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Prevalence of and factors associated with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) in cats of the state of Santa Catarina, Brazil

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ABSTRACT

A cross-sectional study was conducted in 274 cats for determination of FeLV antigenemia and FIV seropositivity and factors associated with those infections in cats presented at the Veterinary Hospital of the Santa Catarina State University - UDESC (Brazil). Apparent prevalence for sick cats at the hospital population was 28.41% (95%CI 21.88–34.94%) for FeLV, 7.65% (95%CI 3.71–11.50%) for FIV and 2.18% (95%CI 0.56–5.47%) for both viruses. For healthy cats, the apparent prevalence was 9.89% (95%CI 3.75–16.02%) for FeLV, 2.20% (95%CI 0.34–7.75%) for FIV by immunoassay (ELISA). Average age for FeLV- and FIV-positive individuals was 38.32 and 64.25 months, respectively. Behavior such as aggressiveness and sex (male) were both associated with increased odds of result positivity test for FeLV and FIV; older animals were also associated with FIV test results. A very small proportion of the animals were vaccinated against FeLV and none against FIV. Most of the animals were adopted from shelters or rescued from streets, living with multiple cats that had access to outdoors. The high prevalence of FeLV suggests a need for better control strategies against this disease.

1. Introduction

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are known to be distributed worldwide [1]. FeLV is mainly associated with anemia, leukemia, and lymphoma in infected felines [2]. It is transmitted through oronasal exposure to the virus present in secretions; direct contact between animals; communitarian use of feeding bowls and through fights [3].

The main consequence of FIV in the infected organism is immunosuppression, but it can also develop neoplasia, blood dyscrasias due to myelosuppression, and neurological disorders [4]. Feline infection by FIV occurs mainly through the parenteral inoculation of viral particles present in the saliva or blood of infected animals [5].

The most widely used test for detecting FeLV and FIV infections is an immunoassay (ELISA), which detects FeLV p27 antigen in the bloodstream [3] and antibodies against the FIV virus protein p24 [1].

Previous studies estimated disease prevalence throughout the world. In Europe, prevalence ranged from 3.6% to 15.6% for FeLV and 3.2% to 8.3% for FIV [6–9]. In North America, prevalence of 2.3% to 7.5% was found for FeLV and 2.5% to 7.5% for FIV [10–14].

Characteristics including age, sex, access to outdoors and aggressive behavior were suggested to be associated with higher rates of positivity for these viruses [9,12,13].

Although Brazil is an extensive country in terms of territory, few studies are available on these viruses and prevalence data vary between 0.33%–31% for FeLV and 5.63%–11.7% for FIV [15–19]. As contamination occurs mainly through direct contact between cats, knowing the prevalence of infection in a cat population, as well as the factors

Abbreviations: FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; OR, odds ratio; CI, confidence interval

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associated to infection, is essential for the prevention of diseases [3]. Therefore, the purpose of this study was to estimate the apparent prevalence of FeLV-positive cats and FIV-seropositive at the Veterinary Hospital of the State University of Santa Catarina, Santa Catarina state, southern Brazil, and to evaluate the main factors associated to infection.

2. Material and methods

2.1. Population sample

A cross-sectional study was conducted at the Veterinary Hospital of the Santa Catarina State University (UDESC) located in the heartland of Santa Catarina state (Brazil). In total, 274 cats were randomly selected. Healthy cats (from routine examination or surgery for elective castration) and sick cats were included in this study, without discrimination of age, sex or breed. All the cats seen at the hospital from November 2015 to October 2016 were given a chance to participate in the study.

In the present study, the sampling size was calculated using the R software (Package Epicalc, *Foundation for Statistical Computing*, Vienna, Austria) [20] with a confidence interval of 90%, expected prevalence of 50%, and maximum error acceptable of 5%. Then, the minimum number of systematically sampled animals was 271 cats.

The present study was approved by the Ethical Committee on Animal Experimentation under the number 7932191015.

2.2. Epidemiological data

To be included in this study, the cat's owner had to sign a free and informed consent form, as well as answering a questionnaire. The information gathered were: breed (mixed-breed or by their specific breed), sex (male or female), reproductive status (intact or neutered), age (in months of life), lifestyle (from breeders or other households – born in the same house where they live and alley cats), outdoor access (yes or no), cat number per habitation (one or two and more animals per household), behavior towards other cats (docile or aggressive).

Also, we collected information about cat care by the owners, such as veterinary visit frequency (once, every month,every quarter, every semester, every annum, or only when sick), cat vaccination against disease (yes or no). In case of "yes" answers, we also asked vaccination frequency (annual, biennial, or sporadically) and if protect against FeLV".

Finally, medical records of each cat were analyzed, and clinical testing and blood collection for prevalence test were made concomitantly.

2.3. Testing protocol

Blood samples were collected by jugular vein puncture and stored in tubes without anticoagulant for serum separation. After blood clot formation (15 to 20 min after collection), the samples were centrifuged in 2000 *g* for 10 min, and the serum obtained was placed in a microtube and stored at −80 °C. The cats were evaluated individually by SNAP Combo Plus for FeLV and FIV – ELISA (*IDEXX Laboratories*®), which detects FeLV p27 antigen (sensitivity of 98.6% and specificity of 98.2%) and antibodies to the FIV p24 (sensitivity of 93.5% and specificity of 100%) [21]. This test was performed according to the instructions provided by the manufacturer; serum samples were taken from the freezer and left for 30 min at room temperature (21 °C), and then centrifuged prior to testing.

2.4. Statistical analysis

The outcome variable was the result of tests (positive/negative). To test the hypothesis of an association between FIV/ FeLV positivity and host-specific information, two models were constructed for statistical analysis. An unconditional logistic regression approach was used. Breed, sex, reproductive status, outdoor access, contact with other cats, and aggressiveness were analyzed as univariate variables.

All terms were checked for collinearity, and no significant correlations were found. Independent variables were tested using a chi-square test, and the Wald test was used for the age variable. All variables with $p < 0.20$ in the univariate analysis were considered for multivariate analysis. All bidirectional interactions between variables in the final models were evaluated for significance level $\alpha = 0.05$. The generated data were analyzed in R statistical software, version 2.13.1.

For the specific age, we also used the means test, where data were initially submitted to a normality analysis by the Shapiro Wilk test, and those with no normal distribution were transformed by Log. Then, the values generated were evaluated by the F and Tukey tests, both at $\alpha = 0.05$, using SISVAR statistical software.

Table 1
Descriptive statistical analysis of prevalence for FeLV and FIV positivity (n = 274) in cats seen at the Veterinary Hospital of the Santa Catarina State University (UDESC).

Factor	Groups	Total number by group (%)	FeLV + (%)	FIV + (%)
Breed	Persian	10 (3.65)	0	0
	Siamese	19 (6.93)	5 (1.82)	1 (0.36)
	Mixed-breed	245 (89.42)	56 (20.43)	15 (5.47)
Sex	Male	115 (41.97)	35 (12.77)	11 (4.01)
	Female	159 (58.03)	26 (9.49)	5 (1.82)
Neutered	Yes	149 (54.38)	31 (11.31)	11 (4.01)
	No	124 (45.26)	30 (10.94)	5 (1.82)
	Unknown	1 (0.36)	0	0
Access to outdoors	No	90 (32.85)	15 (5.47)	2 (0.73)
	Yes	184 (67.15)	46 (16.79)	14 (5.11)
Housing	Single cat per unit	110 (40.15)	24 (8.76)	8 (2.92)
	Multiple cats per unit	164 (59.85)	37 (13.50)	8 (2.92)
Aggressive behavior	No	151 (55.11)	28 (10.22)	3 (1.09)
	Yes	115 (41.97)	31 (11.31)	12 (4.38)
	Unknown	8 (2.92)	2 (0.73)	1 (0.36)

3. Outcome

Total apparent prevalence in the hospital population was 22.26% (95%CI 17.74–27.5%) for FeLV, 5.84% (95%CI 3.63–9.27%) for FIV, and 1.46% (95%CI 0.57–3.69%) for both viruses. Table 1 shows the distribution of positive cats for each variable.

Table 2 displays the test results of descriptive statistics for the factor age.

The feline population of the present study was also characterized regarding veterinary care and immunization. The results showed that only 40.15% (110/274) of the cats had received some type of vaccine, while the majority (59.85% [164/274]) had never been vaccinated. Only 8.39% (23/274) of the animals received the vaccine against FeLV in the last year, but none of them was vaccinated for FIV. Most of the

Table 2
Average age in months of FeLV- and FIV-positive cats seen at the Veterinary Hospital of the Santa Catarina State University (UDESC).

Feline	Average age	Age interval (months)	
		Minimum age	Maximum age
FeLV +	38.32a	5	120
FIV +	64.25b	12	132
Total	44.66a	3	216

For different lowercase letters, a significant difference was found between the means ($p < 0.05$).

cats have been to the veterinarian once [49.27% (135/274)] or only when got sick [32.85% (90/274)].Only 17.88% (49/274) of the owners took the cat to routine visits at least once a year.

Healthy cats represented 33.21% (91/274), of which the apparent prevalence was 9.89% (95%CI 3.75–16.02%) and 2.20% (95%CI 0.34–7.75%) for FeLV and FIV seropositive animals, respectively. Sick cats accounted for 66.79% (183/274), of which 28.41% (95%CI 21.88–34.94%) of them were FeLV seropositive, 7.65% (95%CI 3.71–11.50%) FIV seropositive, and 2.18% (95%CI 0.56–5.47%) seropositive for both. Among the sick FeLV-positive cats, 55.77% (29/52) showed clinical alterations related to the infection, such as anemia (65.51% [19/29]), neurological disorders (20.69% [6/29]), lymphoma (17.24% [5/29]), bacterial coinfections (10.34% [3/29]), and leukemia (6.9% [2/29]). Of the sick FIV-positive cats, 42.86% (6/14) presented clinical alterations associated with virus infection, such as bacterial coinfections (28.57% [4/14]) and gingivostomatitis (14.28% ([2/14]). Yet the seropositive cats for both viruses (50% [2/4]) had clinical alterations related to gingivostomatitis (one case) and leukemia (one case).

With respect to the origin, 46.35% (127/274) of the cats were adopted from the streets, 34.67% (95/274) were obtained from another home, 13.87% (38/274) were born in the same house where they lived, and only 5.11% (14/274) of them were purchased from breeders or veterinary establishments. As for the univariate analysis, FeLV seropositivity was significantly higher in male cats, aggressive cats, and those with access to outdoors ($p \leq 0.20$). The multivariate analysis showed a significantly increased association between sex and aggressive behavior (Table 3).

The univariate analysis showed a significant association for FIV seropositivity with higher age, male sex, outdoor access, and aggressiveness ($p \leq 0.20$). Likewise, the multivariate analysis presented a correlation of positive cases with aggressiveness, where aggressive cats were eight times more likely to be positive for FIV. Male cats were about six times more likely to be positive for FIV. Also, older animals were more likely to be infected, where with one-month increase in age represented 1% rise in the probability of positivity. Table 4 shows the results of univariate and multivariate analyses for factors associated to FIV positivity.

4. Discussion

The highest values observed for FeLV positivity may be related to population density and degree of contact or closeness between cats [22]. No previous studies have been carried out on cat population density for the state of Santa Catarina. However, there must be a high level of proximity between animals, given the fact that most of them have access to the outdoors. (67.15%). Possibly, this fact would facilitate a direct contact between cats and behaviors as grooming, and consequently more virus transmission. On the other hand, the lowest FIV positivity may derive from the lower transmission rate of this pathogen when compared to the FeLV. This is because FIV is mainly transmitted through bites, which, to be effective, must inoculate the virus parenterally. Yet, for FeLV transmission, only contact with oronasal mucosa and contaminated secretions is necessary [3,5,23,25].

When compared to the previous studies, the FeLV prevalence found here was higher than in other regions of Brazil, such as in the states of Bahia, São Paulo, and Rio de Janeiro (3.0%, 0.33%, 8%, and 11.52%, respectively) [15–17,19], but lower than that in the state of Rio Grande do Sul (31%) [18]. Regarding FIV prevalence, our findings were lower than those obtained in the states of São Paulo and Rio Grande do Sul (11.7% and 10.1%, respectively), but similar to other studies in the states of São Paulo and Bahia (5.63 and 6.0%, respectively) [15,16,18,19]. Out of these studies, two selected a sample with the same characteristics as ours, formed for health and sick cats from a hospital population; however, the results were different [15,18]. Two other studies obtained similar results for FIV positivity, despite the differences in recruitment methods. These studies cannot be used as an example because they used animals from a shelter or healthy cats [16,19]. In shelters, cats are submitted to a higher level of contact and kept in higher density of animals than in private residences; thus, low infection rates are expected in healthy cats.

Overall, the prevalence may vary with regions. In developed countries, the prevalence was reported to reach 2.3%–3.3% for FeLV and 2.5%–5.2% for FIV in the United States of America, 3.4% for FeLV and 4.3% for FIV in Canada, 3.6% for FeLV and 3.2% for FIV in Germany, 15.6% for FeLV and 8.3% for FIV in Spain, and 8.4% for FeLV and 11.3% for FIV in Italy [6,8–12]. In developing countries such as Mexico, a prevalence of 7.5% was found for FeLV, and in Costa Rica, it accounted for 16.7% for FeLV and 8.8% for FIV [14,24]. The variations between these studies can be related to differences in recruitment methods, sample sizes, or particularities such as geographic territory and health status of the cats [9,17,22].

In this study, the highest prevalence of FeLV seropositive cats might be related to the increased number of diseased cats, being, therefore, the largest sample (66.79%). Other studies have pointed out a higher prevalence of FeLV and FIV in sick cats when compared to healthy ones [6,7,15]. In addition, control and prevention methods for FeLV and FIV are scarce in the geographic region concerned, which can be confirmed by the small number of FeLV vaccinated animals.

Co-infection with FeLV and FIV have been described in the literature, but with a low prevalence, as observed in the present study. In Brazil, two studies reported co-infection cases with prevalence values of

Table 3
Estimated odds ratios (OR) and p-values of the factors associated with FeLV-p27 positive and negative cats (n = 274) from ELISA results.

Factor	Groups	Univariate analysis		Multivariate logistic regression	
		p-values	OR (95% CI)	*p-values*	OR (95% CI)
Age		0.20	0.99 (0.98-1.00)	0.23	
Breed	Persian	–	–	–	
	Siamese	0,99	–	0,99	
	Mixed-breed	0.99	–	0.99	
Sex	Male	0.005	2.23 (1.25-4.05)	0.003	2.41 (1.33-4.44)
	Female	–	–	–	–
Neutered	No	0.30	1.82 (0.98-2.01)	0.34	–
	Yes	–	–		
Access to outdoors	No	–	–	–	–
	Yes	0.11	1.66 (0.88-3.26)	0.15	
Housing	Single cat per unit	–	–	–	–
	Multiple cat per unit	0,88	1.04 (0.58-1.88)	0,99	
Aggressive behavior	No	–	–	–	–
	Yes	0.10	1.62 (0.90-2.91)	0.05	1.18 (1.00-3.30)

OR: Odds ratio; CI: confidence interval.

Table 4
Estimated odds ratios (OR) and p-values of the factors associated with FIV positive and negative cats (n = 274) from ELISA results.

Factor	Groups	Univariate analysis		Multivariate logistic regression	
		p-values	OR (95% CI)	*p-values*	OR (95% CI)
Age		0.10	1.00 (0.99-1.01)	0.05	1.01 (1.00-1.02)
Breed	Persian	–	–	–	
	Siamese	0.99	–	0.99	
	Mixed-breed	0.99	–	0.99	
Sex	Male	0.02	3.25 (1.14-10.59)	0.002	5.87 (1.81-23.42)
	Female	–	–	–	–
Neutered	No	0.26	1.39 (0.85-2.35)	0.34	–
	Yes	–	–		
Access to outdoors	No	–	–	–	–
	Yes	0.05	3.62 (1.00-23.40)	0.07	
Housing	Single cat per unit	–	–	–	–
	Multiple cats per unit	0.41	0.65 (0.23-1.83)	0.56	
Aggressive behavior	No	–	–	–	–
	Yes	0.002	5.74 (1.77-25.68)	0.001	8.00 (2.31-38.20)

OR: Odds ratio; CI: confidence interval.

0.25% and 4.4% [15,18]. In North America, two studies reported co-infection, both with a prevalence of 0.5% [11,12].

The significant association between FeLV- and FIV-positive cats with male sex found in this study might be related to their higher environmental exposure in relation to females. This might occur because male felines are more prone to aggressive behavior, such as fights for territory protection or courtship [7,25]. The association of FIV seropositive cases in male cats has been demonstrated by other studies in Brazil [15,16] and other countries [7,9,13,24]. Conversely, the cases of FeLV seropositivity in male cats are less common; however, this association has been reconsidered as relevant lately [6,9,26]. In Brazil, one study reported a higher proportion of FeLV-infected males, but not confirmed by statistical analysis [27].

Aggressiveness was also associated with infection by both viruses, but apparently more related to FIV infection [25]. This can be explained by the transmission characteristics of FIV virus, which occurs mainly by parenteral inoculation as a consequence of bites [5]. On the other hand, FeLV transmission generally occurs via oro-nasal contact, after a prolonged contact with infected animals [3]. After more inclusive evaluations of associated factors, animal gender and aggressiveness seem to be synergistic factors. Aggressiveness could be related to territorial behavior of the males, and thus increasing the risk of infection by parenteral inoculation through bites. A study in North America found a correlation between male cats and wounds or abscesses, with infections by both FeLV and FIV, thus demonstrating the relevance of fights for the inoculation of viral particles [28].

A correlation was found between serology and animal age only for FIV, with older cats being more likely to be positive [7,9,25]. Additionally, when evaluating average ages, FIV-positive animals were older than FeLV-positive ones (38.32 months). This increase in age, compared to FeLV-positive cats, might occur due to a longer incubation period compared to FIV-positive animals, within which the feline could remain asymptomatic for years. Distinctly, FeLV-positive cats have more impact on morbidity and mortality rates, and the susceptibility to infection decreases with age [13,29–31]. Similar results were obtained in a retrospective study, where the mean ages for FeLV (48 months) and FIV (96 months) positive cases presented a significant difference [32].

Access to outdoors was one of the evaluated factors that showed an association with FeLV and FIV positivity by the univariate analysis. Unexpectedly, this finding was not observed in the multivariate analysis, which might be due to the difference in sample size between groups. Again, this important factor was also found in several other studies on FeLV and FIV infections [17,28,33].

Unlike this study, the association between intact cats (non-castrated) with FeLV and FIV positivity has already been highlighted in previous studies [12,17,28,33]. This was unexpected for the cat population concerned, as aggressiveness in fighting for territory and during mating is considered an efficient way of transmitting retroviruses [12]. Other factors might have influenced the outcome, such as the old age of cats to be castrated, what increased their reproductive life in months or even years.

5. Conclusion

When associated with information on responsible pet ownership, the factor associated with retrovirus infection, the high prevalence for FeLV and the evidence of FIV circulation in the feline population investigated here demonstrate the high risk of infection by both retroviruses in the cat population of the Planalto Catarinense, southern region of Brazil. It also places emphasis on the need for prophylactic measures to avoid such infections.

The associated factors may vary according to the geographic region and the characteristics of the population evaluated. Adequate epidemiological studies should be implemented, so that prophylactic measures could be taken correctly and effectively.

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Conflicts of interest

None.

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