokines
    - Interferons
- Naturally occurring biologic response modifiers
    - Mammalian- and insect-derived biologic response modifiers
    - Microorganism-derived biologic response modifiers
    - Plant-derived biologic response modifiers
- Synthetic compounds with immunomodulatory activity
    - Antiparasiticytes
    - Other synthetic compounds

Immunomodulators have been used more extensively against FeLV infection than in any other infectious disease in veterinary medicine. However, controlled studies including large numbers of naturally infected cats do not exist for most of these agents, and if controlled studies have been performed, they commonly show disappointing results (Table 20).

Table 20: Summary on studies on efficacy of immunomodulatory drugs for treatment of feline leukaemia virus infection (including EBM grades for judgment of the available efficacy data including expert opinion of the authors)

| DRUG CLASS | DRUG | EFFICACY *IN VITRO* | CONTROLLED STUDY *IN VIVO* | EFFICACY *IN VIVO* | Comments | EBM LEVEL (I - IV) |
|---|---|---|---|---|---|---|
| **Interferons** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | human interferon-α | yes (Jameson and Essex 1983) | yes (SC high dose) | no (Stuetzer et al., 2013) | ineffective in a placebo-controlled blinded field study | I |
| | | | yes (PO low dose) | no (McCaw et al., 2001; Gomez-Lucia et al., 2019; Gomez-Lucia et al., 2020) | ineffective in a placebo-controlled blinded field study, in contrary to some reported effect in uncontrolled studies and case series | I |
| | feline interferon-ω | yes (Rogers et al., 1972) | yes (SC high dose) | yes (De Mari et al., 2004; Gil et al., 2013) | some effect (most likely more on secondary infections), might be an option in cats with chronic gingiva-stomatitis | I |
| **Mammalian- and Insect-Derived Biologic Response Modifiers** | | | | | | |
| | Lactoferrin | n. d. | no | n. d. | might be an option in cats with chronic gingiva-stomatitis | IV |
| | lymphocyte T-cell immune-modulator | n. d. | no | n. d. | some effect based on case series | III |
| | Mellitin | n. d. | no | n. d. | likely ineffective | IV |
| **Microorganism-Derived Biologic Response Modifiers** | | | | | | |
| | *Myco-bacterium* spp. | n. d. | yes | no (Bachman et al., 1982) | not effective in an experimental setting | II |
| | *Staphylo-coccus* spp. | n. d. | yes | yes (McCaw et al., 2001) | some effect on owners' subjective impression on the health of their cats in a placebo-controlled field study | I |
| | *Propioni-bacterium acnes* | n. d. | no | n. d. | some effect based on anecdotal reports and case series | III |
| | *Serratia marcescens* | n. d. | no | n. d. | likely ineffective | III |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | based on an uncontrolled study | |
| | Poxvirus | n. d. | yes | no (Hartmann et al., 1998; Hartmann et al., 1999) | ineffective in large placebo-controlled blinded field studies | I |
| **Plant-Derived Biologic Response Modifiers** | | | | | | |
| | Acemannan | n. d. | no | n. d. | likely ineffective based on uncontrolled studies | III |
| | polyprenyl immuno-stimulant | n. d. | no | n. d. | efficacy unknown, but likely ineffective | IV |
| | RetroMAD1 | n. d. | yes | yes (Huan et al., 2019) | some efficacy on clinical signs in a not well controlled study; further studies needed | IV |
| **Antiparasiticytes** | | | | | | |
| | Levamisole | n. d. | no | n. d. | likely not effective and relatively toxic, but controlled studies missing | IV |
| | Diethyl-carba-mazine | n. d. | yes | no (Nelson et al., 1995) | ineffective in one experimental study | II |

*n. d.*, not determined.
EBM, evidence based medicine (Lloret 2009)
EBM grade I = This is the best evidence, comprising data obtained from properly designed, randomised controlled clinical trials in the target species (in this context cats).
EBM grade II = Data obtained from properly designed, randomised controlled studies in the target species with spontaneous disease in an experimental setting.
EBM grade III = Data based on non-randomised clinical trials, multiple case series, other experimental studies, and dramatic results from uncontrolled studies.
EBM grade IV = Expert opinion, case reports, studies in other species, pathophysiological justification.

***Interferons***

Interferons are polypeptide molecules with various biological functions (Domenech et al., 2011) that were discovered more than 50 years ago and received their name because they were proven to interfere with vaccinia and influenza virus replication. They are a subgroup of cytokines important in both innate and adaptive immunity and play an important role in mediating antiviral and antigrowth responses and in modulating the immune response (Stark et al., 1998). A number of different interferons have been identified that are divided into type I, type II, and type III interferons (Klotz et al., 2017). Mainly type I interferons have been used in veterinary medicine (interferon-α, interferon-β, interferon-ω) with interferon-α and interferon-ω being used against virus infection and interferon-β only against tumours. Among the type II interferons, only interferon-γ that also has antiviral efficacy plays a role in human and veterinary medicine. Besides inhibition of viral

replication, interferon-γ enhances antigen presentation processes and is a critical propellant of the TH1 response (Lee and Ashkar 2018), but has so far only been used to treat atopic dermatitis in dogs.
Several studies suggest that viral protein synthesis itself is not affected by interferons and therefore conclude that the antiviral activity of interferons is related to interference with later stages of the viral replication cycle, such as virion assembly and release (Gomez-Lucia et al., 2009; Domenech et al., 2011). Interferons also trigger virus-infected cells to undergo apoptosis by activating the expression of apoptosis-contributing genes (Goodbourn et al., 2000; Gomez-Lucia et al., 2009). Thereby, interferons prevent the spread of viruses from infected cells and aid in the clearance of virus infections (Goodbourn et al., 2000). Several human interferons have been manufactured by recombinant technology and are available commercially, of which mainly interferon-α has been used in cats. Feline interferon-ω is on the market as a recombinant product in Europe, Asia, and Australia and is licensed for use in cats and dogs.

***Interferon-α*** has antiproliferative, immunomodulatory, and antiviral activity against several DNA and RNA viruses (Gerlach et al., 2009). Feline and canine interferon-α are not commercially available but have been used experimentally in the treatment of different diseases. A variety of recombinant and natural human interferon-α preparations are available, including pegylated forms that have prolonged biologic half-lives. Although interferon-α is relatively species-specific, recombinant human interferon-α (rHuIFN-α) has been used in cats. However, after parenteral use, cats develop antibodies against rHuIFN-α, and thus, after SC administration to cats, rHuIFN-α becomes ineffective after three to seven weeks because of the development of neutralising antibodies (Zeidner et al., 1990a; Zeidner et al., 1990b), while following PO administration, no antibodies develop. RHuIFN-α is commonly used to treat cats with viral infections, mainly in countries in which feline interferon-ω is not available. Two common treatment regimens exist, SC injection of high-dose ($10^4$-$10^6$ IU/kg q24h) rHuIFN-α (Zeidner et al., 1990a; Zeidner et al., 1990b) or oral application of low-dose (1-50 IU/kg q24h) rHuIFN-α (Cummins et al., 1999). RHuIFN-α can be given PO for a longer period, as no antibodies will develop during PO treatment. Given PO however, RHuIFN-α is inactivated by gastric acid and destroyed by trypsin and other proteolytic enzymes in the duodenum (Cantell and Pyhala 1973), and thus, no interferon-α levels can be measured in the blood (Gibson et al., 1985). However, PO RHuIFN-α is still active by stimulating cells of the mucosal lymphoid tissue to produce biologically active interferon-α and interferon-γ and to increase the number of CD4+ and CD8+ T lymphocytes (Dec and Puchalski 2008). Those T lymphocytes can then produce cytokines, such as TNF-α or IL-12, that consequently have systemic effects (Koech and Obel 1990; Cummins et al., 1999; Tompkins 1999).
Several studies have been performed on the use of rHuIFN-α in FeLV-infected cats. FeLV replication is inhibited by rHuIFN-α *in vitro* (Jameson and Essex 1983). High-dose SC rHuIFN-α ($1.6 \times 10^4$ IU/kg to $1.6 \times 10^6$ IU/kg SC) in experimentally FeLV-infected cats with persistent antigenemia resulted in significant decreases of circulating FeLV antigen beginning two weeks after the initiation of therapy (Zeidner et al., 1990a). However, because of anti-interferon-α antibody development, cats became refractory to therapy three to seven weeks after treatment initiation (Zeidner et al., 1990a). In contrast, high dose SC rHuIFN-α ($10^5$ IU/kg SC q24h for 6 weeks) did not lead to a significant improvement of clinical, laboratory, immunologic, or virologic parameters in a placebo-controlled, double-blinded study of naturally FeLV-infected cats (Stuetzer et al., 2013). Effect of low-dose PO rHuIFN-α against FeLV was first evaluated in two older experimental studied (Cummins et al., 1988; Cummins et al., 1999; McCaw et al., 2001). In one experimental study, 21 cats inoculated with FeLV received 0.5 or 5 IU/cat PO rHuIFN-α q24h or were not treated. Treated cats survived longer, and less treated cats developed clinical signs than untreated control cats (Cummins et al., 1988). In the other experimental placebo-controlled study, low-dose PO rHuIFN-α was given (0.5 IU/cat (eight cats) or 5 IU/cat (five cats)) following experimental challenge on seven consecutive days on alternate weeks for a period of one month (Cummins et al., 1999). No difference was found in the development of viraemia between groups; however, treated cats had significantly fewer clinical signs and longer survival times when compared to the placebo group (with a better response in the cats given 0.5 IU/cat) (Cummins et al., 1999). Several uncontrolled studies in the field also reported a beneficial response when cats were treated with low-dose PO rHuIFN-α, but they only included a limited number of cats and no control group, and thus, results are difficult to interpret (Tomkins et al., 1982; Weiss et al., 1991). In a larger study, outcome of 69 FeLV-infected cats with clinical signs that were treated with low-dose PO IFN-α (30 IU/kg for seven consecutive days on a one-week-on one-week-off schedule) was compared to historical controls, and significantly longer survival times were reported in the treated cats (Weiss et al., 1991). In an uncontrolled study, 27 naturally FeLV-infected privately owned cats received low-dose PO rHuIFN-α (60 IU/cat q24h in alternating weeks for four months). Clinical status improved with rHuIFN-α treatment in 15 of 16 symptomatic cats, and plasma antigenemia, reverse transcriptase activity, and proviral load decreased at two months and four months (end of treatment) but increased again after six to 10 months. However, the study did not include a control group (Gomez-Lucia et al., 2019). In a recent study, 27 naturally FeLV-infected received 60 IU/cat rHuIFN-α PO q24h in alternating weeks for four months administered by their owners. Clinical status and anaemia improved during treatment but had deteriorated again when the cats were rechecked four to eight months after the end of treatment suggesting that if FeLV-infected cats are treated with low-dose PO rHuIFN-α it should be long-term. There was, however, no control group included (Gomez-Lucia et al., 2020). In the only published placebo-controlled study, treatment of ill client-owned FeLV-infected cats with low-dose oral rHuIFN-α (30 IU/cat for seven consecutive days on a one-week-on one-week-off schedule) did not result in a significant difference in FeLV status, survival time, clinical or haematologic parameters, or subjective improvement in the owners' impression when compared to a placebo group. Thus, this controlled study was not able to demonstrate any efficacy (McCaw et al., 2001).

***Interferon-ω*** shares a 62% amino acid sequence homology with interferon-α and 33% with interferon-β (Li et al., 2017). Despite the homology between interferon-α and interferon-ω, there is no cross-reactivity of antibodies between the two

interferons, and treatment with interferon-ω can be effective in patients resistant to the effects of interferon-α (Tiefenthaler et al., 1997). Interferon-ω has been found in many species, including humans (Hauptmann and Swetly 1985) and cats (Nakamura et al., 1992), but not in dogs (Himmler et al., 1987). Feline recombinant interferon-ω (rFeIFN-ω) is a recombinant product which is produced by baculoviruses containing the feline sequence for feline interferon-ω that replicate in silkworms after infection. It is licensed for use in veterinary medicine in Europe, Asia, and Australia for SC use at a dose of $10^6$ IU/kg q24h. In healthy dogs, rFeIFN-ω enhances the activities of whole blood cells, macrophages, and natural killer cells (Kuwabara et al., 2006). RFeIFN-ω can be used in cats long-term without antibody development (Domenech et al., 2011). No severe adverse effects have been reported in cats (De Mari et al., 2004). Transient lethargy, fever, vomiting, mild diarrhoea, and anorexia have been documented in some treated cats, especially at higher doses (2.5 x $10^6$ IU/kg). Mild neutropenia, eosinophilia, and reversible increases of AST activity have also been described following treatment (Hampel et al., 2007).
RFeIFN-ω inhibits FeLV replication *in vitro* (Rogers et al., 1972). In FeLV-infected FL47 cells treated with rFeIFN-ω, reverse transcriptase activity was decreased, viability of infected cells was decreased, and apoptosis was increased (Collado et al., 2007). In 16 cats naturally infected with FeLV or FIV infection living in an animal shelter, the acute phase proteins amyloid-A, α-1-glycoprotein and C-reactive protein significantly increased in cats treated with high dose SC rFeIFNω ($10^6$ IU/kg q24h for five days in three cycles starting at day 0, 14, and 60) (Leal et al., 2014). In a placebo-controlled field study, 48 cats with FeLV infection were treated with high dose SC rFeIFN-ω ($10^6$ IU/kg q24h SC on five consecutive days in three cycles starting at day 0, 14, and 60). There was a significant difference in the survival time of treated *versus* untreated cat in a nine-month follow-up period, but not after twelve months. No virologic parameters were measured throughout the study, which again raises the question whether rFeIFN-ω actually had an anti-FeLV effect or rather inhibited secondary infections (De Mari et al., 2004). In 16 cats of a rescue shelter that were infected with FeLV (six cats), FIV (seven cats) or double-infected (three cats) and that received high dose SC rFeIFNω therapy ($10^6$ IU/kg q24h for five days in three cycles starting at day 0, 14, and 60), excretion of concomitant viruses (e.g., FCV, feline herpesvirus, FCoV) was reduced and ten of the 16 cats improved in their clinical scores during; however, no placebo group was included making an assessment of this outcome difficult (Gil et al., 2013).
No studies have been published on the use of low-dose PO rFeIFN-ω in FeLV-infected cats. However, PO rFeIFN-ω has been used successfully to treat feline chronic gingivostomatitis and thus, might be an option in FeLV-infected cats with this condition (Southerden and Gorrel 2007; Hennet et al., 2011; Leal et al., 2013; Matsumoto et al., 2018).

***Mammalian- and Insect-Derived Biologic Response Modifiers***

Some proteins of mammalian or insect origin have been used as biologic response modifiers.

***Lactoferrin*** is a mammalian iron-binding glycoprotein of bovine origin with immunomodulatory properties that is produced by mucosal epithelial cells of all mammals and is present in secretions, especially in milk. It has been used for its local immunomodulatory effect in the oral cavity, and it has been shown to increase cellular phagocytic activity (Sato et al., 1996). It binds iron and thus has antibacterial, but also antifungal, antiprotozoal, and antiviral activity. Adverse effects have not been described. Lactoferrin has been used in cats with FIV infection and chronic gingivostomatitis. Topical application (40 mg/kg q24h for 14 days) to the mouth of a small number of FIV-infected and non-infected cats with chronic gingivostomatitis was associated with improved appetite and reduced oral inflammation, salivation, and pain, and increased neutrophil phagocytic activity (Sato et al., 1996). Lactoferrin also has antiviral activity against FCV *in vitro* (Beaumont et al., 2003; McCann et al., 2003) and was useful as adjunct treatment to reduce the clinical signs in cats with chronic gingivostomatitis (Hung et al., 2014). The efficacy of lactoferrin against FeLV infection has not been evaluated, but it might be a treatment option in FeLV-infected cats with chronic gingivostomatitis, especially in those with FCV coinfection.

***Lymphocyte T-cell immunomodulator*** (LTCI) is a protein derived from thymus epithelial cells and is available in USA. According to the product brochure, lymphocyte T-cell immunomodulator upregulates Il-2 production *in vitro* and regulates CD4 T-cell function. It was also reported to stimulate platelet production in mice with chemotherapy-induced thrombocytopenia. Lymphocyte T-cell immunomodulator has been conditionally approved in USA as an aid for treatment of FeLV (and FIV) infections, and for the associated signs of cytopenias and opportunistic infections. In one clinical field study (published only in a review paper) 22 cats with FeLV or FIV infections were treated with three SC injections (two weeks apart each) of lymphocyte T-cell immunomodulator; four of the 22 cats had cytopenias and those improved during the treatment period (Gingerich 2008). No significant adverse reactions attributable to lymphocyte T-cell immunomodulator treatment were detected. However, controlled independent studies on the efficacy of lymphocyte T-cell immunomodulator in cats are still missing.

***Melittin*** is the major component in the venom of the honeybee. It is a bioactive 26 amino acid peptide that has antiviral activity (Bazzo et al., 1988) against several viruses *in vitro*, including FIV, but when used in a small prospective, placebo-controlled double-blind pilot study in 20 FIV-infected cats that were treated with either melittin (500 µg/kg q12h SC for 6 weeks) or placebo, melittin showed no significant efficacy (Hartmann et al., 2016). Its efficacy against FeLV is unknown.

***Mikroorganism-Derived Biologic Response Modifiers***

Microorganisms and their extracts have been classically used as nonspecific immunostimulators. One example is Freund's complete adjuvant, which is a water-in-oil emulsion containing inactivated whole *Mycobacteria* spp. Injection of this mixture induces cell-mediated immune response and humoral antibody formation to the desired protein. Preparations of other bacteria, including *Propionibacterium acnes*, *Staphylococcus* spp., *Salmonella* spp., and *Serratia marcescens*, are also used as immunostimulators. Some inactivated virus preparations, such as inactivated poxviruses, are also licensed as immunomodulators (Hartmann 2011a).

***Mycobacterium spp.*** are available as Bacille Calmette-Guérin (BCG). Bacille Calmette-Guérin is a cell wall extract of a non-pathogenic strain of *Mycobacterium bovis* that was originally developed in 1908 by Calmette and Guérin as a "vaccine" against tuberculosis in humans. Facultative intracellular organisms, such as *Mycobacteria* spp., have a marked affinity for localizing in and stimulating mononuclear-phagocyte clearance mechanisms (Greene and Watson 1998b). There are no studies on the efficacy against FeLV *in vitro* or *on vivo*, but one study investigated this compound in FeSV-infected kittens. Kittens experimentally infected with FeSV were inoculated with Bacille Calmette-Guérin SC at the same time and site as the FeSV inoculation, at the same site but one week after FeSV inoculation, or with a mixture of viable autochthonous neoplastic cells approximately five weeks after FeSV inoculation. Bacille Calmette-Guérin treatment was not able to prevent tumour development or increase survival rate (Bachman et al., 1982).

***Staphylococcus spp****.* are commercially available as two products, *Staphylococcus aureus* phage lysate and purified staphylococcal protein A (SPA). Staphylococcal phage lysate contains components of *Staphylococcus aureus* and a bacteriophage. Staphylococcal Protein A (SPA) is a bacterial polypeptide product purified from cell walls of *Staphylococcus aureus* Cowan I. *Staphylococcus* spp. products can combine with immune complexes at the Fc (non-antigen-binding) region of certain IgG subclasses and stimulate complement activation, as well as induce T-cell activation, natural killer cell stimulation, and IFN-γ production. A variety of *Staphylococcus* spp. products and treatments have been used in progressively FeLV-infected cats. Interest was first generated when plasma from progressively FeLV-infected lymphoma-bearing cats was passed over *Staphylococcus aureus* columns to remove circulating immune complexes and then returned to the cats. More than 100 cats were treated in this manner, generally undergoing treatments twice a week for ten to 20 weeks (Day et al., 1984; Jones et al., 1984; Liu et al., 1984a; Liu et al., 1984b; Engelman et al., 1985; Snyder et al., 1987; Weiss 1988). In some studies, a high rate of tumour remission and conversion to a FeLV antigen-negative status was observed; in others, responses were less dramatic and short-lived. Subsequently, it was hypothesized that *Staphylococcus* spp. (and other) products might have leached from the filters and columns used for immunosorption and been returned to the cats as contaminants in the treated plasma (Harper et al., 1985). The possibility that these products exerted an immunomodulatory effect caused investigators to treat cats with small doses of Staphylococcal Protein A. In such a study including kittens with experimental FeLV infection, treatment with Staphylococcal Protein A (7.3 mg/kg intraperitoneally twice weekly for eight weeks) did not correct anaemia or improve humoral immune function. In an experimental study involving 17 cats (five FeLV-infected viraemic cats, six FeLV-infected non-viraemic cats, and six uninfected controls), no difference was seen in viraemia and immune response, but a stimulation of bone marrow granulocytic lineage could be detected (Lafrado et al., 1990). In a placebo-controlled field study, treatment of ill client-owned FeLV-infected cats with Staphylococcal Protein A (10 mg/kg intraperitoneally, twice per week for up to ten weeks) did not cause a statistically significant difference in the FeLV status, survival time, or clinical and haematologic parameters when compared with a placebo group, but it caused a significant improvement in the owners' subjective impressions on the health of their pets (McCaw et al., 2001).

***Propionibacterium acnes*** is available for veterinary use and consists of a killed bacterial product. The bacterium *Propionibacterium acnes* itself can have some pathogenicity (if not inactivated), and *Propionibacterium acnes* urinary tract infection in a dog has been described in a case report (Harada et al., 2015). *Propionibacterium acnes* has been shown to stimulate macrophages, resulting in release of various cytokines and interferons, and to enhance T-cell and natural killer cell activity in mice. *Propionibacterium acnes* has been used in FeLV-infected cats, but no controlled prospective studies have been performed. Clinical experience has been documented in roundtable discussions and in anecdotal reports (Lies M 1990; Levy 2000). In one report, 76 symptomatic FeLV-infected cats were treated with *Propionibacterium acnes* (0.1 to 0.2 mg/cat IV twice weekly, then every other week for 16 weeks) and supportive care. Although no specific clinical or laboratory evaluations were discussed, 72% of the cats were reported to become FeLV antigen-negative and survived for an unspecified period. In the other report, 700 field cats with progressive field FeLV infection were treated with *Propionibacterium acnes* (0.2 mg/cat IV every three days, then every week for six or more weeks) in conjunction with supportive care. Approximately 50% of the cats improved clinically, although conversion to FeLV antigen-negative status was rare. All these are, however, uncontrolled studies, and efficacy of this drug must be further evaluated before it can be recommended.

***Serratia marcescens*** is available as a biologic extract (BESM) containing the bacterial DNA and membrane components. *Serratia marcescens* is a motile facultative anaerobic gram-negative bacillus that occurs naturally in soil and water, as well

as in the intestines and that produces a red pigment at room temperature. It can cause nosocomial infections associated with urinary and respiratory tract infections, endocarditis, osteomyelitis, septicaemia, wound and ocular infections, and meningitis in humans. Systemic infections in cats and dogs are also described (Armstrong 1984). Also a case of a cat with dermatitis, cellulitis, and myositis caused by *Serratia marcescens* infection was documented (Kelly et al., 2015). *Serratia marcescens* stimulates feline bone marrow-derived macrophages to release cytokines, such as TNF-$\alpha$, IL-1, and IL-6, leading to elevations in rectal temperature and neutrophil counts (Elmslie et al., 1991). In a study in FeLV-infected cats, weekly treatment with *Serratia marcescens* failed to prevent or reverse FeLV viraemia in cats when initiated before or six weeks after inoculation with FeLV, but induced marrow stimulation through cytokine release leading to neutrophilia and fever (Elmslie et al., 1991).

***Poxvirus*** preparations are commercially available as Parapox virus avis (PIND-AVI) and parapox virus ovis (PIND-ORF) preparations in Europe. These preparations contain inactivated poxviruses and act as so-called “paramunity inducers”. Their proposed mode of action is through induction of interferons and colony-stimulating factors and activation of natural killer cells. They have been used to treat a variety of viral infections in animals, both prophylactically and therapeutically. PIND-ORF was reported to cure 80% to 100% of progressively FeLV-infected cats in uncontrolled reports, when it was administered at a dose of 1 ml SC one to three times a week for four to 30 weeks. However, two placebo-controlled double-blind trials in naturally progressively FeLV-infected cats were not able to repeat these results. In 120 cats treated with either PIND-ORF or placebo, no significant difference was found in terminating viraemia during a six-week treatment period (12% in the treated cats, 7% in the control group). In the second study, 30 naturally infected cats were treated in a placebo-controlled, double-blind trial, and 20 immunologic, clinical, laboratory, and virologic parameters were examined (including FeLV p27 antigen concentration, clinical signs, lymphocyte subsets, and survival time), but no significant differences were demonstrated between PIND-ORF and placebo application in any of these parameters (Hartmann et al., 1998; Hartmann et al., 1999; Proksch and Hartmann 2016).

***Plant-Derived Biologic Response Modifiers***

A few plant-derived biologic response modifiers are also on the market, such as acemannan, as well as mixture products, such as polyprenyl immunostimulant and RetraMAD1.

***Acemannan*** is a mucopolysaccharide derived from aloe vera plant leaves that induces cytokine production and dendritic cell maturation. It stimulates macrophages to secrete a variety of cytokines, including IFN-$\gamma$, TNF-$\alpha$, prostaglandin E2, Il-1, and Il-6. A veterinary product is available for treatment (e.g., intralesional injection) of canine and feline tumours, including feline injection-site sarcomas (Harris et al., 1991; King et al., 1995). It also has been used to treat FeLV infection, either by oral or parenteral administration (Sheets et al., 1991; Yates et al., 1992). In one non-controlled, open-label trial, 50 cats with natural progressive FeLV infection were treated with acemannan (2 mg/kg IP weekly for 6 weeks). It was not described if concurrent medication or supportive care ere provided. After twelve weeks, 71% of the cats were alive, but results for FeLV antigen were still positive. No significant changes in clinical signs or haematologic parameters were found. No control group or clinical and laboratory evaluations were available to document improvement from pre-treatment status (Sheets et al., 1991).

***Polyprenyl immunostimulant*** is a plant-derived veterinary biological that is made up of phosphorylated, linear polyisoprenols. It is marketed in the USA and imported into Europe. *In vitro*, it upregulates synthesis of Th1 cytokines and has antiviral properties (Danilov et al., 1996; Vasil'ev et al., 2008). It has some efficacy in cats with non-effusive feline infectious peritonitis (Legendre and Bartges 2009; Legendre et al., 2017). Based on anecdotal reports, it is also used to help stimulate the immune system of cats with FeLV infection, but controlled trials are missing.

***RetroMAD1*** is commercially available and is a recombinant chimeric protein comprising of MAP30 (ribosome-inactivating protein beta-momorcharin from bitter melon; integrase inhibitor), dermaseptin (cationic antimicrobial peptide from tree frogs), and retrocyclin (primate theta-defensin, virus entry inhibitor) (Huan et al., 2019). For all three components individually some potential for HIV treatment has been suggested (Lee-Huang et al., 1995; Lorin et al., 2005; Pace et al., 2017). Oral administration of 0.4 mg/kg of RetroMad1 PO q24h to symptomatic cats with progressive FeLV infection in a multicentre trial in Brazil found some improvement of clinical signs (gingivitis, anaemia, upper respiratory tract disease) and an increased survival rate after 173 days in treated compared to untreated cats (Huan et al., 2019). However, this trial was not well-controlled, some cats received other drugs (e. g., interferon), and the clinical signs observed were not necessarily associated with FeLV infection. Preliminary data from six symptomatic Malayan cats with progressive FeLV infection that received RetroMad1 demonstrated a reduction in the viral blood RNA load between 50% and 100% (Huan et al., 2019). However, further controlled studies will be necessary to corroborate these results before this drug can be recommended to treat FeLV-infected cats.

***Antiparasiticytes***

Some antiparasiticytes also non-specifically stimulate cell-mediated or humoral immunity, and thus, have immunomodulatory activity. Some of them have been used to treat cats with FeLV infection.

***Levamisole*** is a broad-spectrum anthelmintic, e.g., used for heartworm treatment in cats and dogs (Dillon 1984; Reinemeyer et al., 1995). Levamisole was detected to have immunomodulatory effects, when treatment applied against nematode infection not only killed the parasites, but also improved clinical signs of other infections. It nonspecifically stimulates cell-mediated immunity. It influences phosphodiesterase activity, leading to increased cyclic guanosine 3',5'-monophosphate and decreased cyclic adenosine monophosphate. Increased cyclic guanosine 3',5'-monophosphate in lymphocytes stimulates proliferative and secretory responses. It also potentiates mononuclear cells in phagocytosis, chemotaxis, and intracellular destruction of bacteria. Toxicity of levamisole is relatively high; hypersalivation, vomiting, diarrhoea, and central nervous system signs have been observed, similar to signs observed in nicotine poisoning (Hsu 1980). Based on anecdotal reports, levamisole also has been used in FeLV-infected cats (Cotter 1991), but there are no controlled studies available.

***Diethylcarbamazine*** (N,N-diethyl-4-methyl-1-piperazine carboxamide) is an antiparasitic agent that is widely used, especially in tropical regions, to prevent and treat filariasis in humans. It has been used for treatment of feline Brugia malayi (Chansiri et al., 2005) and also as heartworm preventative in cats and dogs. The antifilarial effect of this drug has been attributed to immunomodulation. Diethylcarbamazine can have severe adverse effects, including hepatotoxicity (Bunch 1993). Uncontrolled studies have suggested that continuous oral diethylcarbamazine treatment started shortly after detection of FeLV infection would prevent or delay FeLV-associated lymphopenia and prolong survival (Kitchen and Cotter 1988; Kitchen et al., 1988; Kitchen and Mather 1989; Kitchen 1996). In one controlled study, its therapeutic effect against FeLV infection was investigated in 24 specified pathogen-free kittens experimentally infected with a lymphoma-causing strain of FeLV. The kittens were divided into four groups and received high dose of diethylcarbamazine (12 mg/kg, q24h PO), low dose of diethylcarbamazine (3 mg/kg q24h PO), zidovudin (15 mg/kg q12h PO), or placebo for ten weeks. Although zidovudin was effective in preventing persistent viraemia, diethylcarbamazine in either dose did not; however, both doses of diethylcarbamazine as well as zidovudin prevented lymphoma development (Nelson et al., 1995).

**Supportive Treatment**

In most cases, secondary diseases in progressively FeLV-infected cats are treated in the same way as they are treated in uninfected cats (Hartmann and Hofmann-Lehmann 2020b). Prompt and accurate identification of FeLV-associated as well as secondary diseases is important to allow early therapeutic intervention and a more successful outcome. The patient should first be evaluated to determine whether the illness is unrelated to the FeLV infection, secondary to immunosuppression from the FeLV infection, or a direct cause of the FeLV infection (Table 21). Patients experiencing illness unrelated to FeLV infection should be managed according to standard protocols for the specific health condition(s). FeLV-infected patients with severe gingivostomatitis are most likely to benefit long-term from full mouth extraction with complete extraction of all tooth roots rather than medical management.
Many FeLV-infected cats respond as well as uninfected cats to appropriate symptomatic treatment and therapy of secondary infection. However, more intensive diagnostic testing and treatment should be performed as soon as an infection has been identified. Secondary infections might require more intensive and prolonged therapy, and the owner should be forewarned that the response to treatment could take longer than expected. FeLV itself does not cause fever, so a search for a concurrent infection is indicated in febrile cats. Fevers of unknown origin that are unresponsive to antibiotics might be caused by a co-infection with a viral, protozoal, or fungal agent. While the disease status in human patients with HIV infection is assessed with various markers such as CD4:CD8 ratio, these markers have not proven reliable in cats with natural FeLV infections (Hartmann et al., 1992b; Hartmann et al., 1999; Hartmann 2005a). Although weight loss can be indirectly related to FeLV infection, it is also associated with many other diseases. Quality of life parameters can include the use of scoring systems such as a modified Karnofsky score, which allows for assessment by both clinician and owner, to detect diminishing quality of life (Hartmann and Kuffer 1998; Taffin et al., 2016).
Glucocorticoids and other immune-suppressive drugs should be avoided whenever possible in progressively FeLV-infected cats, unless clearly indicated for a specific problem. These drugs interfere with granulocyte chemotaxis, phagocytosis, and the killing of bacteria, thus compounding the risk of secondary infections (Cotter 1998). Cats with regressive FeLV infection also should not receive glucocorticoid treatment (unless specifically indicated) because it might increase the risk of reactivation (Rojko et al., 1982; Pedersen et al., 1984). All myelosuppressive drugs should also be avoided in FeLV-infected cats because they can potentiate the myelosuppressive syndromes caused by FeLV.

Table 21: General treatment recommendations for progressively FeLV-infected cats

- **Treatment in patients without clinical signs**
  - If no clinical signs are present, no treatment is necessary.
  - Strict indoor-only lifestyle is recommended.
- **Treatment in patients with various clinical signs**
  - If clinical signs are present, first identification of any underlying diseases (FeLV itself alone is usually not responsible for the clinical signs, e.g., secondary infections might be present) is essential.
  - Underlying diseases should be treated thoroughly.
- **Treatment of FeLV-infected cats with lymphoma**
  - In cats with FeLV-associated lymphoma, antitumor chemotherapy using a recommended protocol can be used similarly to non-infected tumour patients.
  - However, owners should be informed about more guarded prognosis.
- **Treatment of FeLV-infected cats with anaemia**
  - Blood transfusions is recommended if anaemia is severe.
  - Treatment with an erythropoietin derivative (e.g. darbepoetin (long acting recombinant human erythropoietin) 0.25-0.5 µg/kg SC weekly until HCT is normal, then increasing dose interval for maintenance)) can be tried.
  - If there is no effect, glucocorticoid treatment (e. g. prednisolone 2 mg/kg PO q1h as a starting dose, then slowly tapered down to effect) can be considered (anaemia in FeLV-infected cats can have an immune-mediated origin, and some cats might respond).
- **Treatment of FeLV-infected cats with neurologic signs**
  - Underlying diseases (e.g., lymphoma) causing the neurologic signs have to be identified and treated.
  - If no underlying disease is identified (and the neurologic signs are assumed to be caused by FeLV directly), treatment with zidovudin (AZT) 5 mg/kg PO q12h might be an option.
- **Treatment of FeLV-infected cats with recurrent infections**
  - Aggressive treatment (e.g., longer courses of bacteriocidal antibiotics) of recurring infections (e.g., long-term antibiotics after culture and sensitivity) is recommended.
  - If this is not successful, treatment with antiviral or immunomodulatory drugs (e.g., feline interferon-omega $10^6$ IU/kg SC 1x/week (or alternatively $10^6$ IU/kg SC q24h for 5 consecutive days, 3 courses starting on days 0, 14, 60) or raltegravir 20-25 mg/kg PO q12h or AZT 5 mg/kg PO q12h) can be tried.
- **Treatment of FeLV-infected cats with chronic gingivostomatitis**
  - Treatment of choice in cats with severe chronic gingivostomatitis is removal of all teeth (usually in 2 sessions; total removal of all tooth roots must be confirmed by radiographs).
  - If total mouth extraction is not an option, medical treatment can be tried.
    - Glucocorticoids should be avoided.
    - If concurrent FCV infection is present, local oromucosal treatment with feline interferon-omega ($0.1 \times 10^6$ IU/cat q24h) might be an option.
    - If no FCV infection is present or interferon-omega is not effective, treatment trial with azidothymidine (AZT) (5 mg/kg PO q12h) plus antibiotics can be used.
    - In addition, lactoferrin topically (40 mg/kg q24h) to the oral mucosa can be tried.

**Tumours**

Although the prognosis is worse when tumours are associated with FeLV (Vail et al., 1998; Kristal et al., 2001; Teske et al., 2002; Ettinger 2003), antitumor therapy should be considered in FeLV-infected cats because some patients greatly benefit from it. Antitumor therapy should be applied with identical protocols used in non-FeLV-infected cats. In an older report of 38 cats with lymphoma (of which most cats were progressively FeLV-infected, and the most frequent tumour was mediastinal lymphoma) treated with COP protocol, 75% achieved complete remission with a median remission duration of 150 days and a one-year remission rate of 20% (Cotter 1983). In a later report, cats from the same geographic area were treated with the same COP protocol and had a complete remission rate of only 47%, with a median remission duration of 86 days (Moore et al., 1996). In this group, few cats were progressively FeLV-infected, and the alimentary form was the most frequent (Moore et al., 1996). A recent study evaluated the safety and efficacy of a novel multidrug lomustine-based chemotherapeutic protocol (LOPH (lomustine, vincristine, prednisolone and hydroxydaunorubicin) for cats with high-grade multicentric or mediastinal lymphoma, in an area in Brazil endemic for FeLV. Twenty-one cats were included of which 19 (90%) had progressive FeLV infection. Complete response was reported in 17 cats (81%), three had partial remission, and one had no response. The LOPH protocol was well tolerated by cats with lymphoma and progressive FeLV infection and resulted in a better median lymphoma-related survival time (171 days) than similar studies using other protocols (Horta et al., 2020). Therefore, FeLV infection should not prevent lymphoma treatment. Cats with acute leukaemia are difficult to treat because the bone marrow becomes filled with neoplastic blast cells, which must be cleared before the normal haematopoietic precursors can repopulate. This process can take three to four weeks; therefore, neutropenia and anaemia

might not be immediately reversible. The remission rate for cats with acute lymphocytic leukaemia treated initially with vincristine and prednisone is approximately 25%, whereas the rate for cats with AML (treated with doxorubicin or cytosine arabinoside) is close to zero (Cotter 1998). A cat with suspected FeLV-associated chronic lymphocytic leukaemia was successfully treated with a combination of prednisone, chlorambucil, cyclophosphamide, doxorubicin, and lomustine (Kyle and Wright 2010). All these chemotherapeutic drugs are immunosuppressive, and some are also myelosuppressive, so they can increase the risk of FeLV-associated diseases. Owners must be advised to watch for signs of illness. Secondary infections are common and must be treated quickly and aggressively, especially if they occur at the time of the neurophil nadir. Although prophylactic antibiotics are not given routinely in the treatment of leukaemia or lymphoma, broad-spectrum bactericidal antibiotics should be given to progressively FeLV-infected cats receiving tumour therapy, especially if neutropenia, fever or other signs of secondary infection occur.
FeSV-induced feline sarcomas should be treated surgically early, with wide and deep surgical excision. If no metastases are present, but microscopic tumour cells remain at the tumour site after surgery, radiation can be successful in delaying recurrence. Experimentally FeSV-induced fibrosarcomas in kittens occasionally regressed spontaneously or after treatment with anti-FOCMA serum, but this is unlikely to translate into clinical efficacy (Cotter 1998).

**Haematologic Disorders**

Although haematologic disorders are often irreversible in progressively FeLV-infected cats, there might a cyclic course and/or some improvement with treatment. Thus, treatment with blood transfusions (for temporal life support) or bone marrow-stimulating cytokines should be considered. Anaemia can be life-threatening in FeLV-infected cats, and in some cats, blood transfusion is a very important part of the treatment, especially if the anaemia is non-regenerative. Most cats respond after the first transfusion. Of 29 anaemic (with an HCT less than 20%) FeLV-infected cats treated with blood transfusions (over two weeks), the HCT returned to reference ranges in eight cats. This can be explained by the cyclic cytopenias that are occasionally seen in FeLV-infected cats. Glucocorticoid treatment can increase the life span of erythrocytes if the anaemia is immune-mediated, but they should be used very cautiously. If a glucocorticoid treatment trial is started, it should be with a high dose (e.g., 2 mg/kg q12h) and then slowly tapered down to reach the minimal dose that is still effective. Occasionally secondary infections (e.g., haemotropic *Mycoplasma* spp. infections) are responsible for the anaemia which should be treated. This regenerative anaemia has a good prognosis, and therefore, the possibility of such infections should always be examined. Deficiencies of iron, folate, or vitamin $B_{12}$ are rare; therefore, replacement therapy is not likely to be helpful (Cotter 1998). Even though erythropoietin concentrations are often elevated in cats with FeLV-related anaemia, treatment with recombinant human erythropoietin (e.g. darbepoetin (long acting recombinant human erythropoietin) 0.25-0.5 µg/kg SC weekly until HCT is normal, then increasing dose interval for maintenance) can be helpful. Recombinant human erythropoietin treatment not only increases erythrocyte counts but also can increase platelet and megakaryocyte numbers in animals and humans (Ogilvie 1995). In one study, recombinant human erythropoietin also increased white blood cell counts in cats (Arai et al., 2000). No study has been performed involving FeLV-infected cats, but in a study in FIV-infected cats, all recombinant human erythropoietin-treated cats had a gradual increase in erythrocyte counts, haemoglobin concentrations, and HCT, as well as increased white blood cell counts consisting of increased numbers of neutrophils, lymphocytes, or a combination (Arai et al., 2000). A response might not be seen for 3 to 4 weeks, and if it does not occur, iron supplementation might be required in addition. Iron should not be given to cats that have received transfusions because whole blood contains high concentrations (0.5 mg/mL) of iron, and this induces a risk of hemosiderosis in the liver. Anti-erythropoietin antibodies can develop in 25% to 30% of rHuEPO-treated animals after six to twelve months. Binding of these antibodies to the recombinant human erythropoietin and the native erythropoietin nullifies their physiologic actions on erythroid progenitor cells, potentially causing bone marrow failure and refractory anaemia. However, anti-erythropoietin antibodies dissipate after discontinuation of treatment. Based on anecdotal reports, some FeLV-infected cats do not respond to rHuEPO treatment. Reasons for resistance to erythropoietin, other than development of anti-erythropoietin antibodies and iron deficiency, include FeLV infection of bone marrow stromal cells or concurrent infections with other infectious agents in the bone marrow.
Neutropenia can lead to severe immunosuppression, and antibiotics can be necessary in some neutropenic cats to prevent secondary bacterial translocation and development of sepsis. Treatment with filgrastim, a granulocyte colony-stimulating factor (G-CSF) that is on the market as recombinant human product for treatment of neutropenia in humans, has caused transient responses. Filgrastim is used in cats at 5 µg/kg SC q24h for up to 21 days. Potential side effects include bone discomfort, splenomegaly, allergic reactions, and fever (Greene and Watson 1998a; Arai et al., 2000). Short-term increases in neutrophil counts can be followed by neutropenia with continued use of filgrastim because of development of dose-dependent neutralising antibodies to this heterologous product after ten days to seven weeks. Thus, treatment should not be used for more than three weeks (Greene and Watson 1998a; Arai et al., 2000). Another potential risk is the development of persistent antibodies against endogenous feline G-CSF (at higher dosages), resulting in rebound neutropenia. One study suggested that filgrastim is contraindicated in FIV-infected cats because it led to an increased viral load (Arai et al., 2000), but this so far has not been shown for FeLV-infected cats. In one pilot study, a small number of naturally FeLV-infected cats were treated with filgrastim; however, treatment did not result in significant changes in neutrophil counts (Kraft and Kuffer 1995). Anecdotal reports indicate that filgrastim has been used in FeLV-infected cats with cyclic neutropenia with some success (Levy 2000). In some FeLV-infected cats with neutropenia, an immune-mediated mechanism is suspected to lead to a maturation arrest in the bone marrow at myelocyte and metamyelocyte stages. Neutrophil counts can normalise in some of these cats with immunosuppressive doses of glucocorticoids (Stavroulaki et al.,

2020). In animals with myeloid hypoplasia and in the absence of myeloid precursors, direct effects of FeLV are suspected, and glucocorticoids should not be used. Thus, bone marrow aspiration and cytology should ideally be performed before glucocorticoids are considered.

**Management**

It is important to realize that FeLV-infected cats are subject to the same diseases that befall uninfected cats, and that the mere presence of an FeLV-related disease might or might not be caused by FeLV (Levy et al., 2008a; Hartmann 2009b; Little et al., 2020). However, special management has to be considered when owning an FeLV-infected cat. These management protocols should include the individual cat but also housemates of FeLV-infected cats (Table 22) (Levy et al., 2008a; Hartmann 2009a; Hartmann 2012a).

***Individual Feline Leukaemia Virus-Infected Cats***

With proper care and environmental management, FeLV-infected cats can live for many years with good quality of life, even those with progressive infection. In cats with progressive infection, regular veterinary examinations should be encouraged at least semi-annually to promptly detect changes in health status. In cats that develop regressive FeLV infection, subsequent retesting for FeLV antigen during regular annual veterinary examinations is recommended to confirm absence of potential reactivation of the infection and check the health status of the cats.

***Housing and Environment.*** Progressively FeLV-infected cats should be confined indoors to protect the vulnerable immunosuppressed cats from other infectious agents carried by other animals, but also to prevent spread of FeLV to other cats in the neighbourhood. Housing conditions appear to affect outcomes for FeLV-infected cats. In a study of cats in two rescue sanctuaries that group-housed FeLV-infected cats with uninfected cats without separating clinically ill cats from healthy cats, the prevalence of FeLV was more than 20-fold higher than in the general pet cat population. Not only were cats more likely to be infected in the sanctuaries, but they were also more likely to develop the progressive form of infection, leading to poorer long-term prognosis. The benefit of low-density housing can be attributed to reduced environmental stress, infectious pressure, and co-infections (Westman et al., 2019a). Careful management of resources in multi-cat households might assist in reducing these stressors, leading to better prognosis and longer survival. Where possible, FeLV-infected cats should be housed in low-density environments where stressors are reduced, resources are ample, and caregivers can observe patient health status carefully (Little et al., 2020).

***Preventative Healthcare.*** Cats with **progressive FeLV infection** should be regularly followed-up life-long. They should receive preventive health care visits at least every six months for prompt detection of changes in their health status. Veterinarians should obtain a detailed history to help identify changes requiring more intensive investigation and should perform a thorough physical examination at each visit. Special attention should be paid to the oral cavity. Lymph nodes should be evaluated for changes in size and shape. All cats should receive a thorough examination of the anterior and posterior segments of the eye (Willis 2000). The skin should be examined closely for evidence of external parasite infestations, fungal diseases, and neoplastic changes. If clinical or laboratory changes are present, veterinary visits might be necessary more often. FeLV-infected cats should be prescribed appropriate prophylaxis for internal and external parasites. In areas where heartworm is prevalent, cats should be on monthly prophylaxis. Use of routine, consistent parasite control according to the European Scientific Counsel Companion Animal Parasites guidelines (ESCCAP 2018; ESCCAP 2020) will reduce the risk of secondary infection and disease in these potentially immunosuppressed cats. A complete blood count should be performed at least semi-annually in progressively FeLV-infected cats. A serum biochemical analysis and complete urinalysis (urine specific gravity, urine chemistries, and sediment examination) should be performed annually. Urine samples should be collected by cystocentesis so that bacterial cultures can be performed if indicated. Faecal examinations should be performed as needed.
Good nutrition and husbandry are essential to maintain good health. Nutritional support is key to maintaining good health in these patients. A nutritionally balanced and complete feline diet should be fed appropriate to the cat's life stage. Raw meat and raw dairy products should be avoided because the risk of food-borne bacterial and parasitic diseases is likely greater in these potentially immunosuppressed cats. Periodic nutritional assessments should evaluate food intake, body condition score (BCS), muscle condition score (MCS), and quality of nutrition to improve health and alert the clinician to early problems. Unexpected downward trends in body weight or reductions in BCS or MCS should prompt the clinician to investigate further as changes in body weight can precede other signs of illness for months and even years (Little et al., 2020).
Sexually intact male and female cats should be neutered to reduce stress associated with oestrus and mating behaviours. Neutered animals are also less likely to roam away from home and interact aggressively with other cats although no differences were observed in the hormone concentrations between FeLV-infected and noninfected cats with and without clinical signs (Tejerizo et al., 2012). In otherwise healthy, FeLV-infected cats, surgical procedures should be used as required to maintain health and manage disease. FeLV-infected cats should receive the same quality of anaesthetic, analgesic, surgical, and perioperative care given to all feline patients. Preoperative evaluation, including laboratory testing, should follow the same standard of care as for uninfected cats. As for all cats, the use of perioperative antibiotics should be

reserved for those individuals with clear evidence of immunosuppression and/or those undergoing surgeries where the risk of bacterial contamination is moderate to high (Weese et al., 2015). Multimodal analgesia plans should be used in all cats when indicated, especially if they have concurrent painful conditions such as gingivostomatitis.
Vaccination with core vaccines (against FPV, feline herpesvirus, and FCV) should be performed regularly in progressively FeLV-infected cats, even if the cat is kept strictly indoors. If an owner cannot be convinced to keep a FeLV-infected cat inside, rabies vaccinations should be given (in accordance with state and local regulations). FeLV-infected cats might not be able to mount an adequate immune response to administered vaccines, which has been observed for rabies vaccines (Franchini 1990), but this is likely the same for other vaccines as well. Therefore, protection in a FeLV-infected cat after vaccination is not as complete and long-lasting as in a non-infected cat, and more frequent vaccinations (e.g., yearly) have to be considered in FeLV-infected cats (Hartmann et al., 2017), especially if the cat is allowed to roam outside. There is little evidence to suggest modified-live virus vaccines are a risk in retrovirus-infected cats and the response of asymptomatic retrovirus-infected cats can be similar to uninfected cats (Bergmann et al., 2018). FeLV vaccines are not recommended in cats with known progressive or regressive FeLV infections because these vaccines have no effect on the viraemia, carrier state or elimination, or clinical FeLV disease in already infected cat (Helfer-Hungerbuehler et al., 2015a) and do not prevent reactivation of regressive infection. It is yet unclear whether FeLV vaccination in cats with abortive FeLV infection could be omitted.

In cats with **regressive FeLV infection**, subsequent retesting for FeLV p27 antigen during regular annual veterinary examinations is recommended to confirm absence of potential reactivation of the infection and check the health status of the cat.

Table 22: Management of individual FeLV- infected cats

| Management of individual FeLV-infected cats |
|---|
| • FeLV-infected cats should be kept indoors to not be exposed to other infectious agents carried by other animals and to avoid spread of FeLV to other cats.<br>• "Routine vaccination programs" should be maintained in FeLV-infected cats. FeLV-infected cats are not able to mount an adequate immune response to vaccines and protection is not comparable to that in a healthy cat. Therefore, more frequent vaccination (e.g., yearly) should be considered.<br>• FeLV-infected cats should have health care visits at the veterinarian at least semi-annually to promptly detect changes in the health status. A complete blood count should be performed every six months, a biochemistry profiles and urinalyses at least yearly.<br>• Intact male and female cats should be neutered to reduce stress associated with oestrus and mating behaviour and the desire to roam outside the house and interact aggressively.<br>• If FeLV-infected cats are sick, prompt identification of the secondary illness is essential to allow early therapeutic intervention.<br>• Most cats with FeLV infection respond as well as uninfected cats to appropriate medications, although a longer and more aggressive course of therapy (e.g., antibiotics) might be needed<br>• Corticosteroids, other immune-suppressive, or bone marrow-suppressive drugs should be avoided. |

***Management in Veterinary Hospitals.*** FeLV-infected cats might require veterinary care, and special precautions should be taken (Table 22). FeLV is present in body excretions (highest concentrations in saliva) in cats with progressive infection that pose an immediate risk to other cats in their environment. However, FeLV is not very stable outside the host animal under dry conditions (significant reduction of survival within 30 to 60 minutes), although survival of the virus is longer under moist conditions (Francis et al., 1979b). Still, direct contact among cats and immediate fomite transfer are the major risk factors. Progressively infected cats should be physically separated from other cats in the environment. In a veterinary hospital, FeLV-shedding cats can be housed in the same ward with other hospitalized patients as long as they are housed in separate cages (Francis et al., 1977; Francis et al., 1979b) and certain precautionary measures are taken. FeLV is susceptible to all disinfectants as well as common soap; thus, simple precautions (e.g., hand washing) and routine cleaning procedures can prevent transmission in the hospital setting. Progressively FeLV-infected cats should be housed in individual cages and confined to them throughout their hospitalization. They should never be placed in a "contagious ward" with cats that have other infections, such as viral respiratory infections, because of immunosuppression of progressively FeLV infected cats. Animal caretakers and other hospital staff members should be advised to disinfect their hands between direct contacts with patients (primarily to protect the FeLV-infected, immune-suppressed cat) and after cleaning cages and litter boxes. Dental and surgical instruments, endotracheal tubes, and other items potentially contaminated with body fluids of a FeLV-infected cat should be thoroughly cleaned and sterilized between uses. Fluid lines, multidose medication containers, and food can become contaminated with body fluids (especially blood or saliva) and should not be shared among patients.

***Feline Leukaemia Virus-Infected Households***

In a household with one or more progressively FeLV-infected cat/s, all cats should be tested for FeLV antigen and provirus to identify their FeLV status. It is likely, that all cats in that household have been exposed and are progressively, regressively, or abortively infected. If indeed more progressively infected cats are identified, a test and removal system

should be implemented, which involves periodic testing and removal of the antigen-positive cats until all test negative. Cats with regressive or abortive infection are likely protected against new FeLV infection. However, no new naïve cat should be introduced in this household. Shedding of virus generally occurs through salivary glands, and cat-to-cat transmission can occur by allogrooming, sharing of food and water bowls and litter boxes, and fighting and biting behaviour. A naïve newly introduced cat would be at high risk if living together with shedding cats due to high infectious pressure (Hartmann 2009b). It is thus not recommended to introduce naïve cats to such a household, even if the cat has been vaccinated against FeLV. Although protection conferred by FeLV vaccines is good in most situations, ABCD does not recommend reliance solely on vaccination to protect negative cats living together with progressively FeLV-infected cats. If the household is closed to new cats, the cats with negative FeLV antigen test results will tend to outlive the progressively infected cats; thus, after months or years all remaining cats will be antigen-negative and immune to new infection. To monitor the situation in such households, finally proof absence of FeLV shedders or detect cats with potential reactivation of the infection, it is advisable to test all cats in such an environment at least in yearly intervals. For infection control in specific situations (breeders, shelters) see chapter below.

Table 23: Management of multi-cat households with FeLV-infected cats

| Management of multi-cat households with FeLV-infected cats |
|---|
| • FeLV is mainly transmitted through social contact, but also through biting and fighting.<br>• If a progressively FeLV-infected cat lives in an otherwise negative household, most of the other cats have already been infected and are most likely immune to new infection.<br>• The risk of (re-)occurrence of antigenemia in adult FeLV antigen-negative cats is approximately 10% to 15% if they have lived together in a long-term situation with a viraemic cat. This, however, is more likely caused by reactivation of a regressive FeLV infection and not by transmission from the other cats in the household.<br>• If owners refuse to separate housemates, the uninfected cats should receive FeLV vaccination to enhance their natural level of immunity. However, owners should be informed that vaccination does not provide complete protection in these high exposure situations. |

## PROGNOSIS

Despite the fact that progressive FeLV infection is associated with a decreased life expectancy (Levy et al., 2006a; Gleich and Hartmann 2009), treatment of clinical syndromes that accompany progressive FeLV infection is recommended in many cats even with progressive FeLV infection. Survival times vary considerably depending on the stage of infection, host immunity, and the strain of FeLV involved. Under experimental conditions, neonatal cats inoculated with different FeLV strains using plasmid DNA survived in average 49 weeks (Rickard strain pFRA) or 67 weeks (FeLV-A pF6A), respectively (Phipps et al., 2000). In another experimental study, the average life expectancy of the cats infected at the age of 10 weeks to 59 months with FeLV-A/Glasgow-1 and developing progressive infection was 3.1 years (range 0.6 to 6.5 years) (Helfer-Hungerbuehler et al., 2015b). The survival time depended on the disease outcome: cats that developed lymphoma survived an average of 3.6 years (range 1.6 to 6.5 years; n = 14), whereas cats that showed non-regenerative anaemia lived an average of only 0.6 years (range 0.6 to 1.1 years; n = 3). The three cats that suffered from leukaemia survived longest (median: 5.3 years, from 1.8 to 5.3 years; n = 3). The age at the time of infection had no effect on whether the cat developed lymphoma, anaemia, or leukaemia and with that no effect on the survival time (Helfer-Hungerbuehler et al., 2015b). The average survival time of this study was similar to an older field study that estimated that within 3.5 years of progressive infection 83% of the cats would be dead (McClelland et al., 1980). Another field study performed long-term monitoring of a 26-cat household with endemic FeLV and FIV infections and revealed that all progressively FeLV-infected cats died within five years of diagnosis, while FIV infection did not (Addie et al., 2000). These studies all involved group-housed cats in multiple-cat FeLV-endemic environments. Another set-up was used in a large study that compared more than 800 cats diagnosed with progressive FeLV infection with 7,000 age- and sex-matched controls (Levy et al., 2006a; Levy et al., 2008a). The median age of cats in the FeLV-infected group and their controls was two years at the time of diagnosis. Of cats not euthanized near the time of diagnosis, the median survival time after diagnosis was 2.4 years for progressively FeLV-infected cats and 6.3 years for negative controls. The high rate of euthanasia in the first year after diagnosis was likely due to disease conditions that prompted the veterinary visit or due to euthanasia of healthy retrovirus-infected cats (e. g. strays) for purposes of infection control (Levy et al., 2006a). As part of a study on the FIV and FeLV prevalence in owned cats in Germany, a subset of 100 cats (19 FIV-positive, 18 FeLV-positive, 63 uninfected) was evaluated for survival time (Gleich et al., 2009). The mean survival time of progressively FeLV-infected cats (312 days) was significantly shorter compared with uninfected cats (732 days) (Gleich et al., 2009). In an assessment of lifetime medical records for shelter cats classified as FIV-infected (n=63), progressively FeLV-infected (n=22), coinfected (n=4), or uninfected (n=11), cats with progressive FeLV infection and cats co-infected with FeLV and FIV had significantly shorter lifespans as well as a higher incidence of lymphoma (McCallum et al., 2016). Overall, most cats that are progressively infected with FeLV go on to develop FeLV-associated disease. Still, many progressively infected cats, especially adult cats, can live for several years with a good quality of life, and so euthanasia is not recommended solely on the basis of a persistently positive FeLV antigen test. With proper care, FeLV-infected cats can live much longer than this, and, in fact, die at an older age from causes completely unrelated to their FeLV infection (Hartmann 2005a). Owners should be educated in detail about options for care of infected cats, and veterinarians providing an accurate prognosis and careful monitoring of each cat should assist

owners in the care of their cat (Little et al., 2020).

Cats with regressive or focal FeLV infection have a good prognosis and no reduced life expectancy if the infection does not reactivate. Rarely, they can develop FeLV-associated diseases, such as lymphoma or bone marrow suppression, even without reactivation. Cats with abortive FeLV infection have an excellent prognosis and no reduced life expectancy.

## VACCINATION

### Development of Effective FeLV Vaccines

Cats with abortive and regressive FeLV infection can mount an effective immune response and prevent or contain virus replication. This is a unique feature for a retroviral infection and supports the idea that immune protection could also be reached by FeLV vaccines. However, the development of a safe and effective vaccine against FeLV initially presented challenging.

**Live virus vaccines** were based on the ability to maintain FeLV in cell culture and contained FeLV-infected lymphoblasts or fibroblasts. These first experimental vaccines produced strong and long-lasting humoral immune response (Jarrett et al., 1974; Jarrett et al., 1975; Pedersen et al., 1979). In one study, the kittens resisted a subsequent challenge with a large viral dose of a pathogenic FeLV strain (Jarrett et al., 1975) and another live-virus vaccine was found to be both safe and efficacious in protecting the kittens from virulent virus challenge (Pedersen et al., 1979). Immunisation of cats with a low dose of virulent FeLV-A and FeLV-B particles from a cat with thymus lymphoma let do protection against persistent infection in a FeLV-endemic household for two years, while control cats became infected (Hardy 1981b). However, although live virus vaccines were immunogenic and allowed for some protection, there was concern that a live vaccine virus could establish regressive infection with integration into the host genome and later cause FeLV antigen-negative lymphomas (Hardy 1980). Moreover, inoculation of cats with live tumour cells was considered problematic. It was concluded that a live FeLV vaccine, even if highly efficacious, would not be suitable for commercial use.

Further vaccine research focused on **inactivated virus preparations**. Inactivated vaccines contained paraformaldehyde inactivated FeLV producing cells (FL74) or purified whole virus (Jarrett et al., 1975; Olsen et al., 1976a). Some studies claimed that these vaccine preparations were safe and decreased the proportion of cats with progressive infection after challenge exposure (Yohn et al., 1976). However, most of the early inactivated FeLV vaccines were poor immunogens, ineffective in preventing progressive FeLV infection, or they even caused enhancement of tumour development (Olsen et al., 1976a; Olsen et al., 1977; Pedersen et al., 1979; Salerno et al., 1979; Hardy 1981b). Several studies performed vaccination of pregnant queens to protect the progeny from FeLV infection with contradicting results; while some demonstrated protection of kittens against FeLV (Yohn et al., 1976; Schaller et al., 1977; Salerno et al., 1979), another study found no antibodies neither in the vaccinated queens nor the kittens and the latter were not protected from virus challenge at two weeks of age and from FeLV-associated diseases (Hoover et al., 1977). Only when FeLV cell culture conditions were refined by adding cell-free medium to FeLV producing FL74 cells, vaccine antigen recovery was improved, and large amounts of soluble FeLV antigens could be harvested in cell culture supernatants (Olsen et al., 1976b; Wolff et al., 1979). Moreover, it was discovered that FeLV antigen preparations containing merely the virus envelope could provide protection if used in conjunction with potent adjuvants (Olsen et al., 1980; Lewis et al., 1981). The latter preparations had the advantage that no viral genes were introduced in the host cells circumventing the oncogenic potential of FeLV. Moreover, the goal was to produce a subunit vaccine that was free of the immunosuppressive component FeLV p15E (Olsen et al., 1980).
Based on such subunit vaccines, the first FeLV vaccine used in veterinary practices which was based on soluble antigens was introduced in the USA in 1985 (Lewis et al., 1981). Subsequent studies evaluating the efficacy of this early commercial vaccine produced controversial results depending on the situation under which the vaccine was tested, and results varied from vaccines being efficacious in protecting cats from progressive infection (Mastro et al., 1986; Sharpee et al., 1986; Haffer et al., 1987; Pollock and Scarlett 1990) to having insufficient or no efficacy (Pedersen et al., 1985; Osterhaus et al., 1987; Osterhaus et al., 1989; Legendre et al., 1990). Since that time, these original vaccines have undergone major modifications to improve efficacy, and several other products have appeared on the market, which are still commonly used in veterinary practice.

A further approach consisted of **recombinant protein vaccines**. The antigens were produced in *Escherichia coli*, yeast or mammalian cell lines and a defined quantity of antigen could be produced in these systems. Studies using an envelope protein of FeLV-A produced in yeast resulted in protective immunity when followed by consecutive application of a live virus vaccine (Luciw et al., 1986). FeLV envelope antigens presented as immune stimulating complex (ISCOM) (Morein et al., 1984) related protection from viremia under experimental conditions with oronasal challenge infection and lead to development of neutralising antibodies in field cats (Osterhaus et al., 1985; Osterhaus et al., 1987; Osterhaus et al., 1989). A non-glycosylated recombinant FeLV envelope protein (p45) consisting of the SU and the first 34 amino acids of the TM in combination with a potent adjuvant, Quil A, led to a strong humoral immune response and protection against

intraperitoneal challenge infection, while the protein in combination with other adjuvants caused insufficient protection (Marciani et al., 1991). This vaccine led to the development of the first commercial recombinant vaccine in small animal medicine and was confirmed to be highly efficacious in protecting cats from progressive FeLV infection (Clark et al., 1991; Kensil et al., 1991; Lehmann et al., 1991).

Another recombinant vaccine uses a **canarypox virus vector** that carries the *env* and *gag*/part of *pol* genes of FeLV (Tartaglia et al., 1993). After injection, there is a single round of replication by the vector poxvirus resulting in the expression of the inserted FeLV genes. Later, a recombinant canarypox vaccine became available in Europe, which is a slightly different product than that licensed for use in the United States. Recombinant canarypox vaccines are replication-defective live virus vaccines that do not require an adjuvant to induce protective immunity (Poulet et al., 2003). The protective effect of canarypox-vectored vaccines is presumably achieved by stimulating cellular immunity (Poulet et al., 2003; Hofmann-Lehmann et al., 2006). The canarypox-vectored vaccine can also be used to booster a cat that had undergone primary vaccination with an inactivated whole virus vaccine and protection is achieved comparable to two vaccinations with the whole virus vaccine (Grosenbaugh et al., 2006). An improved version of the recombinant canarypox vaccine was produced by a targeted mutation within the FeLV envelope protein immunosuppressive domain p15E (Schlecht-Louf et al., 2014). The introduction of this mutation increased the frequency of vaccine-induced FeLV-specific gamma interferon production and decreased the interleukin-10 producing cells. This shift in the cytokine response associated with a modified immune response toward increased cellular immunity was associated with a slightly increased vaccine efficacy (Schlecht-Louf et al., 2014).

Various other not so efficient FeLV vaccine approaches included synthetic peptides (Elder et al., 1987), a vaccinia virus-vectored vaccine (Gilbert et al., 1987) that lacked immunogenicity, and anti-idiotypic antibody vaccines (UytdeHaag et al., 1986). The idea of the latter was to use large quantities of anti-idiotypic monoclonal antibodies with a paratope mimicking FeLV gp70; however, injection of these antibodies did not lead to induction of FeLV-binding antibodies (UytdeHaag et al., 1986). And finally, molecularly cloned envelope gene recombinants of FeLV subgroups A, B and C and deleted LTR enhancer sequences were constructed in an attempt to produce an attenuated live virus vaccine with high immunogenicity; however, the genetically engineered live virus preparations were not protective against FeLV challenge because they were insufficiently attenuated or antigenic (Luciw et al., 1986). The observation that cats immunized with FeLV transmembrane protein p15E developed neutralising antibodies led to the suggestion that p15E should be included in future FeLV vaccines (Langhammer et al., 2006). In further studies, adjuvanted p15E provided protection from antigenemia in only three of six of the immunized cats, while all three controls became progressively infected (Langhammer et al., 2011).

**Nowadays, there are three types of FeLV vaccines licensed**: 1) classical inactivated whole virus vaccines; 2) genetically engineered recombinant subunit vaccines based on the FeLV envelope protein p45 (Kensil et al., 1991; Marciani et al., 1991); and 3) infectious recombinant canarypox virus engineered to expressing the genes for the envelope glycoprotein and the capsid protein (Tartaglia et al., 1993).

**FeLV Vaccine Efficacies**

The differences between available FeLV vaccines are more significant than those for other feline infectious diseases with differences in performance, particularly efficacy of protection but also with regards to adverse effects. Although most "modern" FeLV vaccines have been found efficacious in protecting cats from progressive infection, controversy exists about the degree of efficacy and about the question on which is the most efficacious vaccine (Sparkes 2003).
Some of the published vaccine efficacy trials were co-authored by the manufacturers (Stuke et al., 2014; Patel et al., 2015a; Sparkes et al., 2015). Few independent studies exist that compare several vaccines (Jarrett and Ganiere 1996; Hofmann-Lehmann et al., 2006; Torres et al., 2010). Nonetheless, comparison of different efficacy studies can be misleading; differences in the protocols can sometimes lead to divergent results from the same vaccine. Differences can include different time spans between vaccination and challenge, use of different challenge viruses and doses and cats of different origin, making meaningful comparison difficult. To overcome the higher resistance to FeLV of older cats, some studies used glucocorticoids for immunosuppression before intranasal challenge or performed parenteral FeLV exposure. These challenges produced a much higher proportion of infected control cats, reducing the overall number of cats needed for efficacy studies. The comparability of these challenges to natural exposure has been questioned (Legendre et al., 1990; Clark et al., 1991; Hines et al., 1991; Hoover and Mullins 1991; Legendre et al., 1991). However, some of the FeLV vaccines proved efficacious even under these severe challenge conditions suggesting that the vaccines will also protect cats against FeLV infection under natural conditions. Some studies have involved natural challenges, in which vaccinated and control cats lived together with FeLV-shedding cats (Legendre et al., 1990; Gruffydd-Jones 1999). This type of challenge might, on the one hand, be more comparable to the natural situation. However, natural challenge situations are often incapable of yielding a high infection rate in unvaccinated control cats, leading to a low calculated efficacy of the vaccine and unsuitable data for vaccine approval.

The European licensing authorities defines **criteria for assessing the efficacy of protection of inactivated FeLV vaccines**. They include a minimum acceptable infectivity rate of more than 80% progressive infections in unvaccinated controls to confirm that an acceptably strong challenge has been provided; a cat is considered persistently infected (progressive infection) when it is FeLV antigen-positive for three consecutive weeks or on five occasions between weeks 3 and 15 post-exposure (European Pharmacopoeia (2005)). The latter differs somewhat from the USDA FeLV vaccine evaluation guidelines, which consider a cat "FeLV-positive" (actively infected) when three consecutive samples had positive antigen or virus isolation results between weeks 3 and 8 post-exposure (Shibley et al., 1991). This difference adds to the discrepancies in interpretation of results of different vaccine studies. A measure for vaccine efficacies subsequent to FeLV exposure as suggested by Pollock and Scarlett is calculated by the so called **"preventable fraction"** (PF) (Pollock and Scarlett 1990). The preventable fraction accounts for the inherent resistance of some unvaccinated cats to development of progressive infection (persistent antigenemia/viremia) after FeLV challenge (Fig. 22). It calculates the proportion of cats with expected progressive infections, as determined in unvaccinated controls, prevented in vaccinated cats by using the following equation:

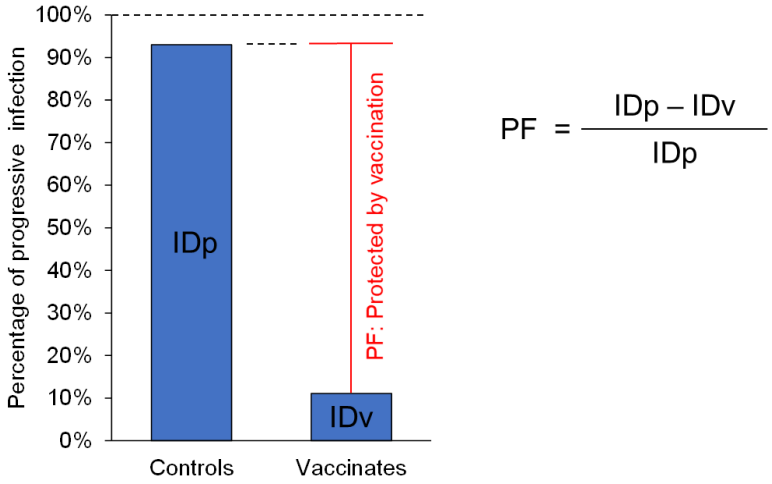


Fig. 22: Measure for vaccine efficacies: preventable fraction (PF) (Pollock and Scarlett 1990). The preventable fraction calculates the proportion of cats that is protected by vaccination and considers the percentage of cats that are naturally protected as determined in the control group. IDp = incidence density of persistent antigenemia/viremia (progressive infection) in placebo (control) cats; IDv = incidence density of persistent antigenemia/viremia (progressive infection) in vaccinates. (Courtesy of R. Hofmann-Lehmann, Zurich, Switzerland)

No accurate pre-challenge measures exist that can predict prior to the virus exposure whether vaccinated cats will be protected from progressive infection. The induction of virus-neutralising antibodies following challenge is a consistent feature in vaccinated cats protected from progressive infection (Willett and Hosie 2013). However, neutralising antibodies develop in only few vaccinated cats prior to FeLV exposure despite the fact that the cats are protected against challenge infection (Lehmann et al., 1991; Jarrett 2001), and many properly vaccinated and protected cats in challenge studies and in the field do not have detectable neutralising antibodies after vaccination prior to virus exposure (Poulet et al., 2003; Sparkes 2003; Hofmann-Lehmann et al., 2006; Englert et al., 2012).

In many experiments it was shown that no FeLV vaccine provides complete (100% efficacy) protection, nor does it prevent infection. Vaccinated and subsequently virus-exposed cats become provirus- and viral RNA positive in the blood, although at very low levels when compared with cats with progressive infection (Hofmann-Lehmann et al., 2006). An independent comparison of FeLV whole virus vaccines available in the United States revealed a very high efficacy in two out of four FeLV vaccines tested (Torres et al., 2010). The study claimed absence of provirus integration and viral RNA in most cats vaccinated with the two whole virus vaccines. These results contradict earlier data showing that neither one of the efficacious whole virus vaccines tested nor the recombinant canarypox vaccine led to "sterilizing immunity" (which in case of FeLV means complete absence of viral RNA, proviral DNA, antigen, and culturable virus after challenge)(Hofmann-Lehmann et al., 2006; Hofmann-Lehmann et al., 2007). FeLV provirus was found to persist for years even after challenge in vaccinated cats, and recurrence of viraemia and disease development was observed in some cats (Hofmann-Lehmann et al., 2008). Thus, vaccination does not protect against development of regressive infection; however, it can effectively protect from progressive infection.

Two studies that compared different FeLV vaccines were discussed rather controversially (Stuke et al., 2014; Patel et al., 2015a). Both studies were co-authored by the manufacturers of one of the included vaccines. The first study claimed that an inactivated whole virus vaccine was superior in protecting cats from FeLV challenge (90% protection from progressive infection) compared to the recombinant canarypox-vectored vaccine (20% protection from progressive infection) (Stuke et

al., 2014). However, the study was criticized, for, among other issues, an intercurrent parvovirus infection that was probably introduced by a contaminated challenge virus (Hofmann-Lehmann et al., 2015). Parvoviruses are highly immunosuppressive, and a study on the immunogenicity of a product was not considered valid under these conditions, particularly since the challenge infection and the parvovirus infection occurred within three weeks after the second vaccination (Hofmann-Lehmann et al., 2015). The second study also compared the recombinant canarypox-vectored vaccine to an inactivated whole virus vaccine (Patel et al., 2015a). Immunosuppression was introduced in the study by using high doses of glucocorticoids during the challenge, and the challenge occurred three months after the second vaccination. Under these circumstances, the whole virus vaccine performed better (preventable fraction 100%) compared to the canarypox-vectored vaccine (preventable fraction 45%). The reasoning for the severe immunosuppression during challenge was questioned, because it can particularly hamper the cell-mediated immune response and thus, specifically the immune response to the recombinant canarypox vaccine (Poulet et al., 2015). On the other side, it was argued that also cats at risk for FeLV infection might be immunosuppressed (Patel et al., 2015b). A third study, also authored by the manufacturer of one of the tested vaccines, compared three current FeLV vaccines: a canarypox vectored vaccine and two inactivated whole virus vaccines (Grosenbaugh et al., 2017). Vaccinated cats and control cats were challenged twice oronasally with a heterologous FeLV isolate. The three tested vaccines showed a similar degree of protection (80-93%), while 75% of the control cats developed persistent infection (Grosenbaugh et al., 2017).

An independent field study compared the antibody response in client-owned cats in Australia that had been vaccinated against FeLV using two commercially available inactivated whole virus vaccines, one in a monovalent and one in a polyvalent formulation (Westman et al., 2021). Cats vaccinated with the monovalent vaccine had higher anti-SU antibodies compared to cats receiving the polyvalent vaccine; however, when cats that had been vaccinated with one of the two vaccines and had been naturally exposed to FeLV later, there was no difference in anti-SU response anymore between the two vaccine groups. The authors postulated that the monovalent FeLV vaccine induced an immediate preparatory antibody response, while the polyvalent vaccine formulation with FeLV primed the humoral immune response prior to FeLV exposure, such that antibody production increased when the animal was challenged (Westman et al., 2021). The study raised further questions concerning the comparable vaccine efficacy of the different FeLV vaccine formulations and correlates of protection.

When considering FeLV vaccination, not only efficacy but also the development of FISS is a concern. An epidemiologic association has been demonstrated between FeLV vaccinations and later development of FISS in early studies (Hendrick et al., 1992; Kass et al., 1993; Hendrick et al., 1994; Macy 1995; Kass et al., 2003; Dean et al., 2006; Hartmann et al., 2015); however, there is no definitive proof of the pathogenesis of FISS, despite extensive research. The most widely accepted hypothesis suggests that a chronic inflammatory reaction at the site of an injection of an irritating substance acts as a trigger for subsequent malignant transformation. In this context, adjuvanted vaccines can be a concern since the adjuvant is added to enhance the immune reaction and with that the inflammation at the injection site. Different adjuvants were identified in histological or ultrastructural investigations of these sarcomas (Hendrick et al., 1992; Madewell et al., 2001; Day et al., 2007; Deim et al., 2008). One study compared the subcutaneous tissue response to different FeLV vaccines. Three groups of 15 cats were injected with one of three FeLV multi-component vaccines, the canarypox-vectored vaccine, a vaccine with a lipid-based adjuvant, and a vaccine adjuvanted with an alum-Quil A mixture. On days 7, 21 and 62 post-vaccination, significantly less inflammation was associated with administration of the canarypox-vectored multi-component vaccine. The inflammation was most severe in cats receiving the multi-component vaccine containing FeLV p45 and an aluminum-based adjuvant (Day et al., 2007). Some studies looked at a possible role of FeLV and its mutant FeSV in the development of FISS, but could not detect either FeLV or FeSV in the tumours (Ellis et al., 1996a), or any other viruses, including FIV, FFV, polyomaviruses or papillomaviruses (Kidney et al., 2000; Kidney et al., 2001a; Kidney et al., 2001b; Kidney et al., 2002). No evidence was found for the replication or expression of endogenous retroviruses being involved in FISS formation (Kidney et al., 2001a; Kidney et al., 2001b). More recently, feline primary cutaneous lymphomas also have been described in association with FeLV vaccination (Roccabianca et al., 2016). A total of 17 cutaneous lymphomas with a history of vaccine injection at the site of tumour development were evaluated. Post-injection time of development ranged from 15 days to approximately nine years in five cats. FeLV gp70 and/or p27 proteins were expressed in ten of the 17 tumours. It was discussed that persistent inflammation induced by the injection and by reactivation of FeLV expression might have contributed to the development of these tumours (Roccabianca et al., 2016).
However, it has to be considered that not only adjuvanted vaccines (Kass et al., 1993; Hendrick et al., 1994; Coyne et al., 1997), but also non-adjuvanted vaccines and injections of long-acting drugs, e.g. glucocorticoids, or injuries, such as foreign bodies or microchip implants have been associated with the development of FISS in some case reports (Hendrick et al., 1994; Lester et al., 1996; Burton and Mason 1997; Coyne et al., 1997; De Man and Ducatelle 2007; Carminato et al., 2011). One study compared associations between vaccine types and other injectable drugs with the development of FISS in a case-control study of 181 cats with soft tissue sarcomas (cases), 96 cats with tumours at non-vaccine regions and 159 cats with basal cell tumours. There was an association between the administration of various types of vaccines (recombinant versus inactivated rabies vaccines) and other injectable products (e.g. long-acting corticosteroids) and FISS development. Of 192 sarcomas, 101 had vaccinations at the site of tumour development during the preceding three years, and 23 had received other injections (Srivastav et al., 2012). General recommendations for prevention of FISS are summarised in the ABCD guideline on feline injection site sarcoma (Hartmann et al., 2015; Hartmann et al., 2019) . With respect to FISS development, FeLV vaccines should be used that cause the least subcutaneous inflammatory reaction, thus, vaccines

without adjuvants should be used rather than adjuvant-containing vaccines, if these are equally effective. Finally, cats should be vaccinated no more than necessary (Day et al., 2007). Therefore, an assessment of the infection risk is recommended and appropriate and, if applicable, long vaccination intervals should be applied in adult animals. Along these lines, no FeLV vaccinations should be administered to indoor-only cats or already immune cats (Hartmann et al., 2015), and FeLV p27 antigen pre-vaccination testing is recommended.

**Vaccination Protocol**

FeLV vaccines are considered non-core vaccines, although the 2020 AAHA/AAFP feline vaccination guideline recommends FeLV vaccines as "core vaccines" for cats younger than one year old (Stone et al., 2020). The latter may be valid in some European countries or areas with a high FeLV prevalence Fig. 5, but it still does not make it a core vaccine (which is for every cat at every age). Generally, FeLV vaccines are recommended for all cats with a potential FeLV exposure risk, such as cats permitted to roam outdoors, or cats brought to or living in multi-cat environments with other cats of uncertain FeLV status. Protection against a potentially life-threatening infection is justified in these cats, and the benefit for considerably outweighs any risk of adverse effects. Cats kept strictly indoors might also be at risk, if kept in multi-cat households in which some cats have outdoor access. It has been shown that FeLV vaccines prolonged life expectancy of vaccinated FeLV-exposed cats (Hofmann-Lehmann et al., 2007), and in areas with high FeLV prevalence, vaccination of all kittens and juvenile cats up to one year of age might be indicated since the environment into which a kitten will subsequently go can rarely be predicted with certainty, and the cat might subsequently be at risk of FeLV exposure (Richards et al., 2006; Scherk et al., 2013; Stone et al., 2020). Additionally, kittens also have a particularly high risk of developing progressive infection after FeLV exposure. In some European countries FeLV has been almost eliminated (Studer et al., 2019), but there can be local variations in the prevalence within countries.

Recommendations for most vaccines are for two subcutaneous doses three to four weeks apart for initial protection followed by a vaccination one year later. FeLV vaccines should be given in the left pelvic limb as distally as possible to enable appropriate surgery with complete cure if FISS should occur. Results of several studies indicate that FeLV vaccine-induced immunity persists for at least twelve months following vaccination, but likely much longer. Some vaccines are licensed for yearly boosters, some for up to three-year boosters. Recommendations by different expert panels differ concerning the booster intervals of adult cats. In adult cats that have received appropriate initial two-dose immunization and a booster after one year, the need for subsequent vaccination and the booster interval is best determined by analysis of the risk factors that the individual is exposed to (Scherk et al., 2013; Stone et al., 2020). Some recommendations, e.g. in the USA, indicate that at-risk adult cats should continue to be FeLV vaccinated annually but cats at low risk of FeLV exposure can be vaccinated at a two year interval (Scherk et al., 2013; Stone et al., 2020). However, ABCD recommends that for adult cats older than three to four years of age, a booster vaccination every two to three years is sufficient (Hosie et al., 2015; Hosie et al., 2020). In a study that addressed the duration of immunity in cats vaccinated with an adjuvanted inactivated whole virus vaccine, ten of twelve cats vaccinated two years prior to the FeLV exposure were protected from progressive infection, while all eleven controls were unprotected (preventable fraction 83%) (Jirjis et al., 2010). A second study with a inactivated whole virus vaccine exposing cats eight, 20, and 36 months after vaccination to FeLV was hampered by the age-related resistance of the cats to the FeLV challenge, and the control cats did not show the required level of progressive infection (in total ten out of 32 cats) (Wilson et al., 2012). Nonetheless, only one of 44 vaccinated cats became progressively infected.

Not all cats respond equally to FeLV vaccination, and immunosuppressed cats can fail to develop immunity (ABCD Guidelines on immunosuppressed cats (Hartmann et al., 2017)). A 5-year field study to control FeLV infection by vaccination in a colony of 30 domestic adult cats naturally exposed to infection suggested that the vaccination was effective in cats not infected with FIV, but failed to protect FIV-infected cats against FeLV (Bandecchi et al., 2006). In contrast, in an experimental study using a recombinant FeLV p45 subunit vaccine, FIV infected cats were successfully immunized and protected from FeLV challenge exposure in the early phase of infection comparable to not-FIV-infected cats (Lehmann et al., 1991). No benefit of FeLV vaccination was observed in healthy cats with progressive FeLV infection concerning virus load or life expectancy; thus, FeLV vaccination is not recommended in progressively FeLV-infected cats (Helfer-Hungerbuehler et al., 2015a). Vaccination of regressively FeLV-infected cats or abortively FeLV-infected cats is also not considered necessary, since the cats most likely have protective immune response against FeLV.

***Primary Vaccination Course***

It is recommended that kittens at risk should be vaccinated at the age of eight or nine weeks and twelve weeks, together with the core vaccinations (Brunner et al., 2006; Kanellos et al., 2008). The primary vaccination course includes an additional vaccine dose given one year later. Combination of different immunogens in one syringe (Brunner et al., 2006; Kanellos et al., 2008) is only legal when the company has registered it for that specific country; therefore, the local veterinary regulations should be consulted.
If a cat's FeLV status is unknown, it should be tested for progressive FeLV infection, preferably also for regressive FeLV infection using DNA PCR prior to vaccination in order to avoid overvaccination. Recently, a new POC test has become available in Europe that does not only test for 27 FeLV antigen, but also for anti-p15E antibodies, and this test is supposed

to detect not only progressively infected cats (antigen-positive), but also regressive and abortive infection (antibody-positive) and thus, could be an excellent option for pre-vaccination testing. However, the value of this test in the field has yet to be evaluated. If FeLV infection prior to vaccination is very unlikely, testing might not be necessary (e.g., kittens from a FeLV antigen-negative queen and tomcat, which had no contact to other cats).

***Revaccinations***

Annual booster vaccinations after a full primary vaccination course is not necessary. In one study it was shown that an adjuvanted inactivated whole virus FeLV vaccine provided immunity for at least two years (Jirjis et al., 2010), and commercial vaccines are available on the market with a claim of a three-year duration of immunity. Combined with the lower susceptibility of adult cats to develop progressive FeLV infection, the ABCD recommends that, in cats older than three years, a booster immunisation every two to three years is sufficient.

## PREVENTION

The control of FeLV is facilitated by the limited survival outside the host and because it is readily inactivated by detergents, heating, drying, or disinfectants. Horizontal transmission among cats usually requires direct contact and can be prevented at home, in veterinary clinics, and in animal shelters by simple segregation and effective disinfection. Identifying and separating FeLV-infected cats is, together with vaccination, the mainstay of preventing further transmission.

## DISEASE CONTROL IN SPECIFIC SITUATIONS

Management of multi-cat households with FeLV-infected cats can be difficult (see also chapter on treatment). Control is especially important in shelters and breeding catteries. When FeLV was first described in the mid-1960s, the highest rate of infection was found in multi-cat households and breeding catteries. In contrast, free-roaming cats had lower rates of infection, and those housed in single-cat households were only rarely infected. Convenient and reliable testing became available in the mid-1970s. Very quickly, cat breeders implemented test and removal programs, which proved to be extremely effective in eliminating FeLV from catteries. The most dramatic example was a mandatory test and removal program in the Netherlands in 1974 (Weijer et al., 1986a). The FeLV prevalence in cats in The Netherlands decreased from 9% in 1974 to approximately 3% in 1985 during such a program. The result of the test and removal program implemented by a cat breeder's society was even better: the prevalence of FeLV in purebred catteries was 11% in 1974 and decreased to less than 2% within four years (Weijer et al., 1986a). Today, FeLV should be considered an abnormality in a well-run cattery. Many stray-cat shelters also implement testing in their conditioning protocols, thus further reducing the rate of FeLV infection (Levy 2000; Hosie et al., 2015).

**Shelters**

Shelters provide special situations with cats severely stressed, exhausted, and unwell, as well as malnourished and parasitized and an extremely high pressure of infectious agents (Squires 2018). There are marked geographical differences in the prevalence of FeLV in rescued cats in Europe (Studer et al., 2019), which can influence policies of testing and vaccination. In some countries, like the northern European countries, the prevalence is low, whilst in others it is noticeably higher, with regional differences within these countries. Wherever possible, in terms of the risk of FeLV infection, cats entering a shelter should ideally be kept in quarantine for at least six weeks, if not (re)homed sooner. All incoming cats (at least in situations with a risk for FeLV and in shelters that allow contact between cats after the quarantine period) should be screened for FeLV antigen, ideally also for FeLV provirus. Antigen-negative but provirus-positive results suggest that the cat is regressively infected and although currently not shedding could reactivate the infection. If only an FeLV antigen test is performed, cats testing negative should ideally be retested six weeks later and kept in quarantine for this time period (Mostl et al., 2013; Mostl et al., 2017), as it can take up to six weeks after infection until the test turns positive. After quarantine, FeLV antigen-negative cats can be introduced into small groups of healthy cats.
Progressively FeLV-infected cats must be kept separate, ideally housed individually, but can be housed together with other progressively infected cats. Progressively infected healthy cats should be adopted out to adequate homes as soon as possible. It must be ensured that such cats cannot pose any risk of infection to other cats. This requires progressively infected cats to be re-homed to households where they will live in isolation indoors or only with other FeLV-infected cats. Ideally, also regressively infected cats (antigen-negative, but provirus-positive) should be placed in a separate place, not together with FeLV-naïve cats, because regressive infection can be reactivated under stressful situations.
The ABCD does not recommend euthanasia of healthy progressively FeLV-infected cats. However, if no adequate home can be found and if separation from the rest of the population is impossible, or if the cat is severely sick, euthanasia should be considered.

A retrospective study in an animal shelter in USA assessed outcomes of cats referred to a specialized adoption program for FeLV-infected cats (Lockhart et al., 2020). Screening for FeLV had occurred at a primary shelter or veterinarian. Upon admission, each cat was re-screened and subsequently deemed infected or uninfected. In total, 801 cats suspected to be infected with FeLV were referred; of these, 149 (18.6%) were ultimately deemed uninfected, and infection was confirmed

in 652 (81.4%) cats. Adoption was the most common outcome for FeLV-infected cats (n = 514 cats; 78.8%), followed by euthanasia or death in care (n = 109; 16.7%). This study demonstrates high demand for lifesaving options for cats diagnosed with FeLV. Progressive FeLV infections could not be confirmed in approximately one in five cats referred to the FeLV adoption program, a reminder of the risk behind basing the fate of a cat on a single positive test result. The majority of cats referred to the FeLV program were adopted, demonstrating that programs centred on adopter education and post-adoption support can create lifesaving outcomes for most FeLV-infected cats, despite uncertainty regarding their long-term prognosis (Lockhart et al., 2020). Survival of progressively infected shelter cats was demonstrated to be associated with viral loads on intake into the shelter: cats with high FeLV p27 antigen and provirus loads had a median survival time of 1.37 years; while the majority of the cats with low viral loads were still alive after 4 years (Beall et al., 2021).

The American Association of Feline Practitioner (AAFP) follows a slightly different approach (Little et al., 2020) concerning management and testing in shelters, especially in areas with high numbers of free-roaming cats, and this situation might also apply to some European countries. In USA, in many shelters limited shelter resources do not permit routine testing of all cats prior to adoption. In such cases if cats are housed individually, shelters might prioritize testing higher-risk cats such as sick cats, cats with bite wounds, and cats from high-risk situations, such as pet hoarding homes. However, if cats are not tested for FeLV infection, a recommendation for post-adoption testing should be clearly explained to the adopter and documented in the cat's file (Little et al., 2020). In such case, all cats entering shelters should be considered potentially infected, regardless of the environment from which they originated, and group-housing of untested cats should be strictly avoided. In facilities in which cats are group-housed, FeLV testing is essential before cats enter the group (Little et al., 2020). Long-term group housing increases the chance of exposure to infected cats inadvertently admitted with negative intake screening tests due to recent infection or regressive infection, and more intensive and common testing such as once per year is required in group-housed cats. Detailed recommendations to shelter management are provided in the ABCD guidelines "Prevention of infectious diseases in cat shelters" (Mostl et al., 2013; Mostl et al., 2017).

**Breeding Catteries**

The prevalence of FeLV infection is now very low in pedigree breeding catteries in most European countries, largely as a result of routine testing and the removal of infected cats a program that began in the 1970s. However, ongoing vigilance is required to prevent introduction of FeLV into a cattery. Certain circumstances in catteries facilitate transmission of infectious diseases, including FeLV infections, such as group living, mingling of kittens with older cats, close contact of cats during mating, the introduction of new cats, and the practice of sending queens to other catteries for breeding (Little et al., 2020).

The FeLV status of all cats in the cattery should be known. Only healthy cats, that are negative for FeLV antigen and provirus should be used for breeding. When testing is performed in the cattery for the first time, all cats should be tested for FeLV antigen. Cats with negative results should be retested no sooner than six weeks later to detect previously negative results from recent infection. Infected cats should be removed from the cattery. All newly acquired kittens and cats should be placed in isolation and tested for FeLV upon arrival. Ideally, they should remain isolated until a second negative antigen test is obtained six weeks later, particularly if they originate from a cattery with unknown FeLV status. Queens sent to another facility for breeding should only be exposed to other cats that have tested negative for FeLV antigen. If FeLV-free status of the other facility is not ensured, the queen should be kept in isolation and be retested in six weeks (Little et al., 2020).

In strictly indoor catteries that follow testing guidelines and maintain a FeLV-negative status, vaccination against FeLV is not necessary if cats have no contact to cats with unknown FeLV status. If any cats of the cattery are allowed access outside, with the opportunity of contact with neighbouring cats of uncertain FeLV status (which is discouraged for pedigree breeding cats), they should be vaccinated. Some catteries do not maintain breeding tom cats and rely on breeding services from other catteries. In such circumstances, vaccination of queens against FeLV can be considered in addition to testing after the queen hast left the cattery for breeding.

Cat shows are not significant sources of FeLV infection, because cats on exhibition are usually housed separately, and the virus has a low tenacity and is susceptible to commonly used disinfectants, and thus, environmental contamination of surfaces is not a risk. Therefore, cats go to cat show do not need to be vaccinated, retested or isolated unless exposure to another cat of unknown FeLV status has occurred (Little et al., 2020).

**Outdoor Cats**

FeLV is a problem particularly in outdoor cats. All cats allowed outdoors should be vaccinated against FeLV, at least in areas where FeLV is still prevalent.

**Blood and Tissue Donors**

FeLV can be transmitted haematogenously; therefore, all feline blood donors should be screened and confirmed to be free of infection before donating blood (Levy et al., 2001; Levy et al., 2008a; Pennisi et al., 2015; Pennisi et al., 2020). Presence of FeLV antigen was still relatively common in healthy, client-owned, indoor cats eligible to become blood donors in Spain and Portugal between 2015 and 2020: 2.9% of the 5105 cats (148) tested positive for FeLV antigen (Mesa-Sanchez et al.,

2021). However, pre-blood transfusion screening should include not only tests to detect FeLV antigen, but also PCR to detect FeLV provirus because it was shown that regressively infected cats (that are antigen-negative, provirus-positive) also can transmit the virus, and blood product recipients can become regressively or even progressively FeLV-infected and develop FeLV-associated disease (Nesina et al., 2015). FeLV was also detected in the corneal tissues of cats by PCR and immunohistochemistry (Herring et al., 2001); therefore, screening potential corneal donors or other tissue donors (such as kidneys for transplantation) for FeLV infection is also generally warranted.

## ZOONOTIC RISK

Although FeLV can replicate in human cell lines (Nakata et al., 2003), no conclusive evidence of natural infection with FeLV has ever been detected in humans. Soon after the first detection of FeLV, concern arose about the possible danger of FeLV to humans due to several facts. First, some FeLV strains grow in human cell cultures (Morgan et al., 1993). Second, lymphoma had been experimentally induced by injection of large doses of virus into neonates of non-feline species, such as in dogs and marmosets (Rickard et al., 1973). Third, one epidemiologic study linked prior contact of humans with sick cats to subsequent development of childhood leukaemia (Bross and Gibson 1970). Fourth, the contact between FeLV-infected cats and children with leukaemia was double that of contact between healthy children and healthy cats (Bross and Gibson 1970; Penrose 1970). Fifth, veterinarians were shown to have a higher death rate from leukaemia than a control population of physicians and dentists (Fasal et al., 1966; Blair and Hayes 1980; Gutenson N 1980; Cotter 1990; Clark et al., 1991), which however could also be explained by their higher exposure rate to radiation caused by fixation of animals during diagnostic imaging procedures.

Epidemiologic studies searching for FeLV or antibodies to any of its components in humans have been inconclusive. Cell-bound antibodies believed to be directed toward FeLV reverse transcriptase were found on malignant cells of humans with chronic myelocytic leukaemia in blast cell crisis. Some old studies detected antibodies against FeLV in humans with leukaemia and owners of progressively FeLV-infected cats (Fink et al., 1971; Caldwell et al., 1975; Olsen et al., 1975; Jacquemin et al., 1978). On the other hand, studies using more specific radioimmunoassays obtained negative results. No human being has ever been found to be viraemic with FeLV. PCR was used without success to find FeLV sequences in blood and bone marrow of young and adult humans with leukaemia (Nowotny et al., 1995). No case of human leukaemia has ever been traced to FeLV infection.

One explanation for the discrepancy between culture of the virus in human cells and the absence of proof of human infection might be related to the lytic action of human complement on the virus. The lytic activity is related to the recognition of carbohydrates in the virus envelope of virus particles produced by cells of mammals other than primates, including humans (Takeuchi et al., 1996). Humans have pre-existing antibodies against these carbohydrates; the virus envelope is decorated by antibodies, which leads to neutralisation of the virus by complement activity. In addition, one study found that human blood cells are uniquely resistant to infection with FeLV-B due to the activity of cellular enzymes that mutate the viral genome, although the biological properties of FeLV-B implicate that this strain would be the most likely candidate for zoonotic spread. A second block was identified that appears to suppress viral gene expression after the viral genome has integrated into the host cell genome. As cells derived from other normal human cell types are fully supportive of FeLV replication, innate resistance of blood cells could be critical in protecting against cross-species infection (Terry et al., 2017).

In conclusion, there is no evidence that FeLV is transmitted from cats to humans, and there are not enough data to truly demonstrate that FeLV is not a risk to humans. Still, if immunosuppressed people live in close contact with FeLV-infected cats, there is a certain risk, not from FeLV itself but from secondary zoonotic infections, e.g. *Mycobacterial* spp. or several fungal agents, that an immunosuppressed cat might acquire and potentially transmit to an immunosuppressed human.

## ABBREVIATIONS

| | |
|---|---|
| AML | Acute myelocytic leukaemia |
| BCS | Body condition score |
| CBC | Complete blood count |
| CTL | Cytotoxic T-lymphocytes |
| ELISA | Enzyme-linked immunosorbent assay |
| enFeLV | Endogenous feline leukaemia virus |
| Env | Envelope (of FeLV) |
| ERV | Endogenous retroviruses |
| ERV-DC | Endogenous retroviruses of domestic cat |
| FcaGHV1 | Felis catus gamma-herpesvirus 1 |
| FCoV | Feline coronavirus |
| FCV | Feline calicivirus |
| FeLIX | FeLV infection "x-cessory" factor, truncated envelope protein encoded by enFeLV |
| FeLV | Feline leukaemia virus |
| FeSV | Feline sarcomavirus |
| FFV | Feline foamy virus, aka feline spumavirus |
| FHV | Feline herpesvirus |
| FISS | Feline injection-site associated sarcoma |
| FIV | Feline immunodeficiency virus |
| FLVCR | FeLV subgroup C receptor-related protein 1, receptor of FeLV-C |
| FOCMA | Feline oncornavirus cell membrane antigen |
| FPV | Feline parvovirus |
| Gag | group-specific antigen |
| G-CSF | granulocyte colony-stimulating factor |
| gp70 | External surface unit (SU) of FeLV envelope, glycosylated protein of 70 kD |
| HCT | Haematocrit |
| HIV | Human immunodeficiency virus |
| ICGN | Immune-complex glomerulonephritis |
| IFA | Immunofluorescence assay |
| IMHA | Immune-mediated haemolytic anaemia |
| LOPH | lomustine, Oncovin (vincristine), prednisolone and hydroxydaunorubicin (doxorubicin), chemotherapy regimen |
| LTR | Long terminal repeat, non-coding part of FeLV genome |
| MCS | Muscle condition score |
| MCV | Mean corpuscular volume |
| MDS | Myelodysplastic syndrome |
| mRNA | Messenger RNA |
| p15E | Transmembrane protein (TM), part of the FeLV envelope, protein of 15kD |
| p27 | Capsid protein of 27 kD |
| PCR | Polymerase chain reaction |
| Pit1, Pit2 | Receptor of FeLV-B |
| POC | Point-of-care |
| Pol | Polymerase |
| PPP | purchasing power parity per capita |
| RD-114 | Endogenous retroviral element of primate origin (in cats genome) |
| rFeIFN-ω | Recombinant feline interferon-ω |
| rHuEPO | Recombinant human erythropoietin |
| rHuIFN-α | Recombinant human interferon-α |
| RT-PCR | Reverse transcription polymerase chain reaction |
| ssRNA | Single-stranded RNA |
| SU | Surface protein of FeLV, part of envelope protein, see also gp70 |

| | |
|---|---|
| THTR1 | Thiamine transporter 1, receptor of FeLV-A |
| TM | Transmembrane protein of FeLV, part of envelope protein, TM is also called p15E (see above) |
| U3, U5 | Unique regions in the long terminal repeat (LTR) of FeLV |
| URE | Upstream region of the enhancer of LTR |

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