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# Interaction of FIV with Heterologous Microbes in the Feline AIDS Model

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## 1. Introduction

Since the emergence of the acquired immune deficiency syndrome (AIDS), there has been a great deal of interest in identifying cofactors that accelerate progression of the disease elicited by human immunodeficiency virus types 1 or 2 (HIV-1, HIV-2). Beside inherent factors and environmental agents, speculations led to the conclusion that infectious diseases frequently occur in HIV infected persons, might augment HIV replication, and consequently facilitate AIDS progression. HIV infection is followed by a long disease-free period, during which a low number of CD4<sup>+</sup> immune cells contains transcriptionally silent provirus. Activation of CD4<sup>+</sup> cells by external factors, including heterologous viruses, terminates latency forcing towards a productive HIV infection. Transactivation of the HIV long terminal repeat (LTR) by cellular, nuclear transcriptional factors (e.g. NF- $\kappa$ B, Sp1) induced by mitogens, cytokines, chemokines, simultaneous virus infections is followed by gene expression including the synthesis of the HIV transactivator protein (TAT), which binds to the transactivating response (TAR) element of the genome, ultimately leading to large scale production of HIV and death of infected cells through apoptosis [1,2]. *In vitro* studies showed that products of immediate early (IE) or early (E) genes of several DNA viruses such as human herpesviruses (HHV) -1, 2, 3, 4, 5, 6A, 8, adenoviruses, as well as hepatitis B virus (HBV) X gene, human T lymphotropic virus type I (HTLV-I) *tax* gene upregulated production of these transcriptional factors and activate HIV-1 or HIV-2 in the same cells. Simultaneous infection in a single cell is relatively rare event *in vivo*; therefore such type of biological effects in nature could be minimal. On the contrary, cross-talk between immune cells carrying different viruses via cytokines, chemokines is more common. Heterologous viruses infect many types of cells, which are not targets of HIV, but release several immunomodulatory mediators, usually in

an abnormal pattern. High level of tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, IL-10, and low level of IL-2, IL-12 were frequently observed in the blood of AIDS patients, only to mention the most important ones. Abundant pro-inflammatory cytokines bind to HIV carrier cells and through consequently activated secondary messenger systems activate the same transcriptional factors for HIV activation. This category of interaction has a more significant impact on HIV infection. This phenomenon called as transcellular transactivation can last lifetime, its intensity may vary depending on the host, the other transiently or chronically coinfecting microbes, etc. [3]. These external confounding factors act in a pleiotropic manner, which is impossible to study *in vitro*. Animal studies are ideal to establish their role in AIDS progression [4].

## 2. The feline AIDS model

Feline AIDS (FAIDS) induced by feline immunodeficiency virus (FIV) is the only natural small animal model of human AIDS [4,5-8]. FIV shares many genetic, structural and biological characteristics with HIV. FIV also shows tropism for CD4+ immune cells, but its receptor is the CD134 molecule with the CXCR4 coreceptor. FIV LTR accommodates multiple enhancer or promoter protein-binding sites (e.g. NF- $\kappa$ B, AP-1). Although FIV lacks TAT and the TAR element, its Orf-2 (also designated as Orf-A) acts as a transactivator gene to some degree and is necessary for productive FIV replication [7,9]. The similarities in the clinical course of infection between HIV and FIV are striking [6,8,10]. Male gender and adult age are known risk factors for both HIV and FIV transmission [6,7,11,12]. Domestic cats infected with FIV develop progressive immune dysfunction characterised by depletion of CD4+ T cells, wasting, cachexia, gingivostomatitis, neuropathological disorders, opportunistic infections, unusual malignancies such as B cell lymphomas, fibrosarcomas [13]. CD4+CD25+hi-FoxP3+ immunosuppressive regulatory T (Treg) cells have been implicated as a possible cause of immune dysfunction during FIV and HIV-1 infection, as they are capable of modulating virus-specific and inflammatory immune responses. Influence of Treg cell suppression during FIV and HIV pathogenesis is most prominent after Treg cells are activated in the environment of established FIV infection [10]. It is important to remember that increased activity of Treg cells promotes immunosenescence in the normal elderly, and AIDS is regarded as an extremely rapid ageing process [14]. Disease progression occurs over a similar time scale to HIV-1 infection in humans [6]. FIV is distributed worldwide; it has several subtypes similarly to HIV [12,15]. Species-specific strains of FIV circulate in many members of *Felidae* family, including endangered big cats in which also induce AIDS-like illnesses [6,12]. Some opportunistic infections in cats also occur worldwide such as feline leukaemia virus (FeLV), feline herpesvirus type 1 (FHV-1), *Toxoplasma gondii* (TG), feline coronavirus (FCoV), *Bartonella henselae* (BH), canine distemper virus (CDV), fungal pathogens or mycoplasmas. Their incidence depends on geographical regions, cat subpopulations (pet, indoor, stray, feral, free-roaming, etc.), gender and age [11,12,16-28]. Incidence of other opportunistic microbes depends on endemic geographical areas and local vectors (e.g. haemoplasma or *Leishmania* species, different helminths) [16,18,29-33]. Some of these microbes establish coinfection in a

particular felid species that carries a specific strain of FIV. Typical examples have been reported recently. A highly virulent FeLV outbreak in FIV<sub>Pco</sub> infected free ranging Florida pumas (*Puma concolor coryi*) threatened this endangered species [12]. In another outbreak in the Serengeti, FIV<sub>Ple</sub> B infected lions (*Panthera leo*) were twice as likely to survive CDV infection compared to lions infected with FIV<sub>Ple</sub> A or FIV<sub>Ple</sub> C [34]. These cases clearly demonstrate that specificities of both microbes might express increased risk for severe synergistic pathogenicity. Recent surveys on coinfections mentioned above were carried out for descriptive epidemiological purposes. In the majority of studies, occurrence of heterologous microbes were judged as opportunistic infections, but some of the investigators have come to the conclusion that FIV and heterologous microbes might mutually aggravate immunosuppression at the level of the organism [12,17]. Both FIV and FeLV independently might predispose animals for toxoplasma infection [21]. In spite of similar clinical manifestations by FIV or FeLV, they might specifically predispose the host for different heterologous microbial pathogens: FIV predisposes to Leishmania [12,16], mycoplasma [25,27,33,35], fungal [26], FCoV [12] infections, while FeLV sensitises the organism to BH infection [36]. Others found that the rate of toxoplasma [16,30,32] or FCV [24] infections occur independently of FIV status. These result, even some of them are contradictory, clearly show that each retrovirus must have its specific way to interact with other microbes at molecular or immunological level. It is conceivable that HIV and human microbes have similar ways for interactions. Both direct transactivation of FIV in the simultaneously infected cells (Figure 1) or cross-talk between cells infected by FIV, and other cells infected by heterologous microbes (Figure 2) can take place in the body of cats.

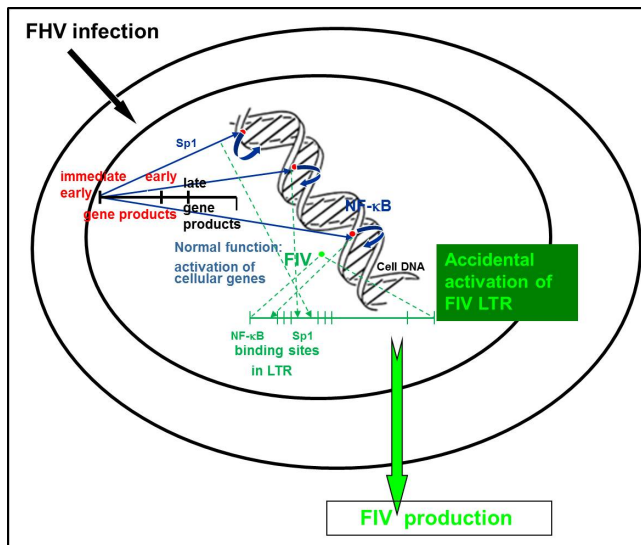
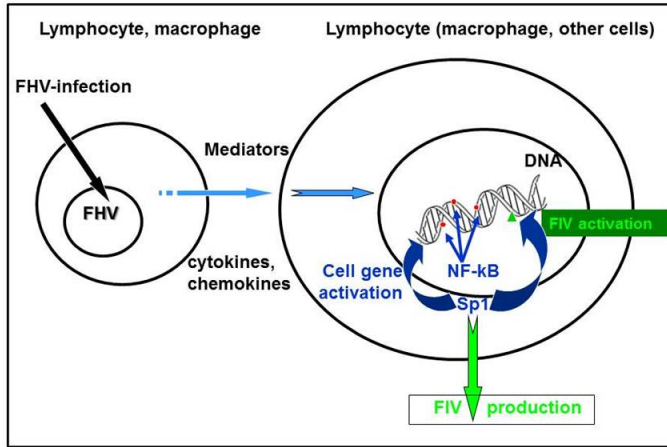


Figure 1. Scheme of intracellular virus transactivation



**Figure 2.** Scheme of transcellular transactivation

Comparison of human clinical observations on opportunistic infections in HIV infected patients and *in vitro* transactivation studies unambiguously demonstrate that several viruses can cause both opportunistic infection and transactivate HIV. The domestic cat is afflicted with multiple microbes that also induce human diseases (e.g. TG, Leishmania, fungi, mycobacteria). Furthermore, domestic cats harbour several feline virus species that are homologous to human viruses: FIV, FeLV, FHV, FCV, FCoV, as well as feline sarcoma virus, feline parvovirus, feline morbillivirus [12]. These feline microbe species provide a panoply of infectious disease models for many devastating human diseases including for studies on the possible interaction between retroviruses and heterologous microbes among natural conditions of FAIDS at molecular, immunological, cellular level in any organ or the whole body of infected cats by any available methodology.

### 3. Simultaneous infections: Both opportunists and transactivators

#### 3.1. Feline herpesvirus

Simultaneous infection by retroviruses and heterogenous microbes and their association with a variety of diseases have come into focus recently. The very sensitive methods to detect nucleic acids of microbes have promoted our understanding that microbial interaction may result in the synergistic induction of more severe disease course. Retroviruses and herpesviruses might be associated with a variety of diseases in animals. Coinfection of chickens with Marek's disease virus (*Gammaparvovirinae*) and retrovirus (avian leukosis virus) increase the incidence of retrovirus associated tumours [37]. As several species of human her-

pesviruses have been implicated as cofactors for AIDS progression, possible interaction of feline herpesvirus 1 (FHV-1, subfamily *Betaherpesvirinae*, a homologue of human cytomegalovirus /CMV/), with FIV has been studied *in vivo* and *in vitro*. FHV-1 might spread in some captive felids with higher frequency than in their free-ranging counterparts demonstrating the importance of human intervention in natural epidemiology [11,29,38]. Single or simultaneous infection by FHV-1 and TG or BH have been implicated as causative agents of feline uveitis [20]. It is of note, that FHV-1 homologue human CMV induces very severe retinitis in HIV infected humans [12], and human CMV was found one of the clinically most frequent and strongest transactivator of HIV. On the contrary to human experience with HSV-1 and adenoviruses [39], FHV-1 could not be implicated in the multifactorial aetiology of gingivitis [24]. FHV-1 also infects T lymphocytes. FHV-1 is a significant pathogen limited to the family *Felidae*, causing an upper respiratory disease in cats. Interaction of acute FHV-1 infection in chronically, experimentally FIV infected specific pathogen-free (SPF) animals results in several immunological abnormalities. FIV infected cats produce less FHV-1 neutralising IgM antibodies during the first 3 weeks of infection than non-FIV infected animals, whereas the IgG antibody response remains unaffected. The ongoing IgG antibody response to FIV is not affected by FHV-1 infection. Lymphocyte blastogenic response to concanavalin A (Con-A) is depressed in FIV-infected and non-infected cats, but response to pokeweed mitogen (PWM) takes longer to return to normal in FIV infected animals. Lymphocytes from FIV infected cats have a greater and more sustained proliferative response to FHV-1 antigen than non-infected cats [40]. Cats with pre-existing FIV infection have more severe signs of disease after exposure to FHV-1 or TG, than FIV-free counterparts. Primary immune responses to all heterologous pathogens are delayed or diminished in FIV infected compared with non-FIV-infected animals. Repeated infections have no significant effect on the level of FIV-specific antibodies or on the production of peripheral blood mononuclear cells (PBMC) containing FIV proviral DNA. FIV infected cats exposed to cofactors have normal levels of IL-2R and major histocompatibility complex (MHC)-II antigen expression on PBMCs, while only-FIV-infected animals have upregulated IL-2R and down-regulated MHC-II expression [41]. By transfection of a recombinant plasmid containing the FIV LTR linked to the chloramphenicol acetyltransferase (CAT) gene, followed by infection of FHV-1 into Crandell feline kidney (CrFK) or *Felis catus* whole fetus 4 (fcwf-4) cells enhancement of CAT activity was demonstrated. Both immunofluorescence and electron microscopy showed productive coinfection of individual T lymphocytes [42]. A series of FIV LTR deletion mutants were constructed and cotransfected with FHV-1 to identify the regions that are responsible for transactivation. It was demonstrated that sequences between -124 and -79, and between -21 and -32 (relative to the cap site) are essential of FIV replication in fcwf-4 cells and that the sequence between -63 and -23 responds to transactivation of FIV LTR by FHV-1 [43]. FHV-1 infected-cell-protein 4 (ICP4) was found to down-regulate FIV LTR directed gene expression via the C/EBP site in the LTR, but after introduction of a site-specific mutation of this site, ICP4 significantly stimulated LTR. These results indicate that FHV-1 ICP4 possesses both abilities to transactivate or down-regulate FIV-directed gene expression. C/EBP and AP-1 regulatory sequences are known to act as both positive and negative regulators [44]. Interestingly enough, plasmids expressing ICP4 homologues of Alphaherpesviruses, namely pseudora-

bies virus (PRV) and equine herpesvirus type 1 (EHV-1) could significantly inhibit FIV LTR-directed gene expression in CrFK and fcwf-4 cells. Moreover, the ICP4 homologues also exhibited a marked suppressive effect on FIV replication in CrFK cells cotransfected with an infectious clone of FIV [45]. These experiments draw attention to the role of other herpesviruses, among them Roseoloviruses [46], which might exist in cats. Similarities and differences between the human or feline AIDS could help elucidating their devastating role in human and feline patients.

### 3.2. Feline adenovirus

Among other DNA viruses, adenovirus (AdV) also might enhance AIDS progression in cats. So far, the only feline adenovirus (FeAdV) isolate (also the first one from the family *Felidae*) was obtained from a fecal sample of a cat with AdV PCR positivity [47] and unknown FIV serostatus [48]. Its hexon [49], fiber [50], and nucleotide sequences published so far suggest that it might be related to human AdV type 1 (HAdV-1). Similar nucleotide sequences have been shown in the feces of a small child suffering with gastroenteritis and her cat in Japan [51], and in one nasopharyngeal aspirate sample of a 468 member cohort of children presenting acute respiratory disease in Brazil [52]. This virus might be widespread all over the world and one can speculate that it could transactivate both HIV and FIV, e.g. in pet owners. Seroepidemiological studies show that approx. 10-20% of free roaming cats are seropositive in Hungary and Europe [53,54]. In a series of consecutive transfer of FIV by intravenous inoculation of experimental SPF casts, four were AdV seropositive, but their serostatus before FIV inoculation remained unknown. One can speculate that accidental transfer of AdV along with FIV occurred [55]. FeAdV would be ideal to compare its unique clinical and molecular effects in the human and the cat both *in vivo* and *in vitro* similarly to studies on the interaction of HHV-6 and SIV in humans and pig-tailed macaques [56,57]. As it was isolated from cat feces, one could use FeAdV and FIV to model intestinal molecular interaction of HAdVs and HIV.

### 3.3. Feline leukaemia virus

Another common interaction in nature is between FIV infection and FeLV. FeLV is of particular significance because it can also induce immunodeficiency [58]. FeLV is a homologue of human T lymphotropic virus (HTLV). It displays a prevalence of 1-8% worldwide [16,24,33,38], but in some geographical areas much higher prevalence of FeLV antigen has been detected (14.2% in Iran, [21], 24.5% in Thailand, [18], 26% in Portugal, [28]). In catteries its prevalence could be much lower or up to zero [17,26,30,32]. Its prevalence has been decreasing in most countries and is regarded as a less important deadly infectious agent in the last 20 years compared to FIV [19]. In confined geographical regions or among captive animals in different facilities, simultaneous decline in the seroprevalence of both FeLV and FIV has also been observed [29,33]. Seroprevalence is higher in intact males than females, and increases with age [17,18]. Transmission is usually by direct contact or saliva wounds. FeLV infection among non-domestic cats of the *Felidae* family is rare, but FeLV has been shown to cross species barriers, especially in case of prior or simultaneous microbial immunosuppres-

sion [12,28]. There are four naturally occurring exogenous FeLV strains: FeLV-A, -B, -C, and -T. FeLV-A is the predominant subgroup circulating in feral cats. Its integrated proviral sequences are transmitted vertically through the germ line. Recombination might occur between FeLV-A and other subgroups. The outcome after exposure depends on several host and environmental factors, but the clinical course is more aggressive than seen with FIV. In approximately one third of exposed cats, viraemia is persistent and eventually results in clinical disorders such as immunosuppression, bone marrow suppression (mainly anaemia), and neoplasm (mainly lymphoma) [12,19]. Like FIV, FeLV infection alone also renders the animals more susceptible to infection, persistence and disease elicited by heterologous microbes [19,28,33] such as TG [21] or BH [36]. Mortality among persistently FeLV infected cats is high as 83% die within 3.5 years [references in 12]. About 10 to 15% of the cats that are clinically ill with FIV infection are coinfecting with FeLV worldwide [59,60]. Upon superinfection with FIV, asymptomatic cats with persistent FeLV infection manifest a more accelerated and exacerbated FIV disease, anticipated death and show a higher FIV load in lymphoid tissues than did naive cats under both natural and experimental conditions, while the blood level of FeLV p27 antigen was not elevated. Interestingly, the synergy between FIV and FeLV is bidirectional: doubly infected cats develop FeLV induced tumours more frequently than did cats infected with either virus alone [61]. Dually infected cats remain more leukopenic than cats infected with FIV or FeLV alone, and their CD4+/CD8+ T lymphocyte ratios become rapidly inverted [62]. This interrelationship is similar to what has been described for HIV and HTLV-I [63].

#### 3.4. Simultaneous *Listeria* and FIV infection

Scarce data are available on the possible effect on FIV and FAIDS exerted by feline bacterial infections. *Listeria monocytogenes* (LM) can infect both humans and cats, and its profound immunomodulatory capabilities are well known. Beside HIV, both FIV and LM impair the innate immune response that fails to gain control of their replication prior to the adaptive immune response. These effects might be additive or synergistic in simultaneously infected individuals. In a series of recent experiments, chronically FIV infected and SPF control cats were challenged with LM, then their whole blood and lymph nodes were collected 3 days after challenge. The number and functions of natural killer (NK) cells (CD3-CD56+), NKT cells (CD3+CD56+), CD4 (CD3+CD4+), CD8 (CD3+CD8+), regulatory T cells (CD4+CD25+Fox3+) and Langerhans cells (CD1a) were evaluated. NK, NKT, CD4+ and CD8+ T cells in the LM challenged lymph node of FIV-infected cats did not increase in number, NK cells did not increase their proliferation, apoptosis was elevated, and perforin expression was not upregulated when compared to SPF control animals. No difference in Treg cell number was found. Delayed control and clearance of opportunistic LM was also observed. In the blood compartment of LM challenged cats, CD4+ T cell proliferation did not differ in SPF-control and FIV positive cats, while CD8+ T cell proliferation increased in FIV-positive animals. The number of Langerhans cells did not decrease in FIV and LM infected animals, but their ability to produce NK-activating IL-15 and other mediators became impaired. Although it is concluded that FIV-infected cats are more vulnerable to LM superinfection [2], it is conceivable that precedent or ongoing *Listeria* infection diminishes the

activity of NK cells, helps promote FIV replication and dissemination in the body of animals. In HIV-infected patients, dysfunction of NK cells with low perforin expression, anergy, furthermore, abnormal antigen presentation and low level of IL-15, IL-12 levels are also known [2]. In humans, infection by LM or other bacteria with immunosuppressive potential might augment impaired immunosurveillance, consequently increase HIV load, and ultimately facilitate AIDS progression.

### 3.5. Feline haemotropic mycoplasmas (haemoplasmas)

Epidemiological surveys on simultaneous infections by FIV and feline haemophilic mycoplasmas suggest that opportunistic infections also promote FIV progression. Feline haemophilic mycoplasmas (*Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutus*) collectively called haemoplasmas can cause anaemia and severe clinical disease of affected cats. *Candidatus Mycoplasma haemominutus* (CMH) is usually not associated with clinical disease, but typically causes anaemia in cats carrying pre-existing FIV or FeLV infection [29,64,65]. Chronic FIV infection appears to modify the acute phase response to feline haemotropic mycoplasmas, which varies with the infecting haemoplasma species, namely CMH and *M. haemofelis* (MH), respectively [64]. A recent study in a Canadian feral cat population found that CMH positive cats were significantly more likely to be concurrently retrovirus positive than were CMH negative animals [33]. Another recent study in Spain found that, FIV seropositivity and male sex were significantly associated with MH, CMH and *Ca. M. turicensis* infection [27]. It is of note, that mycoplasmas in HIV infected patients have been regarded as AIDS promoting factors for a long time, and this phenomenon was proved *in vitro*, either [66]. A meta-analysis of former studies unambiguously demonstrated that genital infection by *M. genitalium* poses high risk for acquisition of HIV. Testing and treatment of *M. genitalium*-positive individuals in high risk groups is recommended as a potential HIV prevention strategy [67]. Bacterial vaginosis-associated microflora in the female genital tract also predisposes for HIV infection. Among flora members, *M. hominis* was shown to activate NF- $\kappa$ B and AP-1. Through mediation of a soluble HIV inducing factor (HIF), these transcriptional factors may subsequently increase genital tract viral load and potentially contribute to HIV transmission [68]. MH infection along with BH infection has been verified by PCR in an HIV infected person [69]. The 34 year old man owned several cats. Haemoplasma DNA is present in saliva and feces of cats, which suggests that aggressive interactions among cats and humans involving biting may lead to transmission of the organism. So far, haemotropic mycoplasma infections have been reported in a patient with systemic lupus erythematosus (SLE) and in an anaemic patient showing that some forms of immunosuppression are required for acquisition of an unusual pathogen [70]. Increasing number of human patients with compromised immune systems living near cats increases the possibility that haemoplasma infections may emerge in this population [69]. Haemoplasma positive cats like mycoplasma positive humans ought to be regarded at increased risk for retrovirus infection. Testing would also be appropriate prior to relocation of felids [29]. Feline mycoplasmas could provide a unique model to clarify the exact role of mycoplasmas in AIDS progression.



### 3.6. Feline mycobacteria

Mycobacteria are important opportunistic infections in HIV infected patients worldwide. Especially in Sub-Saharan Africa very aggressive forms of tuberculosis occur in the immunocompromised population induced by several mycobacterium species. Mycobacteria severely suppress activities of the cellular immune reactions but due to the difficulties in their cultivation and the chronic nature of the disease, it is extremely difficult to study their mutual effects on the immune system. Several mycobacterium species that infect the human and are prevalent in HIV infected patients (*e.g.* *M. bovis*, *M. avium*, *M. microti*), induce disease in cats [23]. This finding raises the idea that FAIDS would be an appropriate model to study intimate relationship between mycobacteria and feline retroviruses *in vivo*.

Repeated exposure of FIV positive cats to bacterial lipopolysaccharide endotoxin (LPS) resulted in lower plasma and brain viremia. In HIV infected humans and FIV infected cats, macrophages produce elevated amount of specific chemokine CXCL10, which in turn damages brain cells. LPS treatment of infected macrophages release IL-10 counteracting CXCL10 expression of brain cells. Suppressed CXCL10 level in the brain of FIV-positive cats and ensuing T cell infiltration is concomitant with reduction in neurovirulence [71]. This observation is in correlation with earlier results, namely that LPS treatment of HHV-6A infected lymphoid cells decrease expression of such soluble mediators that transactivate HIV-1 in other lymphoid cultures [3].

### 3.7. Fungal and parasitic infections in FIV infected cats

Humans and cats can be infected by several common fungal pathogens, such as *Cryptococcus* [22], *Malassezia*, *Microsporum* [26] and others [28]. Dermatophyte fungi were isolated in 29.4% from stray cats in Portugal [28]. *Microsporum gypseum* and, occasionally, *M. canis* were found in an equal ratio of FIV negative and positive cats (8 and 8.5%, respectively). In contrast, *Malassezia* species were more frequently isolated from FIV infected cats than FIV-uninfected cats (84% vs. 28.6%) thus showing a closer biological relationship in the effect of FIV and certain fungal species. The CD4:CD8 ratio for FIV infected cats with cutaneous overall fungal infection was significantly lower than the CD4:CD8 ratio in the FIV infected cats but without cutaneous fungal infection. It is obvious that worsening cellular immunity represents a risk factor to cutaneous fungal colonisation in cats [26], but existence of a cutaneous or systematic fungal infection due to insufficient activity of the cellular immunity acquired by inherent or environmental effects might also make easier to acquire FIV infection. Damaged local immunity in the skin in case of wound FIV infection could be one of the most frequent examples. Cats having human or human homologues of fungal species would be ideal models to study such aspects.

Cats having access to the outdoor world, very easily acquire parasitic infections. Feral cats can serve as a direct or indirect source of infectious diseases for pet cats. Contact with animals of other species, conditions of breeding, food supply and age are considered significant for infections. A recent study conducted in a Brazilian cat colony free from FIV, FeLV and *Neospora caninum* infection, clearly showed these differences. Of cats from urban and rural areas, 10.4% and 27.2% were seropositive for TG, respectively. Cats having access to streets

(17.1%), cats cohabiting with rats (19.6%), and cats feeding on homemade food and raw milk (27.2%) were positive for this protozoon. In addition, 4.2% of cats were positive for *Leishmania* spp. [30]. In several countries, parasitic infections, especially intestinal parasites, of cats are more frequent than infections with FIV or FeLV. In Italy, comparison of FIV, FeLV and TG IgG seropositivity resulted in the same ratio: 6.6%, 3.8% and 30.5%, respectively [11]. Epidemiological studies in Portugal found 24.2% TG seropositivity, 31% carrier state of intestinal parasites (*Toxocara cati*, *Isospora felis*, *Ankylostoma stenocephala*, *Toxascaris leonina*) as compared to FIV and FeLV infection (10.2% and 7.1%, respectively) in the same stray cat population [28]. In a confined region of Canada, 29.8% of cats had TG antibodies, 1.3% excreted oocysts in their feces, while FIV and FeLV seropositivity was significantly lower (5.2% and 3.1%, respectively) [33]. In Thailand, although the ratio between retroviral and parasitic infections were different (FIV, FeLV, heartworm (*Dirofilaria immitis*) and TG IgG (20.1%, 24.5%, 4.6% and 10.1%, respectively) from the other data mentioned above, of the 348 cats sampled for all four pathogens, 3.1%, 2.8% and 0.28% were positive for TG antibodies and FIV antibodies, FeLV antigen or *D. immitis* antigen, respectively. Of the 35 TG seropositive cats, 42.9% were coinfecting with at least one of the other three pathogens [18]. Feral cats from Cairo, Egypt exhibited an extremely high ratio of TG infestation as compared to FIV, FeLV or heartworm seroprevalence: 95.5%, 33.9%, 4.6% and 3.4%, respectively. It is of interest that 57.4% of TG positive cats had very high antibody titre (>1:640) [31]. Oocyst shedding was found to be accompanied by high level antibodies: cats shedding TG had high antibody level (>1:256). In the same cat cohort, *Toxocara cati* eggs were identified in 37%, *Cystoisospora felis* oocysts in 14%, *Taenia* sp. segments in 15% of animals. Most of the fecal samples showed evidence of at least one intestinal parasite, while many samples contained evidence of multiple intestinal parasites. High prevalence of antibodies reflects latent infections. However, it must be emphasized that cats with latent infection can pose a considerable zoonotic risk with respect to the shedding of intestinal parasites in their feces. Stress conditions induce shedding. Intercurrent retroviral infections (FIV or FeLV or both) with consequent declination in systemic and mucosal immunity are regarded as important stress factors [33]. Result of the multivariate logistic regression analysis of another recent survey on simultaneous virus and parasite seropositivity (FIV, FeLV and TG was 19.2%, 14.2% and 32.1%, respectively) in Iran showed that retroviral-associated immunosuppression is a risk factor for activation of toxoplasmosis in cats [21]. Beside toxoplasmosis, Leishmania infection is frequent in many countries. Cellular immunity of normal cats effectively controls *Leishmania infantum* (syn. *L. chagasi*). However, feline immunosuppressive diseases such as FIV and FeLV infection impair the normal response to infection and expose cats to reactivation or new infections by other pathogens, among them Leishmania. Although no statistical evidence of association between TG and Leishmania was found, but in cats coinfecting with FIV, a strong statistical association for the triple coinfection was found [16]. One can conclude from these epidemiological surveys, that parasites cause not only opportunistic infections in retrovirus infected cats, but activation of their latent infection by retroviruses suggests existence of a closer relationship between phylogenetically distant two groups of foreign agents at molecular or immunological level.

Following infection of FIV carrier cats with a secondary pathogen, cytokine dysregulation is more pronounced. TG increases both IFN- $\gamma$  and IL-10, but fails to increase IL-2, IL-12, while TG infected FIV negative cats show an increase in IFN- $\gamma$ , IL-2 and IL-12 [72]. A similar dysregulation has been reported in cats challenged with LM [73]. The increase in IL-10 to IL-12 ratio predicts the loss of cellular immunity in FIV infected cats [72,74]. These *in vivo* studies are in good correlation with *in vitro* experiments. Latent infection in HIV or FIV infected lymphocytes and macrophages can be reactivated and virus production can be increased by chemical immune activators such as phorbol myristate acetate [75], concanavalin A [5], granulocyte macrophage-colony stimulating factor [76]. Both HIV and FIV induce apoptosis not only in the infected cells, but in the vicinity of infected cells as well. Programmed cell death of infected cells is mediated mainly by TNF- $\alpha$  released from infected cells [77]. Cytokine dysregulation affects not only the FIV carrier animal, but damages the fetus by causing a pro-inflammatory placental microenvironment at early pregnancy: increased expression of IL-6, IL-12, decreased expression of IL-1 $\beta$ , SDF-1 $\alpha$ . Similarly to AIDS patients, IL-6 expression correlated with FIV load [78]. The exact role of cytokines and chemokines in the process of simultaneous infections waits for clarification.

#### **4. Diagnosis, treatment and prevention of simultaneous infections**

Preventing exposure of healthy cats to FIV or FeLV infected cats by tests and removal or isolation is an important measure, and is not alternative to vaccination. The most common method for diagnosis of FIV infection is screening for antibodies (typically against p24 and p15) using an ELISA. Several commercial kits are available worldwide. Confirmatory testing for cats with positive results is strongly recommended, Western-blot and immunofluorescent antibody assays (IFA) are used. Infection from queens can be transmitted to kittens, testing for newly acquired cats and kittens is strongly recommended. Vaccinated cats also produce antibodies that cannot be distinguished, by any commercially available antibody test [79], from antibodies due to natural infection, and queens might transmit these antibodies to the litter via colostrum. FIV vaccine induces fewer antibodies for non-structural proteins compared to natural infection. There are tests to discriminate this pattern, but these are unsuitable for routine use. Polymerase chain reaction (PCR) has promoted by some commercial laboratories as a method to determine a cat's true infection status. The test detects FIV RNA or proviral DNA. A real-time PCR assay for FIV quantification of proviral DNA in PBMC has high sensitivity and specificity. This and reverse transcriptase (RT)-PCR are methods to quantitate viral load and dissemination in the body after activation of latent FIV infection. Due to genetically heterologous nature of FIV, tests with concurrent determination of subtype differentiation are recommended. Serological diagnosis of FeLV relies on detection of the core antigen p27 in PBMC using an ELISA. IFA test is also used but discordant results might occur. PCR is offered by a number of commercial laboratories, it can be performed on blood, saliva, bone marrow and tissues. Kittens can be tested for FeLV at any age, as passively acquired maternal antibody does not interfere with testing for viral antigen.

Compliance of cat owners with FIV and FeLV testing is low, in spite of using combined test kits, and professional recommendations by veterinarians (Table 1).

<b>At risk of infection</b>
cats access to outdoors
known exposure to retrovirus infected cats
multicat environment
bite wounds
oral disease
<b>Sick cats</b>
<b>Cats entering new homes, shelters</b>
<b>Newly acquired cats and kittens about to be vaccinated for FIV or FeLV</b>
<b>Cats used for blood or tissue donation</b>

**Table 1.** Major recommendations for FIV and FeLV testing of cats

The owner must also consider the cost of immunisation, fecal parasite testing, de-worming, or blood screening to reveal and eliminate concomitant infections [17]. Further commercially available kits for serological screening, and/or antigen detection including FHV-1 and the most pathogens are widely available for domestic and large cats. These kits used separately depending on the clinical course of the animal can be combined in definitively. Well established, cheap but somewhat laborious and time consuming classical methods have been used for direct detection of common bacteria, fungi and parasites (cultivation, biochemistry, microscopy, etc.). For feline pathogens causing infection in humans, the same methods and kits can be used as in human medicine. With technological advances quantitative real-time or RT-PCR assays have been used subsequently their products are sequenced to determine species or variants. Load of viruses in body fluids can be determined by quantitative molecular methods [5,7,24,36]. Simultaneous detection of nucleic acids of coinfecting microbes would be ideal by using multiple PCR [20, 29]. Development of easily available panels is a financial interest of biotechnology companies. Gene arrays capable of detecting even hundreds of microbes at nucleic acid level could also be marketed in near future.

Aims of detecting simultaneous infection in cats and large felids are very variable. As retroviral infection might represent a considerable risk factor to several bacterial, fungal and parasitic opportunistic infections and activating potential of their latency, demonstration of retroviruses ought to be followed by screening for other pathogens [21]. Vice versa, cats liv-

ing in endemic areas of certain parasites are significantly more likely to be co-infected with FIV and/or FeLV, which may present confounding clinical signs and therefore cats in such areas should be always carefully screened for coinfections [16]. Occasionally, knowing the cat's geographical location can be helpful, while the nature of the clinical presentation might be less informative [23]. Informations concerning risk assessment of viral pathogens to other animal populations are currently an important issue [28]. Screening for multiple infections is appropriate prior to relocation of cats and other felids to prevent introduction of pathogens in host colonies (households, catteries, zoos, national parks, etc.) [29]. As with HIV, careful consideration should also be given to systematic testing and treatment of individuals in high-risk populations, and this may prove to be a potential strategy to prevent FIV transmission [67]. Preventive measures are important, because transmission of pathogens in cluster conditions may occur between asymptomatic cats and immunocompromised animals [25,32]. Furthermore, certain FIV or FeLV strains are able to emerge in new host species. Domestic cat strains of viruses can cross species barriers with potentially devastating consequences to fragile populations of large felids [12]. As seen with feline haemoplasma, it could infect even the immunocompromised human cat owner [69] so; cat owners ought to be protected from common zoonotic infections.

While testing and identification of infected cats is necessary for prevention and transmission, vaccination is also an important tool. Various independent bodies, including the International Vaccination Guidelines Group (IVGG), the European Advisory Board on Cat Diseases (ABCD), the World Small Animal Veterinary Association (WSAVA), or the American Association of Feline Practitioners (AAFP) have developed and regularly issues recommendations for vaccination protocols for cats and kittens. The guidelines encompass the types of antigens used, the types of vaccines available, the frequency of vaccination and the anatomical site used for administration. These guidelines differ from each other and also from the manufacturer's datasheets or recommendations of national authorities. General aspects and details of vaccination of cats are far beyond the scope of recent review; in this regard see a recent publication by Dean et al. 2012. Core vaccines are defined as those vaccines which all cats, regardless of circumstances, should receive to protect animals from severe, life threatening diseases which have global distribution. Only to emphasize those in context with microbes with transactivating and opportunistic potential, FHV-1 vaccination of kittens seems to be important. Vaccination against FCV, FPV also belongs to core vaccines. These vaccinations are recommended to start as early as vaccination with FeLV, applying revaccinations up to 16-20 weeks of age. Most practices routinely give the commonly used antigens annually, although with the exception of the FHV, the FCV and FIV vaccination is recommended every 3 years [80]. Non-core vaccines are those that are required by only those animals whose geographic location, local environment or lifestyle place them at risk of contracting specific infections. This moment, both FIV and FeLV vaccines are regarded as non-core. FIV vaccine development was initiated as a model for HIV vaccination. Vaccination against HIV started with experiments to trigger a specific, potent and long-lasting immunity in a surrogate animal model. Later, several novel approaches were tested in the feline model. Although the quest for a truly effective AIDS vaccine is years away [81], vaccination against FIV became available [82]. This vaccine (Fel-O-Vax FIV®) is marketed in many

countries, but it might not protect cats against all field strains of subtypes A or B. Lifespan of infected cats appears similar to that of uninfected cats, but exposure to other infectious diseases drastically reduces survival of FIV positive animals [17]. Historically, FeLV vaccination has been used for decades, well before FIV vaccination. The combined use of testing and vaccination programs is assumed to have decreased the prevalence of FeLV over the last 20 years [17,19]. Administering the first vaccine to kittens is at 8-10 weeks of age, with regular revaccinations usually every year. Several practitioners stopped revaccinating indoor and older cats, once they reached a certain age ( $\geq 6$  years) assuming that they would not have close contact with a persistently infected cat, or elder cats are less easily infected with FeLV than younger animals. As persistently infected cats have been diagnosed at all ages, due to the decreased susceptibility to FeLV infection, vaccinating at 2-3 years intervals rather than annually seems to be acceptable. For free-roaming cats, annual FeLV vaccination is recommended. To increase revaccination interval assuming the presence of high level antibodies is not feasible because quantitative assays to measure serum FeLV antibodies are commercially available in few countries [80]. A marked difference in vaccination efficacy exists, and suggests that only inactivated whole virus or canarypox-vectored recombinant vaccines should be used. Cats with access to outdoors should be vaccinated, but should have at least one negative FeLV ELISA test before vaccination. Several studies showed that the mean survival time of FeLV positive cats are significantly shorter than that of FeLV-negative cats including vaccinated ones (references in 17). Aspecific measures to prevent FIV and FeLV transmission were set according to the biological characteristics of these viruses. Although retroviruses become inactivated within a few hours on dry surfaces, they may remain viable in dried biological deposits for more than a week. Both viruses are inactivated by common detergents and hospital disinfectants. Spreading via body fluids is best prevented by single set of instruments in clinical practice. To prevent infections by other microbes, including those with transactivation or opportunistic potential, hospitalised cats should not be allowed to have direct contact with one another. It is important not to keep retrovirus-infected cats in contagious disease ward as they are potentially immunosuppressed and carry or acquire other pathogens [17]. A recent survey in the UK showed that several veterinarians are not aware of the guidelines, or they do not adhere to them. Similarly to guidelines in human healthcare, other barriers included lack of agreement or lack of ability to follow guidelines, lack of motivation to follow them, and lack to perceive benefits to patients. The frequency, combination and selection of antigens routinely given to cats by veterinarians remain unknown, as are the anatomical sites to inject cats. Therefore, the impact of the published guidelines on practitioners working also remains unknown [80]. Further work is required to elicit why following guidelines may reduce occurrence and severity of both opportunistic infections and halt onset and slow progression of FAIDS by eliminating infection by other microbes.

The FIV/cat model has provided a unique opportunity to test novel therapeutic interventions aimed at eradicating latent virus, but the use of antiretroviral drugs in FIV infected cats and other felids has not gained grounds in the routine veterinary practice [7,8]. Several conventional microbial coinfections are treated with routine medication.

## 5. Conclusions

Descriptive epidemiological surveys on the simultaneous infection by feline retroviruses, namely FIV and/or FeLV, and heterologous microbes clearly show that progression of FAIDS is facilitated by certain viruses, bacteria and other parasites that also induce opportunistic infections in a vicious circle. Scarce experimental data suggest that FIV transactivating potential of heterologous microbes might increase FIV load, facilitate FAIDS course, and help induce malignancies resulting in considerably impaired quality of life and shorter life span of afflicted domestic cats. Simultaneous cross-species transmission of infection by particular FIV and FeLV strains in endangered big cats may also occur. Emerging infections by FIV transactivating and opportunistic feline microbes in immunocompromised humans have already been described. Additive or synergistic impairing effects on the native immune reactions, activation of negatively affecting Treg cells, depression of cytotoxic T cell activities, and abnormal cytokine pattern exerted by heterologous microbes demonstrate striking similarities between AIDS and FAIDS. Regular vaccination against transactivating microbes (e.g. FeLV, FHV-1) and transactivated microbe (FIV) starting at early age prevents and disrupts deleterious microbial interactions. These result in a complete halt or a significant slow-down of acquired immunodeficiency states. Experience verified in the feline model enables us to continue studying microbial interactions at molecular, cellular, immunological or clinical levels. Further experiments are warranted to better delineate the role of putative cofactors in FIV infection. Further studies should examine concurrent infections as contributing factors in the development and progression of neoplasia in FIV-positive cats. Determination of viral cooperative mechanisms that promote cancer during co-infection would be highly relevant to both FIV- and HIV-related diseases.

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