Since its discovery, feline immunodeficiency virus (FIV) has been the focus of substantial and sustained research efforts, partially in recognition of its potential role as an animal model for human immunodeficiency virus (HIV). Whereas there have been considerable insights into the pathophysiology and immunologic responses to FIV infection, important questions remain regarding the impact of FIV infection on an individual cat and its likely association with specific disease syndromes.

**PATHOPHYSIOLOGY**

FIV-induced immune dysfunction is characterized by a paradoxical state involving immune hyperactivation and immune suppression. As the disease progresses, FIV-infected cats eventually lose the ability to mount an effective cell-mediated immune response against opportunistic pathogens. Early reports of immune dysfunction demonstrated reduced blastogenesis of peripheral blood mononuclear cells following mitogen stimulation in FIV-infected cats compared with uninfected cats. Many studies have since attempted to further characterize the immune dysfunction, concluding that it is multifactorial. Loss of CD4+ T cells and associated reductions in cytokines, chronic antigenic stimulation and anergy, activation of immune regulatory Treg cells, and dendritic cell dysfunction are the major mechanisms by which immune dysfunction can occur in FIV-infected cats.
DIAGNOSIS

Commercially available in-house test kits have been the main method of diagnosing FIV status in domestic cats by identifying feline antibodies to FIV gag (p24 or p15) or transmembrane protein (gp40). The sensitivity (82%–100%) and specificity (98%–100%) of these tests is generally considered to be high, but their predictive value is affected by the prevalence of FIV in the population, and cats testing positive with an in-house test should have their diagnosis confirmed. Western blot studies to identify a range of specific antibodies to FIV have generally been considered to be the gold standard for the detection of FIV antibodies but are technically demanding, and indeterminate results are occasionally seen, at least in people tested for HIV.

The introduction of a vaccine to protect against FIV in domestic cats has complicated the diagnosis of FIV. Vaccine administration results in the production of antibodies that are detected by commercially available in-house tests and Western blots. An enzyme-linked immunosorbent assay (ELISA) has been developed to detect antibodies specifically against formaldehyde-inactivated virus, and this has been suggested as a method of discriminating between vaccinated cats and FIV-infected cats. However, this assay requires further validation, and to the authors’ knowledge is not yet commercially available.

Nucleic acid amplification has been used with increasing frequency to detect viral nucleic acid in blood. Commercial assays qualitatively and more recently quantitatively detect proviral DNA incorporated into the host genome, but experimentally, viral load can also be quantified using real-time polymerase chain reaction (PCR). Following the release of the FIV vaccine, nucleic acid amplification was suggested as an effective way to distinguish between vaccinated and infected cats. However, concerns have been raised about the sensitivity and specificity of some commercially available PCR assays. False-negative results may occur as a result of sequence variation between isolates, and it is important that primers are designed to detect maximally conserved regions of the genome. The assays are generally extremely sensitive and can detect as few as 1 to 10 copies of viral DNA per sample. Consequently, it is imperative that these assays are performed with technical precision, because contamination of samples can easily lead to false-positive results. Sensitivity and specificity of PCR varies between laboratories and is dependent on factors such as primer design, reagents, and the technical proficiency of the laboratory staff. As the expertise and technology in nucleic acid amplification methods grows, the commercial assays will likely improve and become more reliable for practical use in the diagnosis of FIV. Continued surveillance of the sensitivity and specificity of commercial diagnostic tests is required by independent researchers, especially when the methodology remains confidential and therefore not subject to peer scrutiny.

In non-domestic cats nested PCR, western blots, and antibody tests specific to the FIV gag (p24 or p15) have been evaluated for use in the African lion and found to be highly sensitive, however studies evaluating antibody tests centered on transmembrane protein (gp40) are currently lacking. Given the significant genetic differences within and between clades infecting each feline species, extrapolations from findings in domestic cats needs to be done with caution.

The difficulty of evaluating test performance (sensitivity and specificity) in the absence of an obvious gold standard can be addressed statistically. Bayesian analysis combines prior knowledge regarding test performance including uncertainties (prior distribution), and new test results to estimate a distribution of possible values for test performance in the absence of a gold standard. Bayesian analysis has been applied to FIV diagnostic tests including immunomigration, ELISA, and
real-time PCR. Sensitivity and specificity range from 94% to 99.7% and 91% to 98%, respectively, for immunomigration and ELISAs and from 85% to 97% (sensitivity) and 98.8% to 99.9% (specificity) for real-time PCR. The authors have observed, as was demonstrated in this study, equivocal results with the in-house tests and that equivocal results were excluded from the analysis.

**DISEASE ASSOCIATIONS IN DOMESTIC CATS**

Domestic cats experimentally infected with FIV can develop marked immune dysfunction with severe and progressive respiratory and intestinal disease. However, the relationship between seropositivity and disease, especially among naturally infected cats, is less clear. Whereas some surveys have found FIV-positive cats at increased risk of illness, others have demonstrated a similar prevalence of FIV infection among “healthy” and “sick” cats.

Severe clinical signs did not develop in experimentally infected animals even with prolonged follow up (6.5 years), although some clinicopathologic differences were noted between FIV negative and positive cats. Fewer secondary infections could be expected when specific pathogen-free cats are experimentally infected with FIV, but observations of naturally infected cats showed that progression to symptomatic FIV infection, feline AIDS, or even persistent clinical disease was not invariable.

A case-control study that compared disease associations and outcomes in sick cats found an increased likelihood of death in FIV-positive cats compared with FIV-negative cats. Since then, two cohort studies have compared the survival times of naturally infected FIV-positive with FIV-negative cats and found no statistical difference in survival in either a closed household or among pet cats at Canadian teaching hospitals. In the latter study, control cats were age and sex matched, removing these as potential confounders from the survival analysis.

It is difficult to determine the effect FIV infection will have on an individual cats’ survival based on the previous studies. Studies of naturally occurring disease may underestimate the role of FIV because of the potentially prolonged asymptomatic period. Surveys and case control studies are also limited by the inability to determine the temporal relationship between infection and disease. Prospective cohort studies following naturally infected FIV-positive and FIV-negative cats for a period of time would be the ideal way to determine true disease associations, and these studies have been performed, but the asymptomatic period makes this type of study potentially difficult. The proportion of cats that will ultimately develop AIDS or symptomatic FIV infection or with infectious and neoplastic complications of FIV is unknown. For rare diseases, cohort studies are inefficient and potentially expensive.

Hematologic abnormalities are frequently reported in FIV-infected cats, both in the asymptomatic and symptomatic stages of infection. Nonregenerative anemia, leucocytosis, leucopenia, and thrombocytopenia have all been described, but cytopenias affecting multiple cell lineages seems to be most common. In particular, neutropenia is frequently reported in FIV-infected cats, and this may occur as early as 21 days postinfection.

The mechanism of FIV-induced cytopenias is likely to be multifactorial and result from direct or indirect suppression of hematopoiesis or secondary factors such as opportunistic infection and neoplasia. Direct infection of the bone marrow stromal cells with FIV and subsequent changes in cytokine expression can result in suppression of hematopoiesis. Recently, direct infection of bone marrow progenitor cells (as demonstrated by PCR and immunocytochemistry) has also been implicated in the pathogenesis of peripheral blood cytopenia. Myelodysplasia of various cell lines has been reported in association with the hematologic abnormalities in asymptomatic and symptomatic FIV-infected cats. Unlike feline leukemia virus–associated myelodysplastic
syndrome, FIV-associated myelodysplasia does not typically progress to leukemia. One investigator has suggested that the bone marrow changes may be more accurately termed FIV myelopathy rather than myelodysplastic syndrome.30

Oral cavity disease has been identified as an important limitation on quality of life for FIV-positive cats. In a large cohort of cats, diseases identified in FIV-positive cats included pyrexia, gingivitis or stomatitis, and respiratory tract signs.40 A case-control study investigating the prevalence and severity of oral cavity disease in cats from a veterinary teaching hospital and private shelter found that FIV-positive cats were more likely to have oral disease and to have more severe disease than FIV-negative cats. The relationship between FIV infection and disease severity was only significant in cats from the shelter, and there were notable differences in the severity of oral disease between all cats, complicating interpretation of the data.41 Similarly, oral disease of all forms was more common among FIV-positive than age-matched FIV-negative cats.29 In contrast, a comparison of cats with and without gingivostomatitis found no increased risk of FIV infection among affected cats.42 Some of these differences may be due to differences in case definitions: oral disease encompasses a spectrum of disease severity from mild periodontal disease to severe, diffuse gingivostomatitis resulting in anorexia and weight loss. For potentially multifactorial diseases such as those in the oral cavity, determining the contribution of infectious agents such as FIV requires consideration of other potential causes of disease (eg, age, diet, and breed).

The relationship between FIV infection and toxoplasmosis has been investigated repeatedly. Several surveys have identified coinfection of Toxoplasma gondii and FIV in naturally exposed cats but without necessarily confirming a relationship between the two infections or identifying clinicopathologic differences between cats seropositive for T gondii with and without FIV infection.43,44 When regression methods have been used to account for the variability in seropositivity due to age, FIV-positive cats were more likely to be seropositive for T gondii than FIV-negative cats.20,45 Because risk factors for both infections include being male and exposure to outdoors, these surveys cannot determine whether the increased likelihood of both infections is simply due to increased exposure.

Immunologic mechanisms to explain a potential disease association between FIV infection and toxoplasmosis have also been studied. Among naturally infected cats, FIV-positive cats with positive toxoplasmosis serology were more likely to have immunoglobulin M antibodies, lower immunoglobulin G antibodies, and reduced lymphocyte responsiveness to T gondii antigens.46 Variation in experimental methods may be the cause of differences in clinical outcome. For example, differences may be predictable when cats are exposed to T gondii soon after FIV infection7 compared with 12 months after FIV infection,47 when kittens are infected or when animals are infected intravenously with either agent.48 More work is required to clarify if a disease association exists between FIV and toxoplasmosis in naturally exposed cats.

Several descriptive studies identified kidney disease among FIV-positive cats.21,49–52 Because kidney disease is a common disease of all cats, this result was perhaps not surprising and, in the absence of a control population, unremarkable. Renal disease in FIV-positive animals is biologically plausible based on the microscopic renal abnormalities in a small number of FIV-positive cats49,53; the presence of FIV antigen (p24) within tubular, glomerular or interstitial cells50; and the presence of nephropathies in people with HIV.54 In people infected with HIV, patient cofactors play a notable role in disease development, with race being a predictor of the type of renal disease associated with HIV.55

Two case-control studies of naturally infected cats have identified an association between FIV infection and indicators of renal disease including azotemia, proteinuria,53
and small kidneys, but no associations were identified in a third study. Specific pathogen-free cats experimentally infected with FIV were more likely to have evidence of renal disease than FIV-negative control cats.

Observations from case-control studies confirm a complicated relationship between FIV and kidney disease that appears to be age dependent. FIV does not influence initial disease severity but does adversely affect survival times. Among cats with chronic kidney disease, younger cats were more likely to be FIV-positive than cats without chronic kidney disease, and the presence FIV infection increased the hazard of death 2-fold (J. White thesis, unpublished observations, 2011).

Among all the studies attempting to associate FIV infection with disease, the most convincing are those reports describing neurologic disease and lymphoma. The first reported cases of FIV in cats described neurologic abnormalities. These cats displayed behavioral changes with compulsive roaming and abnormal facial movements. Since then, similar reports of neurologic signs have been described in both naturally and experimentally infected cats, independent of secondary infections affecting the nervous system. The majority of reported clinical signs can be attributed to cortical and subcortical neuronal dysfunction, with behavioral changes predominating. Affected cats may show signs of dementia, loss of social behavior, aggression, loss of toilet training, altered sleep patterns, and compulsive roaming behavior. Facial twitching, ataxia, reduced peripheral sensory and motor conduction, seizures, and gait abnormalities have also been described. Cats may lose the ability to learn new tasks, and this has been attributed to impaired cognition.

FIV enters the central nervous system when infected macrophages or monocytes cross the blood-brain or blood-cerebrospinal fluid (CSF) barrier. The neurotropism of FIV has been confirmed following isolation of virus in brain tissue and CSF, the finding of anti-FIV antibodies within the CSF, and CSF pleocytosis in affected cats. Neurotropism is strain dependent, and brain-derived isolates are primarily monocytotropic. The pathogenesis of neurologic disease is likely to be multifactorial, but affected cats have progressive neuronal loss and higher levels of excitatory neurotoxic compounds. It is thought that FIV sensitizes neurons to the effects of glutamate and inhibits glutamate uptake by astrocytes, leading to increased intracellular calcium, neuronal swelling, and death. Neuronal glutamate toxicity can be induced in vitro with purified envelope glycoprotein, suggesting that the mechanism is indirect and does not require whole, infectious virions. FIV-induced neurologic disease seems to parallel overall disease progression and decline in CD4/CD8 ratio, but pathologic changes have also been described in asymptomatic FIV-positive cats.

FIV infected cats with immunodeficiency may develop neoplasia due to reduced immune suppression of the cell-mediated immune response. Recently however, a direct role for FIV in oncogenesis has also been demonstrated. An oncogenic role has been best described in cases of lymphoma, and an association between lymphoma and FIV is now well-recognized. The majority of reports describe predominately B cell lymphoma in FIV-infected cats, with a large proportion of the tumors being extranodal. FIV is thought to induce lymphoma via both direct and indirect mechanisms. Indirectly, chronic antigenic stimulation with FIV leads to activation of B cells, which may undergo malignant transformation if replication errors occur. A direct mechanism has also been proposed whereby the insertion of provirus into the host genome leads to loss of a tumor suppressor gene or activation of an oncogene. One study demonstrated the presence of provirus within a clone of malignant lymphocytes, which is supportive of a direct role of FIV in oncogenesis. Some of the differences in disease expression in the published literature may reflect host, virus, or experimental method variation. As a generalization, it is unusual for an
infectious agent to be a “sufficient cause,” in other words one that, acting alone, always produces disease.\textsuperscript{77} The role of concurrent disease and age has been clearly demonstrated experimentally. Specific pathogen-free cats experimentally infected with FIV developed B cell lymphoma, neurologic disease, and wasting syndromes, whereas cats with a more typical history of exposure to other infectious agents developed chronic stomatitis and upper respiratory disease.\textsuperscript{78} The role of age has also been demonstrated experimentally, with young animals showing greatest susceptibility to disease compared with young adult and older cats.\textsuperscript{79} Variation in virulence due to FIV strain or subtype is possible, and warrants further study, but at least one reported difference in FIV strain virulence\textsuperscript{80} became markedly less apparent when cats of comparable age were infected.\textsuperscript{81} The severity of clinical signs that develops after experimental FIV infection is dependent on the dose of FIV administered.\textsuperscript{82}

Overall, the disease-causing potential of FIV would seem to be less than that of HIV. The diseases that are described in FIV-positive cats also occur in FIV-negative cats, and the existence of any association, let alone the presence of any causative pathway, remains to be confirmed for many diseases, with lymphoma and neurologic disease being the most obvious exceptions.

**DISEASE IN NONDOMESTIC FELIDS**

FIV strains have been present in the nondomestic cat population longer than in domestic cats,\textsuperscript{83} however, the relationship between FIV infection and disease causation or association is less clear. Whereas FIV infects many feline species, selective pressures within each host species have resulted in development of predominantly species-specific strains named in accordance with the infecting felid species such as FIV-Ple (African lion), FIV-Aju (cheetah), FIV-Ppa (leopard), FIV-Pco (puma), FIV-Pon (jaguar), and so on.\textsuperscript{84} Although interspecies transmission among the nondomestic felids is not impossible, it is rare.\textsuperscript{85,86} Therefore, the role of FIV in disease causation needs to be made on an individual felid species level using an evidence-based approach.

There are numerous potential obstacles in accurately assessing the role of FIV in the cause of disease in non domestic felids, making comparisons between infected and noninfected populations in the same environmental settings difficult. For example, prevalence of FIV in African lions in many African countries ranges from 68\% to 100\%, whereas 22\% to 40\% of African cheetahs mainly in the Serengeti population and 26\% to 46\% of the leopards are infected with their respective FIV.\textsuperscript{84,87–89} Second, in the wild it is difficult to monitor infected populations longitudinally, and as a consequence there are limited studies in nondomestic felids.\textsuperscript{90} Finally, the same issues arise as with domestic cats in differentiating the role of FIV as a direct agent of disease, a secondary agent resulting from immune suppression, or simply an incidental finding.

Host-virus symbiosis, or adaptation resulting from the natural selection of resistant felids and the attenuation of FIV over the extended time frame of their relationship, has been suggested as a possible explanation for the absence of obvious clinical manifestations in many FIV-infected nondomestic felids.\textsuperscript{89,91} However, it is unwise to assume this explanation to be true of all FIV subtypes in all felid species, and so the possibility of FIV-associated disease in nondomestic felids has remained a possibility, and researchers in the last decade have actively sought the answer to this question. Indeed, when comparing FIV-Ple subtypes B and E of African lions in Africa for their usage of the important primary attachment receptors (CD134) and coreceptors (CXCR4) on activated CD4\textsuperscript{\textdagger} T lymphocytes, McEwan and colleagues\textsuperscript{92} found that only FIV-Ple subtype E was able to use these receptors, as is the case in domestic cats, indicating differences in their likely in vivo pathogenicity and cell tropism.
Immunologic dysfunction similar to that seen in domestic cats, such as decrease in CD4+ lymphocytes or a reduction in the overall CD4+/CD8+ ratio, has been seen in African lions and pumas infected with FIV-Ple and FIV-Pco, respectively. However, increases in CD5- and CD4-/CD8- cells as well as the CD8+ B2low subset indicated evidence of host adaption to the virus and was suggested as a reason for the asymptomatic infection.

Analogous to the lentiviral encephalopathy in domestic cats and humans, an association between FIV-Ple and neurologic disease was reported in three African lions displaying lymphocytes subset alterations and progressive behavioral, locomotor, and neuroanatomic abnormalities in the absence of other known neuropathogens. Proviral tissue loads were low in brain tissue, suggestive of a non specific encephalopathy rather than the direct effects of viral replication.

Recently, Roelke and colleagues found further evidence to challenge the belief that FIV does not cause apparent pathology in nondomestic felids. In a longitudinal study over 6 years of various clinical, biochemical, histologic, and serologic parameters of FIV-Ple–positive and –negative African lions in Botswana, several important abnormalities similar to those caused by lentivirus infection in HIV were found in the FIV-Ple–positive lions. These abnormalities included lymphadenopathy, gingivitis, and tongue papillomas, whereas clinicopathologic findings included abnormal red blood cell parameters, elevated gamma globulin, depleted lymphoid cells within spleen and lymph node biopsies, and mild elevations of liver indices. These researchers concluded that prolonged FIV-Ple infections in free-range lions could result in adverse clinical, immunologic, and pathologic outcomes. These types of studies would be easy to mirror in the many captive lion and other felids populations in which monitoring for disease is in many places diligent and fastidious, lending themselves to longitudinal comparisons between FIV-positive and -negative populations.

Similarly, Brown and colleagues monitored a group of 28 free-ranging Pallas’ cats in a long-term ecology study in Mongolia from 2000 to 2007, collecting serial blood samples and ultimately necropsy tissues. They found the seroprevalence of FIV-Oma was 25%, and sequence analysis showed a monophyletic virus with little genetic diversity between cats. FIV-positive cats were found to have severe lymphoid depletion in the spleen and moderate lymphoid depletion in the lymph nodes. Continued monitoring of clinical correlates is recommended in this threatened species.

SUMMARY

There are common issues and constraints on our ability to clearly establish the association between FIV infection and the pathogenesis of disease in both domestic and nondomestic felids. Because of the importance of secondary or concurrent infections in the pathogenesis of disease associated with FIV, use of experimental models may not yield answers in domestic cats and is certainly not feasible in non domestic felids, many of which are endangered species. Therefore, researchers might consider early surveillance programs across varied populations and detailed, cohort studies of naturally infected animals to provide further insights. The power of these studies would be enhanced, especially in more unusual presumed disease associations, if a multicenter approach was taken.

REFERENCES


