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Risk factors for feline immunodeficiency virus antibody test status in Cats Protection adoption centres (2004)

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A study was carried out to determine the prevalence of feline immunodeficiency virus (FIV) within a population of cats entering 10 UK adoption centres run by Cats Protection. All cats entering the adoption centres during 2004 were tested for FIV using a rapid enzyme immunoassay antibody test. The overall prevalence of positive test results was 3.1% (95% confidence intervals (CI) 2.7–3.5%), whilst the prevalence at different adoption centres varied from 0.8% (95% CI 0.1–1.5%) to 6.7% (95% CI 4.9–8.5%). Results of the multivariable logistic regression analysis showed that male cats, stray/feral cats and cats in poor health were at a greater risk of testing positive for FIV than female cats, cats that were relinquished by an owner and cats that were in good/fair health, respectively. No evidence was found for an association between neuter status and FIV test results. This study may help to identify cats that are relinquished to rescue centres with an increased risk of FIV for routine FIV testing.

Feline immunodeficiency virus (FIV) was first described by Pedersen et al in 1987,[1] although evidence suggests that FIV has been present in the cat population for many years prior to the first documented report of the disease in 1968.[2] Considerable variation exists in the clinical signs associated with FIV infection and the progression of the disease between individual cats; however, it is clear that FIV infection can be associated with significant disease and is an important cause of mortality, being the most commonly reported cause of mortality (resulting from death or euthanasia) in adoption centres of a UK cat rescue charity.[3]

The factors that influence whether or not and when a cat infected with FIV will develop clinical disease are not fully understood, although it is hypothesised that the presence of concurrent disease[4] and variable pathogenicity associated with different clades[5] may play a role. It is suspected that some cats infected with the virus never develop significant disease, although the reasons why and the proportion of cats that this applies to are unknown. While FIV may directly cause specific clinical disease, for example, neurological signs,[6,7] disease in FIV-infected cats is usually related to immunosuppression that leads to other infectious agents causing illness or, more commonly, to the development of chronic conditions such as gingivitis and rhinitis.

The prevalence of FIV varies greatly between different populations. UK studies have reported the prevalence of antibodies to FIV to be 19% in sick pet cats ($n = 1204$), 6% in healthy pet cats ($n = 1007$)[6] and 10% in a sample of 517 stray cats brought to a Royal Society for the Prevention of Cruelty to Animals (RSPCA) veterinary hospital.[8] Unpublished data collected from Cats Protection (CP) adoption centres that tested all cats admitted during a 1-year period (November 1997–November 1998) revealed that 4.6% (138/3010) of cats over the age of 4 months were seropositive for FIV (M Roberts, personal communication, 2007). The prevalence of FIV is believed to be higher in free-living stray/feral cat populations than in domestic cat populations as suggested by a study conducted in Eastern Australia in which FIV prevalence was reported to be 22% (15/68) among feral cats and 8% (27/340) among pet cats.[9] The prevalence of FIV infection is a particularly important topic for rescue organisations as evidence suggests that rescue cats may have a higher prevalence of FIV infection compared to household cats due to a higher proportion of stray and feral cats in the rescue population.[9] Consequently, some rescue centres have

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policies to routinely test all cats to avoid inadvertently homing FIV-infected cats. In contrast, Levy et al[10] conducted a study of over 18,000 USA cats and reported a significantly higher prevalence of FIV among cats tested at veterinary clinics (3.1%) than cats tested at animal shelters (1.7%). However, the authors suggested that these prevalences should be interpreted with caution due to potential biases associated with the different proportion of sick animals that were tested in both groups. In particular, cats tested at veterinary clinics were likely to be sick cats in which testing was carried out for diagnostic purposes. However, we are not aware of any published studies that have used multivariable analysis to examine risk factors for FIV infection in a UK population that included domestic and stray/feral cats and that were not tested on the basis of their health status. The aim of this study was to estimate seroprevalence of FIV in a population of rescue centre cats and to identify risk factors associated with FIV infection. The results of this study can be used by rescue centres wishing to prioritise groups of cats to be routinely tested for FIV.

Materials and methods

Cats Protection (CP) is the UK's leading feline welfare charity, rehoming or reuniting approximately 55,500 cats each year. Data collected by 10 CP adoption centres that tested all cats aged 6 months or more for antibodies to FIV during 2004 were included in the study. Kittens that were known or estimated to be less than 6 months of age were excluded from testing as antibody tests may not reliably detect infection in young kittens due to the possible presence of maternally derived antibodies. The Idexx snap combo test was used as a screening test for FIV antibodies at CP adoption centres and the results of this study are, therefore, based on the FIV-antibody test results.

Prevalence

The prevalence of FIV-antibody positive cats (based on the Idexx test results) was calculated and 95% confidence intervals (CI) were reported for the sample of 7098 cats that were tested. Prevalence for individual groups of cats (eg, male cats, stray/feral cats) was not calculated since the data required to do this were not easily retrievable from the individual centres, as data relating to potential explanatory variables were often not stored electronically. (Data for the 879 cats included in the case–control study were retrieved manually by adoption centre managers once controls had been randomly selected by their unique identification numbers.)

Chi-squared analysis was used to compare the prevalence of cats that tested positive for antibodies to FIV on admission to CP adoption centres during 1997–1998 and during 2004. Details on the testing protocols that were used during 1997–1998 were unavailable; however, it is likely that the protocols used may have differed. Statistical significance was set at $P < 0.05$.

Case–control study

A matched case–control study design with a ratio of three controls per case was used to test associations between the variables of gender, neuter status and health of the cat when it was admitted to CP and the cat's background (previously owned or stray/feral) and the risk of testing positive for antibodies to FIV. The use of a 3:1 control to case ratio was used to maximise the power of the study for the number of cases that could be identified in the 1-year data collection period. Control cats were individually matched to case cats by CP adoption centre to facilitate retrospective data collection.

A sample size of 576 cats (144 FIV positive cats and 432 FIV negative cats) was calculated to provide the case–control study with 80% power to detect an odds ratio (OR) of 2.0 or more. These calculations were based on a 0.05 probability of a type-I error (95% confidence) and assuming that 15% of controls were exposed to risk factors (Epi-Info 6, CDC, USA). The final multivariable model was based on 723 cats (180 FIV positive cats and 543 FIV negative cats).

Case definition and selection

A case cat was defined as a cat that had tested positive for antibodies to FIV on admission to one of the study adoption centres during 2004. All cats fulfilling the case definition were included as cases.

Control definition and selection

A control cat was defined as a cat that had tested negative for antibodies to FIV on admission to one of the study adoption centres during 2004. Three control cats were selected, using randomly generated numbers, from all eligible control cats at the centre from which their matched case was identified.

Outcome

The outcome under investigation in this study was the FIV test result, based on Idexx snap combo test results. Ideally a polymerase chain reaction (PCR) test would have been used as a confirmatory test for all samples, but the retrospective nature of this study meant that confirmatory results were not available for all samples. However, the results of this study are based on field-based data and facilitate comparison of results with other studies based on FIV-antibody test results (eg, see Levy et al[10]).

The manufacturers reported that, at the time of testing (2004), the Idexx snap combo test had a sensitivity of 100% and a specificity of 99.6% (Idexx, personal communication 2004). Hartmann et al[11] also reported the sensitivity and specificity (and 95% CI) of the Idexx snap combo test to be 100% (93.1–100%) and

99.6% (98.5–99.9%), respectively, when using Western blot as the gold standard. However, difficulties are recognised in assessing the sensitivity and specificity of tests in the absence of a generally agreed confirmatory gold standard, as is the case with FIV testing, hence the potential for false positive and false negative results within the dataset is small but still exists.

Potential risk factors

Data relating to the variables of gender, neuter status, previous history (owned, stray or feral) and current health (good, fair, poor) were collected from adoption centre records for each cat in the study. The 'health' categories (good, fair, poor) were subjectively assigned by each adoption centre manager, based on data relating to the cat's health that had been collected at the time of admission to the centre. Although prospective records of the cat's health at the time of admission were used to minimise bias associated with the cat's FIV test result, it is possible that some bias might still be present due to adoption centre managers' knowledge of the cat's FIV test result and previously identified risk factors for FIV. The variable of age was not considered for analysis due to the lack of reliable information available for stray and feral cats.

Data were obtained for 219 cases and 660 controls; however, missing data existed for some cases and controls.

Statistical analysis

Variables listed in Table 1 were tested for association with FIV test result using univariable conditional logistic regression models. The statistical package Egret (Cytel Software Corporation, USA) was used for data analysis. Variables with a univariable P-value <0.3 were considered for inclusion in a multivariable model, which was built using the technique of backward elimination. The effect of biologically plausible interactions between variables was also tested for in the model.

Table 1. Description of variables included in a study of risk factors for FIV positive status in cats tested at CP adoption centres during 2004

Variable	Description
Gender	Gender of cat (male, female)
Neuter status	Neuter status of cat at time of admission to CP (neutered, unneutered)
Previous history	Previous history of cat at time of admission to CP (owned, stray or feral)
Health	Health of cat at time of admission to CP (good, fair, poor)

Population proportional attributable risk (PPAR)

PPAR represents the fraction of cases that would not have occurred if they had not been exposed to the risk factor.[12] The PPARs were calculated for each of the explanatory variables included in the final multivariable model by the method outlined by Bruzzi et al.[13]

Results

Details of the participating CP adoption centres and the numbers of cats tested are listed in Table 2. The overall prevalence of cats testing positive for antibodies to FIV was 3.1% (95% CI 2.7–3.5%); however, the prevalence of FIV positive cats ranged from 0.8% (95% CI 0.1–1.5%) to 6.7% (95% CI 4.9–8.5%) at different adoption centres.

The prevalence of FIV positive test results was significantly ($P = 0.01$) lower (3.1%) in cats admitted to CP adoption centres in 2004 than in 1997–1998 (4.6%, 95% CI 3.8–5.3%).

The results of the univariable conditional logistic regression analysis are summarised in Table 3.

The variables of gender, previous history of the cat and health on admission to CP remained significantly ($P < 0.001$) associated with the risk of testing FIV positive in the multivariable analysis (Table 4). The variable of the cat's health on admission to CP was reduced from three categories (good, fair, poor) to two categories (good or fair, poor) to significantly ($P < 0.05$) improve the fit of the model. The variable of the previous history of the cat was reduced from three categories (owned, stray or feral) to two categories (owned, stray/feral) in an attempt to better represent the difference between free-living cats (stray/feral) and owned cats whilst significantly improving the fit of the model. In addition, the use of two categories was believed to

Table 2. CP adoption centres included in the study of risk factors for FIV positive status

CP adoption centre	Number of cats tested for antibodies to FIV	Number of cats that were positive for antibodies to FIV	Percentage of cats that were positive for antibodies to FIV (95% CI)
1	974	36	3.7 (2.5–4.9)
2	759	34	4.5 (3.0–6.0)
3	973	29	3.0 (1.9–4.1)
4	673	19	2.8 (1.6–4.1)
5	390	5	1.3 (0.2–2.4)
6	616	5	0.8 (0.1–1.5)
7	730	49	6.7 (4.9–8.5)
8	270	8	3.0 (0.9–5.0)
9	883	23	2.6 (1.6–3.7)
10	830	11	1.3 (0.6–2.1)
Total	7098	219	3.1 (2.7–3.5)

Table 3. Univariable conditional logistic regression results of OR, 95% CI and *P*-values of risk factors associated with FIV positive cats tested by CP during 2004

Variable	Number of cases (%)	Number of controls (%)	OR (95% CI)	*P*-value
Gender				
Female*	57 (26)	340 (53)	1.00	<0.001
Male	162 (74)	299 (47)	3.25 (2.29–4.63)	
Neuter status				
Neutered	130 (60)	361 (64)	1.00	0.73
Entire	87 (40)	206 (36)	1.07 (0.74–1.54)	
Previous history				
Owned*	76 (35)	343 (55)	1.00	<0.001
Stray	107 (49)	196 (32)	2.84 (1.95–4.12)	
Feral	35 (16)	81 (13)	2.68 (1.45–4.96)	
Health				
Good*	71 (39)	265 (48)	1.00	<0.001
Fair	28 (16)	187 (34)	0.59 (0.31–1.11)	
Poor	82 (45)	100 (18)	3.67 (2.12–6.34)	

*Reference category.

reduce misclassification that might have arisen when adoption centre managers recorded a cat as stray or feral. No statistically significant interactions were found between variables in the multivariable model.

Population proportional attributable risk

The PPARs were calculated for each of the explanatory variables included in the final multivariable model (Table 5). The PPARs were derived from multiple logistic regression and, therefore, were not additive.

Discussion

FIV is an important cause of feline disease; however, there is limited information on the prevalence of the disease in the UK and to the authors' knowledge this study is the first to use multivariable analysis to examine risk factors for FIV infection in a population of UK cats that were tested for FIV regardless of their health status.

The results of this study provide evidence that male cats, stray/feral cats and cats in poor health were at an increased risk of testing positive for antibodies to FIV in 2004 at the adoption centres included in this study (Table 4). The prevalence of FIV positive cats was shown to be significantly lower in this study (3.1%) compared to the 4.6% found in the 1997–1998 study of cats tested at CP adoption centres. The reason for the differences in the prevalence may be due to a drop in the true prevalence, may have resulted

Table 4. Multivariable conditional logistic regression model of OR, 95% CI and *P*-values of risk factors associated with FIV positive cats tested by CP during 2004

Variable	Number of cases (%) ($n = 180$)	Number of controls (%) ($n = 543$)	Adjusted* OR (95% CI)	*P*-value
Gender				
Female†	43 (23)	292 (54)	1.00	<0.001
Male	137 (76)	251 (46)	3.31 (2.11–5.20)	
Previous history				
Owned†	57 (32)	282 (52)	1.00	<0.001
Stray/feral	123 (68)	261 (48)	3.09 (1.88–5.08)	
Health				
Good/fair†	98 (54)	447 (82)	1.00	<0.001
Poor	82 (46)	96 (18)	4.22 (2.54–7.01)	

*Adjustment is for all variables shown.
†Reference category.

Table 5. PPAR values of explanatory variables for risk factors associated with FIV positive cats tested by CP during 2004

Explanatory variable	PPAR
Gender	
Female	0.00
Male	0.53
Previous history	
Owned	0.00
Stray/feral	0.46
Health	
Good/fair	0.00
Poor	0.35

from different test protocols that were used or may have resulted from different proportions of 'high risk' cats that were admitted to the centres during the two study time periods. Unfortunately, test protocol details and data are not available to compare the proportions of 'high risk' cats included in each study, so the latter two hypotheses cannot be explored further. The prevalence observed amongst stray cats tested at CP centres (9.8%) in 1997–1998 (unpublished data) was similar to the prevalence rate reported by Muirden[8] for stray cats sent to a RSPCA hospital during a 5-month period in 1997 (10.4%). More recently published figures of the prevalence of FIV in UK cats are not available for comparison with this study.

In agreement with a previously published study,[8] the results of this study indicated that male cats were approximately three times more likely than female cats to be FIV positive. The reason for this association is believed to be linked to the predominant transmission route of FIV through bite wounds,[14] as males are believed to be more likely than females to bite during displays of territorial aggression. There is a widely held belief that entire males are at an increased risk of FIV infection when compared to neutered males. In order to explore further whether entire males were at a higher risk of FIV infection than neutered males in our study, we combined the two variables of gender and neuter status into a single variable with four categories (neutered females, neutered males, entire females, entire males). Neutered males and entire males were four times more likely to test positive for FIV antibodies (OR 4.21 and 4.10, respectively) when compared to the reference group of neutered females. However, the fit of the multivariable model was not significantly ($P = 0.25$) improved when compared to the final model presented in Table 4, indicating that it is indeed the gender of the cat rather than the effect of neuter status on gender that influences FIV-antibody status. Levy et al[10] also reported no significant ($P > 0.26$) association between the combined variable of a cat's gender and neuter status and the risk of FIV seropositivity following multivariable analysis. Similarly, Hopper and Muirden[8,15] reported that male cats (regardless of their neuter status) were at an increased risk of testing positive for FIV antibodies when compared with female cats. Our results and those of Levy's, Hopper's and Muirden's studies suggest that entire or neutered male cats are just as likely to test seropositive for FIV when compared to female cats. Hence, the widely considered belief that entire males have a higher risk of FIV seropositivity than neutered males appears to be a misconception that is not supported by published studies.

The previous history of the cat was also shown to be associated with FIV status. Cats that were admitted to CP from stray or feral backgrounds were approximately three times more likely to be FIV positive than cats that were relinquished by their owners. An increased opportunity and tendency for stray/feral cats to fight with other cats is thought to explain this association. Data related to the previous history of the cat may have been subject to misclassification. For instance, it is possible that some cats that were classified as 'owned' cats had previously been stray or feral cats before being taken in by a household. In addition, it is possible that some owners were reluctant to admit to relinquishing their cat to an adoption centre and may have claimed that the cat was a stray in order to reduce their embarrassment. Both of these sources of misclassification would result in the association between stray/feral cats and the risk of FIV positive test results being underestimated.

As the health of the cat at the time of infection and the time since infection are not known, no assumptions can be made about whether the cat's health is a cause or an effect of FIV infection. The health of the cat at the time of admission to the CP shelter may be more likely to be related to the effect of FIV status rather than acting as a contributing risk factor for FIV infection; however, a prospective study would be needed to explore this association further. The results of this study indicate that cats in poor health were approximately four times more likely to have a positive FIV test result when compared with cats in good or fair health. Levy et al[10] used two categories (healthy or sick) for their variable of health status and reported that sick cats were 2.7 times more likely to have a positive FIV test result when compared to healthy cats.

The variable of age was not included in the analysis due to the difficulty in reliably assessing the age of stray and feral cats. However, previous work has suggested that increasing age is associated with an increased risk of testing positive for FIV. This association might be expected, as there is a long incubation period before disease develops and once a cat has been infected with FIV the cat will test positive for the remainder of its life. Therefore, the proportion of cats testing positive for FIV will increase as the age of the cat increases. A future study, including age as a categorical variable is recommended; however, the accuracy of data relating to the age of stray/feral cats may be difficult to establish.

Controls were matched to cases by adoption centre to facilitate retrospective data collection; however, this had the disadvantage of excluding analysis of adoption centre as a potential risk factor. The reasons for the variation in FIV prevalence between adoption centres (0.8–6.7%) are not clear, but are likely to be related to geographical variations in prevalence and to the proportion of stray/feral cats, male cats and cats in poor health that are admitted to the centres. Valuable information could be obtained from a further study designed to enable the location of the adoption centre to be assessed as a potential risk factor and as a possible confounder for other risk factors identified in the analysis reported in this study.

Many rescue organisations have limited resources and consequently need to consider which groups of cats to prioritise for FIV testing, to ensure that their funds are used as effectively as possible for the cats they care for. The PPARs for variables included in the multivariable model (Table 5) may be used to inform future policy decisions relating to FIV testing in rescue centres. This study showed that if a policy exists to test all stray/feral cats for FIV, then it is even more important that all male cats should also be tested for FIV, regardless of their previous history, health status and neuter status, as the PPARs (Table 5) indicated that 53% of the FIV positive tests recorded in this study were associated with male cats and 46% were associated with stray/feral cats. However, consideration should also be given to the implications of false positive and false negative results and the costs associated with FIV testing and laboratory confirmation of results.

The results of this study were based on screening test results, as PCR confirmatory test results were not available for all screening tests. Samples that tested positive for FIV antibodies were sent to Langford Veterinary Diagnostics at the University of Bristol for confirmation of diagnosis by PCR assay. However, occasionally cats with positive FIV-antibody test results were euthanased on humane grounds without a confirmatory test result due to their very poor health. Negative FIV-antibody test results were checked only if there was a high index of suspicion of FIV infection. Ideally a confirmatory test would have been used for all samples, but the retrospective nature of this study meant that confirmatory results were not available for all samples. However, the results of this study are based on field-based data generated through the routine practices employed by adoption centres and the use of these data is justified by the production of results that are of more benefit to adoption centres than results produced from data generated by non-routine practice.

Although laboratory confirmation of all test results (positive and negative) is recommended to increase confidence in the diagnosis, the choice of a confirmatory gold standard test remains difficult for retroviruses such as FIV which integrate into the host genome.[16] The specificity of the Idexx Snap Combo test was unlikely to have been 100%; hence, it is possible that some false positive results were present within our dataset. The presence of false positives would have led to an overestimation of the prevalence of FIV infection; however, we consider it unlikely that the results of the multivariable analysis will have been biased as the accuracy of the test is unlikely to have varied in the presence of the different risk factors under investigation. Since the positive predictive value of the test decreases and the negative predictive value of the test increases as the true prevalence of disease decreases, an adoption centre with a low prevalence of FIV is expected to incorrectly detect a higher proportion of FIV positive cats (ie, more false positives) when compared with an adoption centre with a higher prevalence of FIV. In addition, test results should always be interpreted with caution as a cat with a negative antibody test result may still be infected with FIV and test positive with other test modalities such as PCR.[15,17]

In addition to the variables identified in this study, there may be other variables that were not included in this study that are associated with an increased risk of testing positive for FIV. Other variables that might merit investigation include the previous health of the cat, presence of abscesses and fighting wounds.

The results of this study indicated that within a population of cats entering 10 CP adoption centres during 2004, male cats, stray/feral cats and cats in poor health had a greater risk of testing positive for FIV than female cats, cats that were relinquished by an owner and cats that were in good/fair health, respectively. No evidence was found to support the common belief that an association exists between neuter status and FIV test results. This study may help rescue centres to prioritise cats that have an increased risk of FIV for routine FIV testing.

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