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Review article

Retrovirus Infections and Brazilian Wild Felids

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Abstract

Feline leukemia virus (FeLV) and *Feline immunodeficiency virus* (FIV) are two retroviruses that are deadly to the domestic cat *(Felis catus)* and important to the conservation of the threatened wild felids worldwide. Differences in the frequencies of occurrence and the existence of varying related viruses among felid species have incited the search for understanding the relationships among hosts and viruses into individual and population levels. Felids infected can die of related diseases or cope with the infection but not show pathognomonic or overt clinical signs. As the home range for eight species of neotropic felids and the home to hundreds of felids in captivity, Brazil has the challenge of improving its diagnostic capacity for feline retroviruses and initiating long term studies as part of a monitoring program.

Key Words: Feline retrovirus, FeLV, lentivirus, FIV, wild felids, conservation, wildlife diseases

Introduction

In 1964 in Glasgow, Scotland, W.F.H. Jarrett and co-workers published a pair of articles in Nature reporting the probable transmission of lymphossarcoma and the presence of virus-like particles in tumor and cultured cells of domestic cats *(Felis catus)* (32, 33). Soon after its discovery, these virus-like particles, further denominated *Feline leukemia virus* (FeLV), were shown to be a deadly pathogen of domestic cats. The discovery of FeLV caused great concern among small animal veterinarians and cat owners, initially throughout Europe and North America, and it incited great interest in understanding the underlying pathogenic mechanisms of this virus, the development of appropriate diagnostic methods, and the elaboration of adequate management protocols to control the dissemination of the pathogen. An equal concern arose in subsequent years among wildlife veterinarians: "What if this deadly virus spread and killed the threatened wild felids worldwide?" Actually, wild felid specimens have so far been reported to suffer from diseases usually associated with FeLV (34; 42). Most wild felid species, fortunately, were found to be only rarely infected with FeLV, except for the European wildcat *(Felis silvestris),* a naturally occurring species from continental Europe and the British Isles, that presents FeLV infection rates similar to those of the domestic cat (17, 22, 37, 43). Almost half a century after its discovery, FeLV can still be considered a potential threat to wild felid species, although the exact role of FeLV as a pathogen, e.g., in the European wildcat population, is not completely understood.

Similar concerns arose after 1987, when N.C. Pedersen and co-workers in California, USA, described in Science a new retrovirus found in cats presenting hallmark symptoms of immune suppression (53). This virus was soon after named *Feline immunodeficiency virus* (FIV), and it proved to be an important emerging and fatal virus for domestic cats. Preliminary screenings in the beginning

of the last decade (51) showed that serum samples from several wild felid species worldwide presented antibodies that cross-reacted with FIV and that the prevalence surpassed 90% in free-ranging Serengeti African lions *(Panthera leo)*. Again, the concern of wildlife veterinarians dealing with wild felids became high a few years after the discovery: "What if the wild felid species succumb to FIV and disappear?" The subsequent history showed that many seropositive wild felids are infected with closely related lentiviruses distinct from FIV and have not exhibited overt clinical signs. Much older lentiviruses related to the emergent FIV have so far been detected in African lions and North American pumas *(Puma concolor),* and long term co-evolutionary processes might have provided asymptomatic host-virus relationships (7, 12, 13). Otherwise, the history has not reached an end, and the role of lentiviruses in wild felids will depend, for example, on how naïve felid host species react when exposed to FIV from domestic cats and how other felid hosts and their co-adapted lentiviruses continue to evolve.

The Viruses

The FeLV and FIV viruses are both members of the family *Retroviridae* and the sub-family *Orthoretrovirinae*. These viruses, however, belong to different genera. FeLV is one of the 17 virus species of the genus *Gammaretrovirus.* There are four subgroups of FeLV (A, B, C, and T), which are distinguished by the receptors that they require for entry into the cell (2). FIV belongs to the genus *Lentivirus* with the *Puma lentivirus* (PLV), which comprise the two species of feline lentiviruses officially accepted by the International Committee on the Taxonomy of Viruses (ICTV) (http://www.ictvonline.org/virusTaxonomy.asp). Nevertheless, other closely related variants of FIV have been characterized, such as those from lions [lion lentivirus (LLV)] and from the Pallas'cat *(Felis manul)* (FIV-O). These viruses, however, have not been designated to formally represent viral species, but they are so far classified as infra-specific entities. Like other natural retroviruses, FeLV and FIV are not single genomic species but are a genetically complex group of closely related viruses, and they are subject to selective pressures in their natural hosts.

Retroviruses are broadly divided into endogenous and exogenous viruses. The former are ancient genomic derivatives of retroviruses that have entered the germ line cells of vertebrate ancestors and remain today as fossil records present in virtually all animal genomes analyzed. Despite their ubiquity, endogenous retroviruses usually comprise silently defective genomic entities that do not accomplish viral particles and do not harm the host. The cat endogenous FeLV (en-FeLV) is supposed to have originated from rodent retroviruses through prey ingestion (4, 5). The exogenous retroviruses, on the other hand, are those viruses that we recognize as transmissible agents of diseases, the category into which FeLV and FIV fall. The exogenous retroviruses infect somatic cells and are transmitted from the mother to her offspring and through horizontal contact, differently from the endogenous viral elements that are exclusively transmitted as mendelian genes.

FeLV and FIV share similar biologic properties as members of the *Retroviridae* family of viruses. They have an envelope partially derived from the host cell membrane and contain specific outer proteins coded by the viral genome that binds to cell surface receptors and allows viruses entry into the cell they infect. Inside the envelope, the virus particles are composed of a few different proteins, including the reverse transcriptase (RT) enzyme and an RNA genome. The *pol* gene codifies the RT enzyme, which converts the viral RNA genome into a DNA copy suitable for integration into the cellular chromosomes, such as with proviruses. The proteins that compose the architecture of viral particles are codified by the so-called structural *gag* gene, and the outer envelope proteins are codified by the *env* gene (49). The long terminal repeat (LTR) regions are regulatory sequences at both ends of the virus genome that play a critical role in virus replication and pathogenesis (1).

Transmission

As with other enveloped viruses, retroviruses are very labile. Under environmental conditions, the envelope suffers desiccation, and, subsequently, the virus becomes non-infectious. Thus, viruses remain as complete infectious particles (virions) for only a short period of time in the environment after they have been shed by an infected host. Environmental contamination has a minor epidemiological importance in FeLV and FIV transmission. Felids get infected with FeLV by close contact and with FIV by biting. The viruses can be spread through saliva, blood, breast milk, and other fluids, and transplacental transmission has been reported.

The oronasal route is particularly important for FeLV transmission in comparison to FIV (38). FeLV is commonly spread horizontally among cats that live together, sharing eating and drinking bowls and mutually grooming, or that fight, and it is spread vertically from mothers to their offspring (38). For lentiviruses, aggressive behavior largely influences the rates of transmission because the most important means of transmission for the viruses are inoculation through bites and scratches. Intact male domestic cats are more prone to acquire FIV than neutered males and females, as they are more aggressive and fight with each other more often. Fights during reproductive seasons provide opportunities for retrovirus transmission among wild felids that naturally exist at low densities and are solitary. Vertical transmission has a lesser epidemiological role on FIV transmission than horizontal transmission through the lifetime (67). FeLV and FIV can potentially be transmitted via needles, syringes, darts, and

other equipments, but routine disinfection procedures are effective in preventing iatrogenic transmission of viruses if carefully undertaken (30; 67). Fleas have been considered potential vectors for virus transmission (68). FeLV provirus may also be infectious, and it is recommended to test blood donors for provirus accordingly (38).

Domestic cats harboring retroviruses are potential sources of infection to wild felids; interbreeding is suspected as a FeLV transmission route between domestic cats and European wildcats (17, 22, 37, 43), and a FeLV infected puma was shown to have ingested a domestic cat (34). In captive settings, there is a high possibility of cross-transmission of different feline lentiviruses among different felid species, including cross-transmission of FIV of the domestic cat (66). Outside captive settings, a free-ranging leopard cat *(Prionailurus bengalensis)* has been documented to have harbored a virus genetically similar to the FIV circulating in the domestic cat population in the region (48).

Prevalence

FeLV and FIV occur worldwide and are associated with domestic cats in rates that vary depending on the geographical region and the clinical condition of the evaluated animals (3). These viruses are also present in urban domestic cat populations in São Paulo and Rio de Janeiro in seroprevalences reported between 0 and 17.5% for FeLV and 12.9% and 21% for FIV (21, 44, 61). However, none of the 102 domestic cats sampled in rural areas of the Pontal do Paranapanema, São Paulo, in proximity to fragmented areas of Atlantic Forest where jaguars, ocelots, and other neotropic felids live, tested positive (47).

Currently, the European wildcat *(Felis silvestris, silvestris* group*)* is the only wild felid species in which FeLV appears to be endemic, similar to the domestic cats in the considered regions (17, 22, 37). Additionally, FeLV has been found in pumas in North America (16, 34), an Asiatic wildcat *(Felis silvestris, ornata* group), and two sand cats *(Felis margarita)* (52). Other reports referring to captive felids have included a leopard cat (55), a puma (45), a clouded leopard *(Neofelis nebulosa)* (15), a bobcat *(Lynx rufus)* (60), Iberian lynxes (*Lynx pardinus)* (39), and cheetahs *(Acynonyx jubatus)* (8, 42). Concerning South American felids, one margay *(Leopardus wiedii)* and two Pampas' cats *(Leopardus colocolo)* were found to be infected with FeLV in a survey conducted in North American zoos (35). No evidence of FeLV infection was detected in 104 neotropic small felids housed in 19 zoos and three conservation centers in São Paulo state, Brazil, in samples collected from 1996 to 1998 (19). In another survey in Brazil, one free-ranging ocelot in very poor condition and presenting notoedric mange, and another 10 captive felids, including jaguars *(Panthera onca)*, pumas, margays, and a Pampas' cat, were found to be FeLV seropositive (59). Recently, FeLV exposure has been detected in two free-ranging Brazilian pumas that were found to be FeLV antibody positive (20). Active replicating viruses were detected in captive jaguarundis *(Puma yagouaroundi)* (Filoni et al., unpublished).

Antibodies to feline lentiviruses have been documented in 27 of 37 nondomestic felid species worldwide. All the South American felid species have been reported lentivirus positive at low frequencies (36, 65). The different feline lentiviruses seem to group more by geographic region than in groupings concordant with the phylogenetic relationships of the host species (66), explaining why genetically similar viruses occur in different hosts in the same areas and vary across different geographic regions. In Brazil, molecular evidence of FIV infection has been reported for jaguars, pumas, jaguarundis, ocelots, oncillas *(Leopardus tigrinus)*, margays, and Pampas' cats (36), and antibodies cross-reacting to FIV were detected in captive lions from a Brazilian zoological park (14) and in a Brazilian free-ranging puma. Antibodies to PLV were detected in that puma and another one also in Brazil (20).

Disease Outcome

FeLV

As an oncogenic and immunosuppressive virus, the effects of FeLV in domestic cats range from cytoproliferative diseases, such as lymphomas and myeloproliferative disorders, to degenerative and immunosuppressive illnesses, such as anemia and leucopenia (30). Until recently, the outcome of FeLV infection was categorized according to parameters obtained by virus isolation and serological tests, in which detection of the FeLV soluble structural protein p27 (antigenemia) was used as a measure of viremia (40, 41). According to this, in a multicat environment only a third of the exposed cats developed a progressive infection with persistent antigenemia, the presence of replicating virus in bone marrow, and a lack of FeLV-specific immunity (31). Most infected cats were considered regressors, presenting transitory antigenemia that become undetectable within a few months. Many regressor cats were believed to mount an effective immune response and clear the infection (abortive infection), although a latent infection defined by the presence of non-replicating virus in bone marrow was recognized in some non-antigenemic cats. Also, a minor proportion of cats continued to present antigenemia in the absence of replicating virus in the bone marrow, explained as a focal infection in tissues like the spleen, lymph nodes, small intestine, or mammary glands. Newer research has suggested that most infected cats do not clear the infection, remaining infected for life following exposure, in which FeLV provirus (DNA) can be detected in the blood by polymerase chain reaction (PCR). The continuous presence of provirus may explain the long persistence of virus-neutralizing antibodies. Virus replication may be transient or persistent in cats that are provirus positive as detected

by reverse transcriptase (RT)-PCR to viral RNA even in those cats that are not antigenemic (25, 26, 29, 38, 64). Some cats may develop an abortive infection, in which viral DNA or RNA cannot be detected. It is not known if FeLV DNA or RNA can be detected in the blood of cats presenting focal infections.

The worst prognosis is recognized for cats developing progressive infection. These cases present extensive virus replication in lymphoid tissues, bone marrow, and mucosal and epithelial tissues, and the cats succumb to FeLV-associated diseases within a few years. Conversely, development of FeLV-associated diseases is unlikely when an effective immune response contains virus replication prior to or at the time of bone marrow infection, as observed in the regressive infection outcome (38).

In addition to the cases explained above, the course of infection in a cat can also be associated with the FeLV subgroup that is present. As reviewed by Tandon and co-workers (62), FeLV-A is the dominant subgroup and is found in all FeLV-infected cats; though low in pathogenicity, it is the most contagious subgroup. FeLV-B is poorly infectious, not being present in all infected cats and only detected in the presence of FeLV-A. FeLV-B arises via recombination of FeLV-A with en-FeLV, while FeLV-C and FeLV-T develop from FeLV-A via mutations or insertions. Cats infected with FeLV-A usually exhibit a prolonged clinical asymptomatic phase; subsequently, they may develop lymphoma mainly of T-cell origin. FeLV-B is associated with lymphoid malignancies. FeLV-C is associated with the development of aplastic anemia, and FeLV-T is associated with lymphoid depletion and immunodeficiences.

European wildcats and leopard cats were demonstrated to harbor en-FeLV (6, 56, 63). The European wildcat presented higher en-FeLV loads than domestic cats (63). The authors hypothesized that the chance of integration of en-FeLV in the genome of wild felid species that prey frequently upon rodents may be higher than for the domestic cat, which usually receives a rodent-free diet. Thus, it can be speculated that the *modus vivendi* of wild felids potentially can increase the pathogenicity of FeLV for wild species, since higher en-FeLV loads increase the possibility of recombinations with FeLV-A. However, studies addressing this matter have not been performed.

The high prevalence of FeLV infection in European wildcat populations suggests that FeLV is self-sustained in the natural populations and that it is not of high pathogenicity (17, 22, 37). For every other felid species other than wildcats, FeLV is considered pathogenic. It has been associated with poor body condition and death in wild felids, and it may be important for the survival of endangered non-domestic felids (69). For instance, FeLV association with diseases has already been demonstrated for one North American free-ranging puma that had lymphoproliferative disease and anemia (34) and for one captive cheetah that developed multi-centric T-cell lymphoma (42*)*. A seropositive FeLV captive jaguarundi in Brazil developed a tumor suggestive of FeLV origin (Filoni et al., unpublished).

Feline lentiviruses

FIV causes immunodeficiency and neurological signals in domestic cats (69). Acute FIV infection in domestic cats is associated with fever, lymphadenopathy, and leucopenia. The virus is detected in high concentrations in the blood by culture and PCR within two weeks of infection. Within the first weeks of FIV infection, both CD4+ (helper) and CD8+ (cytotoxic-suppressor) T-lymphocytes decline. This initial lymphopenia is followed by production of antibodies against the virus, suppression of viral load in the blood, and a rebound of CD8+ T-lymphocytes in excess of pre-infection levels, resulting in inversion of the CD4+/CD8+ ratio that persists for life (Levy et al., 2008). The cat then enters a prolonged asymptomatic phase that lasts for years, with minimal clinical symptoms, while progressive dysfunction of the immune system occurs (18). Over time, both CD4+ and CD8+ T-lymphocytes decline (38). The cell-mediated immune response is more affected than humoral activity, and chronic inflammatory conditions, infections with intracellular organisms, and neoplasia are more common than those infections controlled by antibodies (38). The terminal phase of the disease is marked by immunological decompensation, exacerbation of plasma viral loads, and clinical symptoms of immunodeficiency, and the cat generally dies of opportunistic infections (18). Lymphoid alterations include thymic depletion, lymphoid hyperplasia, plasmacytosis, and terminal lymphoid depletion. Neurological manifestations are also evident, including auditory and visual changes and alterations in sleep patterns. Many early symptoms resolve during the asymptomatic phase of the disease (18).

Lentivirus seropositive wild felids do not show overt clinical signs (14, 35). Evidence in free-ranging populations of lions and pumas has supported the hypothesis that co-evolutionary processes might have provided apathogenic virus-host relationships, as suggested by studies that have not associated mortality or reproductive impairment with seropositivity in lions and pumas in the wild (27, 67). However, the stress associated with sample collection precludes meaningful interpretation of hematological data, and supplementary analyses have not been performed for wild species. In addition, some reports have been published describing captive lions that developed lymphoma (10, 54), neurological disease, and CD4+ T-cell depletion, similar to that noted in domestic cats infected with FIV (67). Seropositive captive lions in Brazil showed episodes of anemia and/or leucopenia during their lifetime (14), although these signs are unspecific and also common to seronegative felids.

Diagnosis

The diagnosis for FeLV, FIV, and other related feline lentiviruses cannot be solely based on hematological or macro- and microscopic changes. The findings are unspecific, and largely concordant with opportunistic infections and related diseases that may develop. Specific laboratory tests are required for a feline retrovirus diagnosis. However, measurements of plasma viral loads, changes in T-cell lymphocytes, as detected by flow cytometry (for lentivirus infections), and the diagnosis for any morbid presentation in retrovirus positive animals are important to establish the actual pathogenic role of these viruses in wild felid species (9, 28, 57, 67).

Routine diagnostic screening for FeLV has traditionally relied on detection of the viral protein p27. Several serological tests, including enzyme linked immunosorbent assays (ELISA) and commercial immunochromatographic assays, can be used to detect p27 free in serum, plasma, and, in the case of commercial assays, also in whole anticoagulated blood. Indirect immunofluorescence tests (IFA) have been used to detect p27 antigen within infected cells, using blood or bone marrow smears (24, 38). Real-time PCR tests can be the most sensitive test methods when performed under optimal conditions. Depending on how the PCR is performed (RT-PCR or PCR), it can detect viral RNA or cell-associated DNA (provirus), respectively, and it can be performed on blood, bone marrow, and tissues (25, 26). In addition, PCR testing of saliva has been shown to have a high correlation with blood antigen tests (23, 38). Antibody detection assays have been traditionally used for FIV diagnosis in domestic cats. The infection is life-long, and antibodies in peripheral blood persist, while viral loads are frequently below the limit of detection of virus antigen tests. Most serological tests detect antibodies against the conserved structural proteins TM and p24 of lentiviruses (11, 38). Western blot (WB) analysis is considered more specific, as proteins other than p24 are used, and a positive result is given only if antibody binding occurs for more than one antigen. Different lentiviruses naturally occurring in wild felid species may present different proteins, and thus the diagnostic sensibility of serological assays may be enhanced if specific antigens are used instead of those of the FIV. To date, a puma lentivirus peptide used in an ELISA detected two pumas as positive in a sample collection tested, while the FIV WB just detected one of these as positive (20). Similar to FeLV, PCR has been promoted as a method to determine the true status of infection in a cat, but widely variable results obtained from different laboratories have indicated that the diagnostic accuracy of PCR for FIV should first be improved before being adopted (38). For wild felids, PCR-based tests should be directed to species-specific viruses, and attempts to isolate and characterize new virus strains are encouraged, not only to understand the natural history of the feline lentiviruses but also to understand the pathogenesis and to anticipate the perils for the survival of these threatened species.

Control Measures

Control measures for domestic cats have largely relied on testing and removing infected/sick cats from contact with susceptible uninfected cats. In addition, intense efforts have culminated with the development of vaccines for FeLV and FIV for domestic cats.

A number of ethical/conservationist questions preclude the adoption of similar measures for wild felids and would simply be unfeasible for free-ranging animals. Utilization of recombinant vaccines for non-domestic felids at risk of exposure could be evaluated, but their effective utilization is controversial. Presently, due to the very low prevalence of FeLV in most wild felid species, vaccination of captive felids is not suggested. Considering the existence of several feline lentiviruses presenting low or absent pathogenicity to their hosts and the interference of the vaccine in some diagnostic tests, vaccinating captive felids for FIV is not recommended.

As for the possibility of wild felids acquiring FeLV or FIV from domestic cats, any conservationist initiative is encouraged to advertise against keeping cats in proximity to natural reserves where wild felids naturally occur. Similarly, all zoological parks and other reproduction and conservationist centers that keep wild felids should maintain policies of capturing and removing domestic cats that enter their facilities. In Brazil, feral cats are commonly encountered in zoos.

It is essential to identify and characterize the viruses and detect variations in the frequencies of occurrence, both for free-ranging and captive felid populations. In a previous report (19), we suggested the creation of a surveillance program, consisting of periodic serologic testing for retrovirus infections, and more sensitive assays, such as those based on PCR, for the purposes of monitoring felid populations in and among zoos and prior to translocations. Such tests should be included in routine quarantine procedures, whenever an animal enters or leaves the population, is immobilized for examinations, or presents ill. Long term epidemiological studies, in addition to cross-sectional studies and detailed case reports, would certainly help to elucidate the actual pathogenic role of these viruses for the different wild free-ranging and captive felid populations.

Retroviruses and Conservation of Threatened Wild Felids in Brazil

Considerable progress related to many aspects of FeLV and FIV infections has been made during the last few decades. In the case of wild felids, however, many questions remain unanswered. The consequences of co-evolution of pathogen and host are dynamic, as there are constant changes in the virulence of the agent and resistance of the host. Species with reduced genetic variation, particularly in genes involved with disease resistance, may be less able to mount an effective immune

response against an emerging pathogen. It is suspected that lentivirus infections have played a supporting role in the deadly epidemics of *Canine distemper virus* (CDV) that killed a third of a free-ranging African lion population in 1994 (50, 58). On a similar note, we do not have a clear idea about what have extinguished the great saber-toothed tigers about ten thousand years ago (49). We cannot dismiss the fact that FeLV, FIV and closely related lentiviruses continue to evolve and change, presenting a potential threat to the present-day wild felids.

For the South American neotropic felids, we consider it of utmost importance to insert the matter of infectious disease into conservation management strategies. Feline retroviruses are present in domestic cats and in neotropic felid species in Brazil. To what extent this poses risks to the endangered neotropic felids we still do not have a clear idea.

Detailed long term studies are required to provide a better understanding of infectious diseases in neotropic felids in Brazil. Ideally, zoological parks all over the country should follow unified strategies for disease monitoring. In addition, conservationists should include the importance of infectious diseases into their projects and efforts, as has been occurring in Brazil for jaguars by the Jaguar Conservation Fund, and also for other felids by the Associação Mata Ciliar, Instituto de Pesquisas Ecológicas – Ipê, and the Associação Pró-Carnívoros.

In Brazil, unfortunately most zoos lack resources for adopting efficient infectious diseases protocols. Thus, we consider that constant funding and adequate laboratory support should be made available to serve this demand. Central storage facilities for biomaterials already exists for felids as represented by the Centro Nacional para Pesquisa e Conservação de Predadores Naturais (CENAP), which are supported by the government. Systematizing and centralizing data, using standardized protocols for laboratorial analysis, inviting specialists to examine cases in their respective areas of expertise and reporting the occurrence of diseases and control measures to the scientific community (46) would certainly result in great progress and benefit the conservation of wild felid species in Brazil.

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