

Risk factors for feline leukemia virus (FeLV) infection in cats in São Paulo, Brazil

Fatores de risco da leucemia viral felina em São Paulo, Brazil

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Abstract

The aim of this study was to identify risk factors for feline leukemia virus (FeLV) infection in cats (n = 812) from the city of São Paulo. Information on age, gender, outdoor access, reproductive status, origin and number of potential contacts were obtained for each animal. Direct immunofluorescence test was used to identify the infected cats. Fifty cats (6.2 %) were positive for FeLV infection. The risk factors identified were "outdoor access" (OR = 47.2; p < 0.001), "having been rescued from the street" (OR = 3.221; p = 0.008) or being three to six year old (OR = 3.046; p = 0.009); the most important risk factor was free outdoor access. A predictive model for FeLV infection was built based on the results of the multivariate analysis. Cats with free outdoor access are more predisposed to infection, with 18% more chances of becoming infected. If the animal is one to three year old, the probability increases, reaching 34%. If the cat is exposed to these three risk factors, the probability of infection is even higher (63%). When analyzed together or as isolated risk factors, the age and being rescued from the street represent less impact on the spreading of the FeLV, as the probability of infection for cats exposed to each of these risk factors is 1 and 4%, respectively. Thus, free roaming and outdoor access are the most important risk factors associated to FeLV infection among cats in São Paulo city and must be taken in consideration in the prevention of this retrovirus infection.

Keywords: Cats. Feline leukemia virus. Risk factors. Epidemiology. FeLV.

Resumo

O objetivo deste estudo foi o de identificar os fatores de risco para a leucemia viral felina (FeLV) em uma amostragem de 812 gatos da cidade de São Paulo, Brasil. Informações sobre idade, sexo, acesso à rua, estado reprodutivo, origem e número de contactantes foram obtidos para cada animal. Teste de imunofluorescência direto para a pesquisa de antígeno viral em esfregaço de sangue periférico foi utilizado para a identificação dos felinos infectados. Foram encontrados 51 gatos (6,2%) positivos para a infecção. Os fatores de risco identificados foram "acesso a rua" (OR = 47,2; p < 0,001), "ter sido resgatado da rua" (OR = 3,221; p = 0,008) e "ter idade entre 3 e 6 anos" (OR = 3,046; p = 0,009), sendo o "livre acesso à rua" o fator de risco mais importante. Foi elaborado um modelo preditivo para a infecção pelo FeLV, baseando-se nos resultados da análise multivariada. Gatos com acesso livre à rua apresentam 18% mais chances de se infectarem e se estiverem na faixa etária entre 3 e 6 anos de idade o risco aumenta para 34%. Se o gato estiver exposto aos três fatores de risco, a probabilidade de infecção é ainda mais alta, de 63%. Quando analisados em conjunto ou como fatores de risco isolados, a idade e o fato de "ter sido resgatado da rua" possuem menor impacto na disseminação de FeLV, já que a probabilidade de infecção para os gatos expostos a cada um desses fatores é de 1% e de 4%, respectivamente. Assim, o acesso livre a rua e ter sido resgatado da rua foram os fatores de risco mais importantes associados com a infecção pelo FeLV na cidade de São Paulo e devem ser levados em consideração na profilaxia da leucemia viral felina.

Palavras-chave: Gatos. Leucemia viral felina. Fatores de risco. Epidemiologia. FeLV.

Introduction

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are amongst the most common infectious agents of domestic cats throughout the world^{1,2,3,4,5}. Feral cats^{6,7} or endangered wild felids as lynx and panthers⁸ are also under risk of FeLV infection. Both retroviruses affect the immune system

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and are causes of significant morbidity and mortality⁹ or predispose to infections such as hemotropic mycoplasmas¹⁰ or feline infectious peritonitis¹¹. Co-infection with FIV and FeLV might occur, increasing the risk for developing severe disease⁹. FeLV is primarily transmitted by saliva and respiratory secretions¹² but it is also secreted by urine, faeces, tears and milk^{13,14}. The spreading occurs by “friendly” contact among cats, usually licking and grooming of one another⁹ or by fighting and biting^{3,15}. Transmission may also occur through the sharing of food and water pans⁹. In contrast to FIV infection, primarily transmitted by biting and found more commonly among male cats, FeLV infection is found almost equally in male and female cats⁶.

The prevalence of FeLV in healthy cats is similar throughout the world, ranging from 1% to 8%⁹. The most recent studies report a prevalence of 2.3-3.3% in North America^{7,16}, 0-2.9% in Asia¹⁷ and 3.5-15.6% in Europe¹. However, if only sick cats are considered, the prevalence is as high as 38%^{1,2}. For both FeLV and FIV infection, prevalence was higher among cats tested at veterinary clinics^{1,10,11,18}.

Young cats are highly susceptible to infection and are more predisposed to develop FeLV-related diseases as lymphoma, myeloproliferative or myelosuppressive diseases and immunosuppression¹¹. Until recently, it was believed that while estimated 30% of FeLV-exposed cats developed progressive infection and become ill, the remaining cats experienced abortive infection, with the absence of infected cells in the peripheral blood and tissues. Newer researches suggest that most cats remain infected showing regressive or latent infections without persistent circulating antigens. Yet, FeLV proviral DNA can be detected in the blood by polymerase chain reaction (PCR)^{19,20,21}. The provirus is integrated into the cat's genome, so it is unlikely to be cleared over time¹³. Following infection, regressive and progressive infections can be distinguished by repeated testing for viral antigen in peripheral blood or molecular assays for detection of FeLV proviral DNA²¹.

To protect susceptible cats against FeLV infection, several injectable inactivated adjuvanted vaccines have been produced and widely used, because protection against persistent viremia may prevent the development of FeLV-associated fatal disease. Testing for FeLV-infection and vaccination protocols, beside pet owners educational efforts are recommended for maximizing prevention of retrovirus infection²² among owned cats. The prevalence of FeLV infection has reportedly decreased during past 20 years, presumably as a result of implementation of widespread testing programs and development of effective vaccines^{3,16}.

Identification followed by segregation of infected cats is considered the single most effective method for preventing new infections with FeLV or FIV²². Thus, the purpose of the present study was to identify risk factors for FeLV infection in cats in the city São Paulo, SP, Brazil and to elaborate a predictive model based on the results.

Material and Method

From January 2003 to December 2004, samples from 812 cats were submitted by veterinary practitioners of São Paulo city (Brazil) to us. An identification chart filled out by the veterinary practitioners was sent together with the blood smear slides. Information regarding age, sex, origin, reproductive life, contacts (in the household/cattery/pet shop), outdoor access and information about the health status and previous history including the familial information were obtained. The blood samples were collected from the jugular or cephalic vein with the owner's consent. Duplicate blood smears were prepared, properly packed and immediately sent to the Laboratory of Immunodiagnosis of the Department of Medical Clinics, School of Veterinary Medicine, University of São Paulo. Immediately after their arrival, the slides were fixed in acetone/methanol, dried and stored at -70°C until evaluation.

Two circular areas with diameter of approximately 1 cm were outlined in each blood smear slide, 10 µl PBS buffer with 1% bovine serum albumin were placed in the left area as a negative control, 10 µl FeLV antibodies (Primary reagent for FeLV IFA, VMRD, Inc., Pullman, WA, USA) were placed in the right area. For each group of slides being tested, a positive (blood smear from FeLV infected cat) and negative control were also included. The slides were incubated at 37 °C in a moist chamber during 30 minutes removed and rapidly immersed in washing buffer and then in distilled water. After drying, 10 µL of the anti-IgG FITC Conjugate (Secondary reagent for IFA, VMRD, Inc., Pullman, WA, USA) were added to each site, the slide incubated, washed once again and dried. The blood film was covered with a drop of buffered glycerin and coverslip and examined under high power magnification (x 400) in a microscope for immunofluorescence (Olympus BX60-Olympus Optical Co, Ltd., Japan). Positive and negative controls were included in each set of tests. Samples were tested in duplicate. The sample was considered positive when leukocytes and platelets were clearly fluorescent²³.

The status in relation to FeLV infection (FeLV+ and FeLV-) was considered as the dependent variable. Sex, age group (less than three years, between three and six years, more than six years), reproductive life (inactive and active), origin (household, cattery, pet shop, street), contacts in the household (none, from one to five, from six to fifteen, more than fifteen), and access to the street were the independent variables analyzed by chi-square test (χ^2)²⁴. If $p \leq 0.20$ the variable was included in the logistic regression analysis²⁵.

The multivariate model was built according to the stepwise forward method. The risk factors for the FeLV infection were identified by the multivariate model, and the strength of the association between the independent variable and the dependent variable was assessed by the odds ratio (OR) calculation with the software SPSS version 9.0. Variables with a signifi-

cance value of $p < 0.05$ were maintained in the final multiple model. A predictive model was built according to identified risk factors to calculate the probability of infection by FeLV.

Results

Fifty cats were positive for FeLV infection (6.2%). All variables analyzed by the χ^2 test had a significance value of $p < 0.05$ (Table 1). Most positive animals (74%) were three to six years old. The infection rate was higher in males compared to females ($p = 0.015$), as well as in the group of cats rescued from the street compared to the groups of cats born in home or cattery ($p = 0.001$). A larger number of infected cats was found in the group that had outside access compared to cats without access to the street ($p < 0.001$) and in the group with sexually intact cats ($p < 0.001$). Regarding to the number of contacts, the infection rate was higher in cats living alone or in small groups up to five animals in the household. The logistic regression analysis showed that outside access (OR = 47.252; $p < 0.001$), cat rescued or adopted from the street (OR = 3.221; $p = 0.008$), and age between three and six years (OR = 3.046; $p = 0.009$) remained as the main risk factors for FeLV infection among cats (Table 2).

A predictive model for the infection was built based on the results of the multivariate analysis and showed that cats with outside access have 18% more chances of becoming infected than those that are kept indoors (Table 3). If the cat has outside access and is three to six years old, the probability increases to 34%. If the cat has outside access and has been rescued from the street, the probability of being infected is 42%. If the cat is exposed to all factors, the probability of infection is even higher (63%).

Discussion

The current study revealed an overall prevalence of 6.2% for FeLV infection among cats from the city of

Table 1 - Frequency of FeLV infected cats from São Paulo city, Brazil according to gender, age, origin, number of cats sharing the same environment, reproductive life and access to the street - São Paulo - 2003 and 2004

		VLF (-)		VLF (+)		Total		P
		No.	%	No.	%	No.	%	
Sex	Female	409	95.8	18	4.22	427	100.0	0.015
	Male	353	91.6	32	8.31	385	100.0	
	< 3 yrs	300	93.2	15	4.8	315	100.0	
Age	3-6 yrs	249	89.9	22	10.14	271	100.0	0.042
	> 6 yrs	213	94.8	13	5.17	226	100.0	
Origin	Born in the house	183	95.8	8	4.18	191	100.0	0.001
	Shelter	197	98.5	3	1.50	200	100.0	
	Pet shop	50	94.0	3	6.00	53	100.0	
	Street	332	89.2	36	10.84	368	100.0	
Number of cats in the same household	1	84	84.8	15	15.15	99	100.0	<.0001
	2 - 5	161	92.0	14	8.00	175	100.0	
	6 - 15	153	96.8	5	3.16	158	100.0	
	> 15	364	95.6	16	4.21	380	100.0	
Reproductive status	Neutered	522	97.6	13	2.43	535	100.0	<.0001
	Intact	240	86.6	37	13.36	277	100.0	
Life style	Indoor	617	99.2	5	0.80	622	100.0	<.0001
	Outdoor	145	76.3	45	23.70	190	100.0	
Total		762	93.8	50	6.2	812	100.0	

VLF(-):negative for FeLV antigen., VLF(+):positive for FeLV antigen

Table 2 - Variables maintained in the final multiple model for identification of risk factors for FeLV infection in cats of S. Paulo (n = 812) - São Paulo - 2003 and 2004

Variable	Category	FeLV (+)/n	OR	p	95% CI	
					Lower	Upper
Age (years)	Less than 3	15/315	0.953	0.871 0.009	0.365 1.038	1.974 5.327
	3 to 6	22/271	3.046			
	More than 6	13/226	1.000			
Origin	Household	8/191	1.000	0.687 0.849 0.008	0.315 0.212 1.403	5.891 3.783 7.744
	Cat	3/200	1.353			
	Shelter	3/53	0.868			
	Pet shop	36/368	3.221			
Outside access	No	5/622	1.000	< 0.001	17.158	122.874
	Yes	45/190	47.252			

Table 3 - Probability of feline leukemia virus infection (Prob FeLV+) according to the presence or absence of the significant risk factors - São Paulo - 2003 and 2004

Prob FeLV+	Age group 3 to 6 years	Rescued from the street	Outdoor access
1%	1	0	0
2%	0	1	0
18%	0	0	1
42%	0	1	1
34%	1	0	1
4%	1	1	0
63%	1	1	1

1 = presence of the risk factor, 0 = absence

São Paulo, Brazil. As the aim of this study was to determine risk factors for FeLV infection among cats, a mixed sample comprised by healthy or sick cats, stray or household owned cats was studied. Therefore, the prevalence was lower than the previously reported in Brazil (12.5% to 32.5%)^{11,18} among sick cats tested at clinics or housed in large groups²⁶. Almost the same prevalence as observed in this study has been found on cat samples collected in other states of the country⁵.

FeLV infected cats were found more frequently among male, intact cats, 3 to 6 years old, rescued from the street and owned as single pets or in groups up to five cats (Table 1). As in other reports^{1,4,16} adult cats were found to be at risk to develop persistent FeLV infection ($p < 0.042$). The association between infection and active reproductive life observed in this study ($p < 0.001$) has also been previously reported¹. Outdoor access was evidenced as the main risk factor, with the strongest association with FeLV infection. All these risk factors have previously been reported by others^{14,27}.

Male cats were found to be more exposed to the risk. This finding was unexpected because earlier studies could not revealed association between FeLV infection and gender⁶ although similar results to ours were observed by other authors^{1,3,27}. For a long time, FeLV transmission is thought to occur between infected queens and their littermate, and among cats living in prolonged close contacts^{9,28}. As occur in FIV infection, aggressive behavior more common in male cats might play a greater role than previously thought. Cats presenting for fighting injuries were FeLV-positive in a higher rate compared to normal cat population¹⁵. Therefore, as suggested recently³, the older statement that FeLV is the disease of friendly cats should be reconsidered. Once infected, sharing the same environment with their housemates may represent a serious risk for naïve cats without outdoor access.

Another risk factor identified in our study was age. A high number of adults aging between three and six years was found in the positive group as previously

observed^{3,4,27}. At this stage of life, cats have more physical vigor and higher sexual activity, and, therefore, they have more probability to outdoor access. FeLV is a fatal disease with a relatively short course and cats persistently infected normally survive three to five years after infection^{9,29} or less³. This could explain the high proportion of FeLV-infected cats found in the young adult group (three to six years old) and the low prevalence among older cats. If those older cats experienced a regressive or latent infection is not known. FeLV infection may result in different scenarios, including latent infection with the presence of FeLV provirus DNA in peripheral blood and/or bone marrow cells, without antigenaemia¹⁹.

As FeLV is considered a common disease in cats living in groups and maintaining friendly contact, the infection rate might be expected to be higher in cats living in large groups, as reported previously^{6,30}. However, living in groups and sharing the same household was not associated with the infection in the present study. Similar results were found in a large study done in Germany³. This finding was unexpected because it is known that transmission takes place during prolonged close contacts among infected cats shedding the virus and susceptible cats⁹. The increasing awareness about FeLV infection leading to the adoption of preventive measures and the confinement of cats observed in many groups included in this study might have accounted for the low prevalence when compared to that found among single owned cats.

The importance of free access to the street with high possibility of contact with cats of unknown health status was highlighted by the strong association between outside access and FeLV infection ($OR = 47.2$). Several authors have already mentioned the outdoor access as one of the most important risk factors for FeLV infection^{17,30}. Cats rescued from the street might have had the opportunity of contact with free roaming FeLV infected cats, thus explaining the outdoor origin as a risk factor for FeLV infection. The frequency of FeLV

infection in stray cats (abandoned or feral) in Brazil is not known. In other countries, studies performed with unowned free roaming or feral cats showed infection rate of FeLV around 3.5 to 4.3%, similar to the figures found for owned cats^{31,32,33,34}.

Several studies showed sex and reproductive status as factors associated with FeLV^{29,30,35}. Usually, intact young male adult cats are more prone to outside access than females. However when analyzed in the multivariate model, outside access was more powerful than the gender or the reproductive status. Thus, both variables (being male and sexually active life) highlighted in the univariate model were not included in the final multivariate model.

A predictive model built based on the results of multivariate model stressed the importance of origin (rescued from the street), age and free outdoor access. When analyzed as single risk factor, age and the origin showed less impact on the spreading of the disease than the free outdoor access that means, the contact with other cats of unknown FeLV-status is the most

important risk factor. Life style and outside free roaming has been mentioned as the main risk factor^{1,16}. The knowledge of probability of being infected by FeLV has a practical importance for the prevention of the infection. Avoiding outdoor access along with the vaccination of high FeLV-risk cats may protect them against FeLV infection and FeLV-related diseases.

In conclusion, free roaming and outdoor access are the most important risk factors associated to FeLV infection among cats in São Paulo city and must be taken in consideration in the prevention of this retrovirus infection.

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